2014 KRIBB Article Abstracts
First or corresponding articles indexed in SCIE, Scopus, and PubMed
Contents

01 Genome Institute (Article 1~50)
- Genome Structure Research Center
- Epigenomics Research Center
- Functional Genomics Research Center

27 Aging Research Institute (Article 51~76)
- Aging Intervention Research Center
- Infection and Immunity Research Center
- Immunotherapy Research Center

41 Bioconvergence Research Institute (Article 77~102)
- Biochemicals & Synthetic Biology Research Center
- BioNanotechnology Research Center
- Super-Bacteria Research Center

55 Future Biotechnology Research Division (Article 103~132)
- Biomedical Translational Research Center
- Plant Systems Engineering Research Center
- Industrial Bio-materials Research Center

71 Division of Biological Infrastructure (Article 133~195)
- Microbial Resource Center
- Laboratory Animal Resource Center
- International Biological Material Research Center
- Human Derived Material Center
- Korea National Primate Research Center
- Bio-Evaluation Center
- ABS Research Support Department
105 Division of Research & Business Development (Article 196~197)
- Biotechnology Process Engineering Center

107 Division of KRIFF Strategic Projects (Article 198~219)
- Viral Infectious Disease Research Center
- Stem Cell Research Center
- Korean Bioinformation Center

119 Bio-Therapeutics Research Institute (Article 220~252)
- Natural Medicine Research Center
- Chemical Biology Research Center
- Incurable Disease Therapeutics Research Center

137 Integrated Biorefinery Research Institute (Article 253~295)
- Sustainable Bioresource Research Center
- Bioenergy and Biochemical Research Center
- Industrial Microbiology and Bioprocess Research Center
- Eco-friendly Biomaterial Research Center
- Other Articles (Article 296~303)

161 Indexes
- Author Index
- Journal Index
- Keyword Index
Genome Institute

- Genome Structure Research Center
- Epigenomics Research Center
- Functional Genomics Research Center
Carbon monoxide confers protection in sepsis by enhancing Beclin 1-dependent autophagy and phagocytosis

Antioxid Redox Signal. 20(3):432-42.

*Co-first: Seon-Jin Lee(sjlee@kribb.re.kr)

AIMS: Sepsis, a systemic inflammatory response to infection, represents the leading cause of death in critically ill patients. However, the pathogenesis of sepsis remains incompletely understood. Carbon monoxide (CO), when administered at low physiologic doses, can modulate cell proliferation, apoptosis, and inflammation in pre-clinical tissue injury models, though its mechanism of action in sepsis remains unclear.

RESULTS: CO (250 ppm) inhalation increased the survival of C57BL/6J mice injured by cecal ligation and puncture (CLP) through the induction of autophagy, the down-regulation of pro-inflammatory cytokines, and by decreasing the levels of bacteria in blood and vital organs, such as the lung and liver. Mice deficient in the autophagic protein, Beclin 1 (Beclin1(+/-)) were more susceptible to CLP-induced sepsis, and unresponsive to CO therapy, relative to their corresponding wild-type (Beclin1(+/-)) littermate mice. In contrast, mice deficient in autophagic protein microtubule-associated protein-1 light chain 3B (LC3B) (Map1lc3b(-/-)) and their corresponding wild-type (Map1lc3b(+/-)) mice showed no differences in survival or response to CO, during CLP-induced sepsis. CO enhanced bacterial phagocytosis in Beclin1(-/-) but not Beclin1(+/-) mice in vivo and in corresponding cultured macrophages. CO also enhanced Beclin 1-dependent induction of macrophage protein signaling lymphocyte-activation molecule, a regulator of phagocytosis.

INNOVATION: Our findings demonstrate a novel protective effect of CO in sepsis, dependent on autophagy protein Beclin 1, in a murine model of CLP-induced polymicrobial sepsis.

CONCLUSION: CO increases the survival of mice injured by CLP through systemic enhancement of autophagy and phagocytosis. Taken together, we suggest that CO gas may represent a novel therapy for patients with sepsis.

Keywords: Apoptosis regulatory proteins; Autophagy; Carbon monoxide; Cell proliferation; Inflammation; Liver; Lung; Phagocytosis; Punctures; Sepsis

PMID: 23971531

NSC26188 induces apoptosis of prostate cancer PC-3 cells through inhibition of Akt membrane translocation, FoxO3a activation, and RhoB transcription


Won KJ, Kim BK, Han G, Lee K, Jung YJ, Kim HM, Song KB, Chung KS, Won M.
*Co-corresponding: Misun Won(misun@kribb.re.kr), Kyung-Sook Chung(kschung@kribb.re.kr)

We previously reported that NSC26188 caused apoptosis of cancer cells by inducing expression of RhoB. We here present that NSC26188 induces apoptosis of prostate cancer PC-3 cells by inhibiting Akt/FoxO3 signaling, which mediates RhoB upregulation. The apoptosis and Akt dephosphorylation caused by NSC26188 was not substantially relieved by overexpressing wild-type Akt but was relieved by overexpressing constitutively active Akt (CA-Akt) or myristoylated Akt (myr-Akt). Furthermore, overexpression of CA-Akt or myr-Akt downregulated RhoB expression, indicating that RhoB expression is regulated by Akt signaling. Interestingly, membrane translocation of GFP-Akt by insulin exposure was abolished in the cells pretreated with NSC26188 suggesting that NSC26188 directly interfered with translocation of Akt to the plasma membrane. In addition, NSC26188 activated FoxO3a by dephosphorylating S253 via Akt inhibition. Activated FoxO3a translocated to the nucleus and increased transcription of RhoB and other target genes. PC-3 cells transiently overexpressing FoxO3a exhibited increased RhoB expression and apoptosis in response to NSC26188. Conversely, FoxO3a knockdown reduced NSC26188-induced RhoB expression and cell death. These results suggest that RhoB may be a target gene of FoxO3a and is regulated by Akt signaling. Taken together, NSC26188 induces apoptosis of PC-3 cells by interfering with membrane recruitment of Akt, resulting in Akt dephosphorylation and FoxO3a activation, which leads to transcription of RhoB.

Keywords: Antineoplastic agents; Apoptosis; Forkhead transcription factors; FoxO3a; Gene expression regulation; NSC26188; Piperazines; Prostatic neoplasms; Protein transport; Proto-oncogene proteins c-akt; RhoB GTP-binding protein

PMID: 24085402
P300 cooperates with c-Jun and PARP-1 at the p300 binding site to activate RhoB transcription in NSC126188-mediated apoptosis


*Corresponding: Misun Won(misun@kribb.re.kr)

The anti-cancer agent NSC126188 induces apoptosis of stomach carcinoma NUGC-3 cells by inducing RhoB expression. Here, we present that the p300 binding site in the RhoB promoter is crucial for the binding of p300 and its partner transcription factors to activate RhoB transcription in NSC126188-mediated apoptosis. NSC126188 increased expression of p300 and c-Jun. Conversely, knockdown of p300 decreased RhoB expression in the presence of NSC126188. We found that poly(ADP-ribose) polymerase-1 (PARP-1) was associated with the p300 binding site and that PARP-1 knockdown inhibited NSC126188-mediated RhoB expression. In the cells treated with NSC126188, p300, PARP-1, and c-Jun interacted and bound the p300 binding site. Furthermore, chromatin immunoprecipitation (ChIP) analysis revealed strong p300 binding and weak c-Jun binding at the p300 binding site of RhoB promoter in cells treated with NSC126188. We also demonstrated that c-Jun played a crucial role in p300 binding. However, PARP-1 did not directly bind the p300 binding site, suggesting a bridging role between p300 and c-Jun. Electrophoretic mobility shift assays demonstrated a complex comprising p300/c-Jun/PARP-1 that bound wild type, but not a mutated, p300 binding site. In addition, overexpression of p300, PARP-1, or c-Jun dramatically enhanced RhoB promoter activity when it contained the wild type sequence but not mutated sequences, indicating the crucial role of the p300 binding site in NSC126188-induced transcription of RhoB. Taken together, these data suggest that p300 is recruited and cooperates with c-Jun and PARP-1 at the p300 binding site to activate RhoB transcription during NSC126188-mediated apoptosis.

Keywords: Apoptosis; NSC126188; PARP-1; RhoB; c-Jun; p300

Inhibition of STAT3 activation by KT-18618 via the disruption of the interaction between JAK3 and STAT3


Shin DS, Jung SN, Yun J, Lee CW, Han DC, Kim B, Min YK, Kang NS, Kwon BM*.
*Corresponding: Byoung-Mog Kwon(kwonbm@kribb.re.kr)

The constitutive activation of STAT3 in human cancers causes the abnormal proliferation and survival of cancer cells, and thus, STAT3 is a therapeutic target of antitumor drugs. We screened a small-molecule library of 8600 synthetic compounds from the "Korea Chemical Bank" to identify inhibit STAT3 activity using a cell-based luciferase assay system. KT-18618 ((Z)-(4-chlorophenyl)-N-methyl-2-[1,3,3,3,-tetrafluoro-2-(thiophen-2-yl)prop-1-enyloxy]-acetamide) was selected as a novel inhibitor of the JAK3/STAT3 pathway. KT-18618 inhibited STAT3 phosphorylation and the expression of STAT3-regulated genes. The inhibition of STAT3 phosphorylation led to the apoptosis of MDA-MB-468 cells. We postulated that the inhibition of the JAK family of proteins or c-Src inhibited STAT3 phosphorylation. Interestingly, the phosphorylation of these kinases was only mildly inhibited, but the phosphorylation of STAT3 was completely inhibited. This result implies that the inhibition of STAT3 phosphorylation by KT-18618 is an independent event that occurs through the phosphorylation of upstream kinases. Co-immunoprecipitation experiments revealed that KT-18618 inhibited the JAK3-STAT3 interaction. Moreover, JAK3 molecules were captured by biotinylated KT-18618, implying that KT-18618 bound to JAK3 molecules. Additionally, 1μM KT-18618 inhibited JAK3 kinase activity by approximately 28% in an in vitro kinase assay. From these results, we suggest that KT-18618 binds to JAK3 molecules and disrupts the JAK3-STAT3 interaction, which leads to the inhibition of STAT3 phosphorylation. KT-18618 is the first inhibitor of the JAK3-STAT3 interaction.

Keywords: Apoptosis; Breast cancer; JAK3; STAT3

PMID: 24607275
APPEX: analysis platform for the identification of prognostic gene expression signatures in cancer

Bioinformatics. 30(22):3284-6.

Kim SK, Hwan Kim J, Yun SJ, Kim WJ, Kim SY. *Corresponding: Seon-Young Kim(kimsy@kribb.re.kr)

SUMMARY: Because cancer has heterogeneous clinical behaviors due to the progressive accumulation of multiple genetic and epigenetic alterations, the identification of robust molecular signatures for predicting cancer outcome is profoundly important. Here, we introduce the APPEX Web-based analysis platform as a versatile tool for identifying prognostic molecular signatures that predict cancer diversity. We incorporated most of statistical methods for survival analysis and implemented seven survival analysis workflows, including CoxSingle, CoxMulti, IntransSingle, IntransMulti, SuperPC, TimeROC and multivariate. A total of 236 publicly available datasets were collected, processed and stored to support easy independent validation of prognostic signatures. Two case studies including disease recurrence and bladder cancer progression were described using different combinations of the seven workflows.

AVAILABILITY AND IMPLEMENTATION: APPEX is freely available at http://www.appex.kr.

**Keywords**: Analysis workflow; APPEX; Cancer diversity; Dataset; Prognostic signature

PMID: 25091586

REGNET: mining context-specific human transcription networks using composite genomic information

BMC Genomics. 15:450.

Chi SM, Seo YK, Park YK, Yoon S, Park CY, Kim YS, Kim SY* Co-corresponding: Seon-Young Kim(kimsy@kribb.re.kr)

BACKGROUND: Genome-wide expression profiles reflect the transcriptional networks specific to the given cell context. However, most statistical models try to estimate the average connectivity of the networks from a collection of gene expression data, and are unable to characterize the context-specific transcriptional regulations. We propose an approach for mining context-specific transcription networks from a large collection of gene expression fold-change profiles and composite gene-set information.

RESULTS: Using a composite gene-set analysis method, we combine the information of transcription factor binding sites, Gene Ontology or pathway gene sets and gene expression fold-change profiles for a variety of cell conditions. We then collected all the significant patterns and constructed a database of context-specific transcription networks for human (REGNET). As a result, context-specific roles of transcription factors as well as their functional targets are readily explored. To validate the approach, nine predicted targets of E2F1 in HeLa cells were tested using chromatin immunoprecipitation assay. Among them, five (Gadd45b, Dusp6, Mll5, Bnap2 and E2F3) were successfully bound by E2F1. c-JUN and the EMT transcription networks were also validated from literature.

CONCLUSIONS: REGNET is a useful tool for exploring the ternary relationships among the transcription factors, their functional targets and the corresponding cell conditions. It is able to provide useful clues for novel cell-specific transcriptional regulations. The REGNET database is available at http://mgrc.kribb.re.kr/regnet.

**Keywords**: Composite gene-set analysis; Gene ontology; KEGG; Microarray; TFBS; Transcription network

PMID: 24912499
EGR1-dependent PTEN upregulation by 2-benzoyloxyccinnamaldehyde attenuates cell invasion and EMT in colon cancer


Kim J, Kang HS, Lee YJ, Lee HJ, Yun J, Shin JH, Lee CW, Kwon BM', Hong SH.
*Co-corresponding: Byoung-Mog Kwon(kwonbm@kribb.re.kr)

There has been little evidence to support EGR1 and PTEN function on the EMT of cancer cells. We tried to evaluate how these genes affect cancer cell invasion and EMT through investigating the molecular mechanism(s) of 2'-benzoyloxyccinnamaldehyde (BCA). Matrigel invasion and wound healing assay, and in vivo mice model were used to evaluate the effect of BCA on colon cancer cell migration. The molecular mechanism(s) of BCA were evaluated by knock-down or overexpression of EGR1 and PTEN. BCA at 50 nM increased E-cadherin and EGR1 expression without cytototoxicity. Cell migration was inhibited significantly by BCA both in vitro and in vivo. Moreover, BCA inhibits Snail and Vimentin expression, as well as β-catenin nuclear accumulation. Suppression of EGR1 by siRNA attenuated the inhibition of matrigel invasion by BCA, indicating that EGR1 is responsible for BCA effect. PTEN was upregulated by BCA treatment or EGR1 overexpression. In addition, shPTEN transfection stimulated EMT and cell invasion in vitro. Our data suggest that BCA leads to a remarkable upregulation of EGR1 expression, and that EMT and invasion is decreased via EGR1-dependent PTEN activation. These data showed a critical role of EGR1-PTEN signaling pathway in the EMT of colon cancer, as well as metastasis.

**Keywords**: 2'-Benzoyloxyccinnamaldehyde; Colon cancer; Early growth response protein-1 (EGR1); Epithelial–mesenchymal transition (EMT); Metastasis; PTEN

PMID: 24704156

SH3RF2 functions as an oncogene by mediating PAK4 protein stability

Carcinogenesis. 35(3):624-34.

*Co-corresponding: Young Il Yeom(yeomyi@kribb.re.kr), Kyung Chan Park(kpark@kribb.re.kr)

SH3RF (SH3-domain-containing RING finger protein) family members, SH3RF1-3, are multidomain scaffold proteins involved in promoting cell survival and apoptosis. In this report, we show that SH3RF2 is an oncogene product that is overexpressed in human cancers and regulates p21-activated kinase 4 (PAK4) protein stability. Immunohistochemical analysis of 159 colon cancer tissues showed that SH3RF2 expression levels are frequently elevated in cancer tissues and significantly correlate with poor prognostic indicators, including increased invasion, early recurrence and poor survival rates. We also demonstrated that PAK4 protein is degraded by the ubiquitin-proteasome system and that SH3RF2 inhibits PAK4 ubiquitination via physical interaction-mediated steric hindrance, which results in the upregulation of PAK4 protein. Moreover, ablation of SH3RF2 expression attenuates TRADD (TNFR-associated death domain) recruitment to tumor necrosis factor-α (TNF-α) receptor 1 and hinders downstream signals, thereby inhibiting NF-κB (nuclear factor-kappaB) activity and enhancing caspase-8 activity, in the context of TNF-α treatment. Notably, ectopic expression of SH3RF2 effectively prevents apoptosis in cancer cells and enhances cell migration, colony formation and tumor growth in vivo. Taken together, our results suggest that SH3RF2 is an oncogene that may be a definitive regulator of PAK4. Therefore, SH3RF2 may represent an effective therapeutic target for cancer treatment.

**Keywords**: Apoptosis; DNA primer; Immunohistochemical analysis; Oncogene protein; PAK4; p21-Activated kinases; SH3RF2 protein; TNF-α receptor

PMID: 24130170
Tumorigenesis is a consequence of failures of multistep defense mechanisms against deleterious perturbations that occur at the genomic, epigenomic, transcriptomic and proteomic levels. To uncover previously unrecognized genes that undergo multilevel perturbations in gastric cancer (GC), we integrated epigenomic and transcriptomic approaches using two recently developed tools: MENT and GENT. This integrative analysis revealed that nine Hippo pathway-related genes, including components \([\text{FAT, JUB, LAT2, TEA domain family member 4 (TEAD4) and Yes-associated protein 1 (YAP1)}]\) and targets \([\text{CRIM1, CYR61, CTGF and ITGB2}]\), are concurrently hypomethylated at promoter CpG sites and overexpressed in GC tissues. In particular, \textit{TEAD4}, a link between Hippo pathway components and targets, was significantly hypomethylated at CpG site cg21637033 \((P = 3.8 \times 10^{-10})\) and overexpressed \((P = 5.2 \times 10^{-10})\) in 108 Korean GC tissues compared with the normal counterparts. A reduced level of methylation at the \textit{TEAD4} promoter was significantly associated with poor outcomes, including large tumor size, high-grade tumors and low survival rates. Compared with normal tissues, the \textit{TEAD4} protein was more frequently found in the nuclei of tumor cells along with YAP1 in 53 GC patients, demonstrating the posttranslational activation of this protein. Moreover, the knockdown of \textit{TEAD4} resulted in the reduced growth of GC cells both \textit{in vitro} and \textit{in vivo}. Finally, chromatin immunoprecipitation-sequencing and microarray analysis revealed the oncogenic properties of \textit{TEAD4} and its novel targets \([\text{ADM, ANG, ARID5B, CALD1, EDN2, FSCN1 and OSR2}]\), which are involved in cell proliferation and migration. In conclusion, the multilevel perturbations of \textit{TEAD4} at epigenetic, transcriptional and posttranslational levels may contribute to GC development.

**Keywords:** Gastric cancer; Hippo pathway-related gene; Integrative analysis; \textit{TEAD4} protein; YAP1

**PMID:** 24325916
Human Noxin is an anti-apoptotic protein in response to DNA damage of A549 non-small cell lung carcinoma


'Corresponding: Misun Won(misun@kribb.re.kr)

Human Noxin (*hNoxin*, C11Orf82), a homolog of mouse *noxin*, is highly expressed in colorectal and lung cancer tissues. *hNoxin* contains a DNA-binding C-domain in RPA1, which mediates DNA metabolic processes, such as DNA replication and DNA repair. Expression of *hNoxin* is associated with S phase in cancer cells and in normal cells. Expression of *hNoxin* was induced by ultraviolet (UV) irradiation. Knockdown of *hNoxin* caused growth inhibition of colorectal and lung cancer cells. The comet assay and western blot analysis revealed that *hNoxin* knockdown induced apoptosis through activation of p38 mitogen-activated protein kinase (MAPK)/p53 in non-small cell lung carcinoma A549 cells. Furthermore, simultaneous *hNoxin* knockdown and treatment with DNA-damaging agents, such as camptothecin (CPT) and UV irradiation, enhanced apoptosis, whereas Trichostatin A (TSA) did not. However, transient overexpression of *hNoxin* rescued cells from DNA damage-induced apoptosis but did not block apoptosis in the absence of DNA damage. These results suggest that *hNoxin* may be associated with inhibition of apoptosis in response to DNA damage. An adenovirus expressing a short hairpin RNA against *hNoxin* transcripts significantly suppressed the growth of A549 tumor xenografts, indicating that *hNoxin* knockdown has *in vivo* anti-tumor efficacy. Thus, *hNoxin* is a DNA damage-induced anti-apoptotic protein and potential therapeutic target in cancer.

**Keywords**: Anti-apoptotic protein; Apoptosis; DNA damage; *hNoxin* knockdown; *Noxin*; Target; UV

PMID: 24214091

Synthesis and structure-activity relationship study of chemical probes as hypoxia induced factor-1α/malate dehydrogenase 2 inhibitors


'Co-first: Misun Won(misun@kribb.re.kr), Hyun Seung Ban(banhs@kribb.re.kr)

A structure-activity relationship study of hypoxia inducible factor-1α inhibitor 3-aminobenzoic acid-based chemical probes, which were previously identified to bind to mitochondrial malate dehydrogenase 2, was performed to provide a better understanding of the pharmacological effects of LW6 and its relation to hypoxia inducible factor-1α (HIF-1α) and malate dehydrogenase 2 (MDH2). A variety of multifunctional probes including the benzophenone or the trifluoromethyl diazirine for photoaffinity labeling and click reaction were prepared and evaluated for their biological activity using a cell-based HRE-luciferase assay as well as a MDH2 assay in human colorectal cancer HCT116 cells. Among them, the diazirine probe 4a showed strong inhibitory activity against both HIF-1α and MDH2. Significantly, the inhibitory effect of the probes on HIF-1α activity was consistent with that of the MDH2 enzyme assay, which was further confirmed by the effect on *in vitro* binding activity to recombinant human MDH2, oxygen consumption, ATP production, and AMP activated protein kinase (AMPK) activation. Competitive binding modes of LW6 and probe 4a to MDH2 were also demonstrated.

**Keywords**: Cell-based HRE-luciferase assay; Colorectal cancer; HIF-1α activity; LW6; MDH2 enzyme assay

PMID: 25356789
Collagen Triple Helix Repeat Containing 1 (CTHRC1) acts via ERK-dependent induction of MMP9 to promote invasion of colorectal cancer cells

Oncotarget. 5(2):519-29.

'Co-corresponding: Hee Gu Lee(hglee@kribb.re.kr)

Collagen triple helix repeat-containing 1 (CTHRC1) is known to be aberrantly upregulated in most human solid tumors, although the functional roles of CTHRC1 in colorectal cancer remain unclear. In this study, we investigated the occurrence of CTHRC1 upregulation and its role in vivo and in vitro. The expression profile and clinical importance of CTHRC1 were examined by reverse transcription-polymerase chain reaction and immunohistochemical analyses in normal and tumor patient samples. CTHRC1 was detectable in normal tissues, but also was highly expressed in tumor specimens. CTHRC1 upregulation was significantly associated with demethylation of the CTHRC1 promoter in colon cancer cell lines and tumor tissues. Clinicopathologic analyses showed that nodal status and expression of CTHRC1 (95% CI 0.999-3.984, p=0.05) were significant prognostic factors for disease-free survival. Promotor CpG methylation and hypermethylation status were measured by bisulfit sequencing and pyrosequencing analysis. Furthermore, we showed that overexpression of CTHRC1 in the SW480 and HT-29 cell lines increased invasiveness, an effect mediated by extracellular signal-regulated kinase (ERK)-dependent upregulation of matrix metalloproteinase 9 (MMP9). Consistent with this, we found that knockdown of CTHRC1 attenuated ERK activation and cancer cell invasivity. These results demonstrate that CTHRC1 expression is elevated in human colon cancer cell lines and clinical specimens, and promotes cancer cell invasivity through ERK-dependent induction of MMP9 expression. Our results further suggest that high levels of CTHRC1 expression are associated with poor clinical outcomes.

**Keywords** : Clinicopathologic analyses; Colorectal cancer; CTHRC1 promoter; ERK-dependent induction; Invasion; MMP-9

PMID: 24504172

The EF-hand calcium-binding protein tescalcin is a potential oncotarget in colorectal cancer

Oncotarget. 5(8):2149-60.

'Co-corresponding: Hee Gu Lee(hglee@kribb.re.kr)

Tescalcin (TESC) is an EF-hand calcium binding protein that is differentially expressed in several tissues, however it is not reported that the expression and functional roles of TESC in colorectal cancer. Levels of messenger RNA (mRNA) and protein expression of TESC in colorectal cancer tissues were assessed using RT-PCR, real time PCR, immunohistochemistry, and clinicopathologic analyses. Quantitative analysis of TESC levels in serum specimens was performed using sandwich ELISA. Colorectal cancer cells transfected with TESC small interfering RNA and short hairpin RNA were examined in cell proliferation assays, phospho-MAPK array, and mouse xenograft models. Here we demonstrated that TESC is overexpressed in colorectal cancer (CRC), but was not expressed in normal mucosa and premalignant dysplastic lesions. Furthermore, serum TESC levels were elevated in patients with CRC. Knockdown of TESC inhibited the Akt-dependent NF-κB pathway and decreased cell survival in vitro. Depletion of TESC reduced tumor growth in a CRC xenograft model. Thus, TESC is a potential diagnostic marker and oncotarget in colorectal cancer.

**Keywords** : Cell growth; Colorectal cancer; Diagnostic marker; NF-κB; Serum TESC level; Tescalcin; Tumor growth

PMID: 24811141
nc886, a non-coding RNA of anti-proliferative role, is suppressed by CpG DNA methylation in human gastric cancer


nc886 is a 101 nucleotide long non-coding RNA that has been designated as a precursor microRNA or a vault RNA based upon its sequence. nc886 has also been suggested to be a tumor suppressor, mainly inferred by its expression pattern as well as its genomic location at human chromosome 5q31, a locus for a tumor suppressor gene(s). However, legitimate data based on nc886’s correct identity for its functional cellular roles as a tumor suppressor have not been provided yet. Here we have investigated nc886 in gastric cancer where its expression is suppressed due to CpG DNA hypermethylation at its promoter region in a cohort of paired tumor/normal tissues from 88 gastric cancer patients. CpG hypermethylation of nc886 and thus its diminished expression is significantly associated with poor survival in these cancer patients. nc886 inhibits cell proliferation when ectopically expressed in gastric cancer cells. nc886’s tumor suppressive role is corroborated by the induction of well-known oncopgenes such as FOS, NF-κB, and MYC upon its knockdown. All these activities of nc886 are undoubtedly independent of mature microRNA or vault RNA. Our data indicate that nc886 is a putative tumor suppressor and could potentially be used as a diagnostic marker in gastric cancer.

**Keywords**: Cell proliferation; CpG DNA methylation; Diagnostic marker; Gastric cancer; nc886; Tumor suppressor

PMID: 25003254

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Reactive oxygen species-mediated activation of AMP-activated protein kinase and c-Jun N-terminal kinase plays a critical role in beta-sitosterol-induced apoptosis in multiple myeloma U266 cells

Phytother Res. 28(3):387-94.


Although beta-sitosterol has been well known to have anti-tumor activity in liver, lung, colon, stomach, breast and prostate cancers via cell cycle arrest and apoptosis induction, the underlying mechanism of anti-cancer effect of beta-sitosterol in multiple myeloma cells was never elucidated until now. Thus, in the present study, the role of reactive oxygen species (ROS) in association with AMP-activated protein kinase (AMPK) and c-Jun N-terminal kinase (JNK) pathways was demonstrated in beta-sitosterol-treated multiple myeloma U266 cells. Beta-sitosterol exerted cytotoxicity, increased sub-G1 apoptotic population and activated caspase-9 and -3, cleaved poly (ADP-ribose) polymerase (PARP) followed by decrease in mitochondrial potential in U266 cells. Beta-sitosterol promoted ROS production, activated AMPK, acetyl-CoA carboxylase (ACC) and JNK in U266 cells. Also, beta-sitosterol attenuated the phosphorylation of AKT, mammalian target of rapamycin and S6K, and the expression of cyclooxygenase-2 and VEGF in U266 cells. Conversely, AMPK inhibitor compound C and JNK inhibitor SP600125 suppressed apoptosis induced by beta-sitosterol in U266 cells. Furthermore, ROS scavenger N-acetyl L-cysteine attenuated beta-sitosterol-mediated sub-G1 accumulation, PARP cleavage, JNK and AMPK activation in U266 cells. Overall, these findings for the first time suggest that ROS-mediated activation of cancer metabolism-related genes such as AMPK and JNK plays an important role in beta-sitosterol-induced apoptosis in U266 multiple myeloma cells.

**Keywords**: AMPK; Apoptosis; Beta-sitosterol; JNK; ROS; U266

PMID: 23640957
Epigenomics Research Center

Article 17

Epigenetic silencing of BTB and CNC homology 2 and concerted promoter CpG methylation in gastric cancer


‘Co-corresponding: Yong Sung Kim(yongsung@kribb.re.kr)

BTB and CNC homology 2 (BACH2) is a lymphoid-specific transcription factor with a prominent role in B-cell development. Genetic polymorphisms within a single locus encoding BACH2 are associated with various autoimmune diseases and allergies. In this study, restriction landmark genomic scanning revealed methylation at a NotI site in a CpG island covering the BACH2 promoter in gastric cancer cell lines and primary gastric tumors. Increased methylation of the BACH2 promoter was observed in 52% (43/83) of primary gastric tumors, and BACH2 hypermethylation was significantly associated with decreased gene expression. Treatment with 5-aza-2'-deoxycytidine and/or trichostatin A restored BACH2 expression in BACH2-silenced gastric cancer cell lines, and knockdown of BACH2 using short hairpin RNA (i.e., RNA interference) increased cell proliferation in gastric cancer cells. Clinico-pathologic data showed that decreased BACH2 expression occurred significantly more frequently in intestinal-type (27/44, 61%) compared with diffuse-type (13/50, 26%) gastric cancers (P<0.001). Furthermore, BACH2 promoter methylation paralleled that of previously identified targets, such as LRRC3B, LIMS2, PRKD1, and POPDC3, in a given set of gastric tumors. We propose that concerted methylation in many promoters plays a role in accelerating gastric tumor formation and that methylated promoter loci may be targets for therapeutic treatment, such as the recently introduced technique of epigenetic editing.

Keywords: BACH2 hypermethylation; Cell proliferation; Concerted methylation; Gastric cancer; RLG5

PMID: 24858026

Identification of body fluid-specific DNA methylation markers for use in forensic science


‘Co-corresponding: Yong Sung Kim(yongsung@kribb.re.kr)

DNA methylation, which occurs at the 5’-position of the cytosine in CpG dinucleotides, has great potential for forensic identification of body fluids, because tissue-specific patterns of DNA methylation have been demonstrated, and DNA is less prone to degradation than proteins or RNA. Previous studies have reported several body fluid-specific DNA methylation markers, but DNA methylation differences are sometimes low in saliva and vaginal secretions. Moreover, specific DNA methylation markers in four types of body fluids (blood, saliva, semen, and vaginal secretions) have not been investigated with genome-wide profiling. Here, we investigated novel DNA methylation markers for identification of body fluids for use in forensic science using the Illumina HumanMethylation 450K bead array, which contains over 450,000 CpG sites. Using methylene data from 16 samples of blood, saliva, semen, and vaginal secretions, we first selected 2986 hypermethylated or hypomethylated regions that were specific for each type of body fluid. We then selected eight CpG sites as novel, forensically relevant DNA methylation markers: cg06379435 and cg08792630 for blood, cg26107890 and cg20691722 for saliva, cg23521140 and cg17610929 for semen, and cg01774894 and cg14991487 for vaginal secretions. These eight selected markers were evaluated in 80 body fluid samples using pyrosequencing, and all showed high sensitivity and specificity for identification of the target body fluid. We suggest that these eight DNA methylation markers may be good candidates for developing an effective molecular assay for identification of body fluids in forensic science.

Keywords: Aging; Body fluid identification; CpG site; DNA methylation marker; Epigenetics; Forensic

PMID: 25128690
De novo assembly and characterization of the complete chloroplast genome of radish (Raphanus sativus L.)


Jeong YM, Chung WH, Mun JH, Kim N’, Yu HJ.

Radish (Raphanus sativus L.) is an edible root vegetable crop that is cultivated worldwide and whose genome has been sequenced. Here we report the complete nucleotide sequence of the radish cultivar WK10039 chloroplast (cp) genome, along with a de novo assembly strategy using whole genome shotgun sequence reads obtained by next generation sequencing. The radish cp genome is 153,368 bp in length and has a typical quadripartite structure, composed of a pair of inverted repeat regions (26,217 bp each), a large single copy region (83,170 bp), and a small single copy region (17,764 bp). The radish cp genome contains 87 predicted protein-coding genes, 37 tRNA genes, and 8 rRNA genes. Sequence analysis revealed the presence of 91 simple sequence repeats (SSRs) in the radish cp genome. Phylogenetic analysis of 62 protein-coding gene sequences from the 17 cp genomes of the Brassicaceae family suggested that the radish cp genome is most closely related to the cp genomes of Brassica rapa and Brassica napus. Comparisons with the B. rapa and B. napus cp genomes revealed highly divergent intragenic sequences and introns that can potentially be developed as diagnostic cp markers. Synonymous and nonsynonymous substitutions of cp genes suggested that nucleotide substitutions have occurred at similar rates in most genes. The complete sequence of the radish cp genome would serve as a valuable resource for the development of new molecular markers and the study of the phylogenetic relationships of Raphanus species in the Brassicaceae family.

Keywords: Brassicaceae family; Chloroplast genome; Next-generation sequencing; Raphanus sativus; Simple sequence repeat

Dynamic changes in DNA methylation and hydroxymethylation when hES cells undergo differentiation toward a neuronal lineage


DNA methylation and hydroxymethylation have been implicated in normal development and differentiation, but our knowledge is limited about the genome-wide distribution of 5-methylcytosine (5 mC) and 5-hydroxymethylcytosine (5 hmC) during cellular differentiation. Using an in vitro model system of gradual differentiation of human embryonic stem (hES) cells into ventral midbrain-type neural precursor cells and terminally into dopamine neurons, we observed dramatic genome-wide changes in 5 mC and 5 hmC patterns during lineage commitment. The 5 hmC pattern was dynamic in promoters, exons and enhancers. DNA hydroxymethylation within the gene body was associated with gene activation. The neurogenesis-related genes NOTCH1, RGMA and AKT1 acquired 5 hmC in the gene body and were up-regulated during differentiation. DNA methylation in the promoter was associated with gene repression. The pluripotency-related genes POU5F1, ZFP42 and HMGAI acquired 5 mC in their promoters and were down-regulated during differentiation. Promoter methylation also acted as a locking mechanism to maintain gene silencing. The mesoderm development-related genes NKX2-8, TNFSF11 and NFATC1 acquired promoter methylation during neural differentiation even though they were already silenced in hES cells. Our findings will help elucidate the molecular mechanisms underlying lineage-specific differentiation of pluripotent stem cells during human embryonic development.

Keywords: 5 hmC; DNA methylation; hES cell; Hydroxymethylation; Neuronal lineage; Promoter

PMID: 24087792
A nineteen gene-based risk score classifier predicts prognosis of colorectal cancer patients

*Co-corresponding: Yong Sung Kim(yongsung@kribb.re.kr)

Colorectal cancer (CRC) patients frequently experience disease recurrence and distant metastasis. This study aimed to identify prognostic indicators, including individual responses to chemotherapy, in CRC patients. RNA-seq data was generated using 54 samples (normal colon, primary CRC, and liver metastases) from 18 CRC patients and genes associated with CRC aggressiveness were identified. A risk score based on these genes was developed and validated in four independent CRC patient cohorts (n = 1063). Diverse statistical methods were applied to validate the risk scoring system, including a generalized linear model likelihood ratio test, Kaplan-Meier curves, a log-rank test, and the Cox model. *TREM1* and *CTGF* were identified as two activated regulators associated with CRC aggressiveness. A risk score based on 19 genes regulated by *TREM1* or *CTGF* activation (TCA19) was a significant prognostic indicator. In multivariate and subset analyses based on pathological staging, TCA19 was an independent risk factor (HR = 1.894, 95% CI = 1.227-2.809, P = 0.002). Subset stratification in stage III patients revealed that TCA19 had prognostic potential and identified patients who would benefit from adjuvant chemotherapy, regardless of age. The TCA19 predictor represents a novel diagnostic tool for identifying high-risk CRC patients and possibly predicting the response to adjuvant chemotherapy.

**Keywords**: Adjuvant chemotherapy; Colorectal cancer; CRC aggressiveness; Marker; Metastasis; Prognosis

PMID: 25049118
AGO2 and SETDB1 cooperate in promoter-targeted transcriptional silencing of the androgen receptor gene

Nucleic Acids Res. 42(22):13545-56.

Cho S, Park JS, Kang YK*. *Corresponding: Yong-Kook Kang(ykkang@kribb.re.kr)

In mammals, RNA interference is primarily a post-transcriptional mechanism. Evidence has accumulated for additional role in transcriptional gene silencing (TGS) but the question for a good paradigm for small interfering antigene RNA (agRNA)-induced chromatin modification remains unanswered. Here, we show that SETDB1, a histone H3-lysine 9 (H3K9)-specific methyltransferase, cooperates with Argonaute-2 (AGO2) and plays an essential role in agRNA-induced TGS. The androgen receptor (AR) gene was transcriptionally silenced by agRNA targeted to its promoter, and we show that this repression was mitigated by knockdown of SETDB1 or AGO2. Chromatin immunoprecipitation demonstrated that agRNA-driven AGO2 was first targeted to the AR promoter, followed by SETDB1. SIN3A and HDAC1/2, the components of the SIN3-HDAC complex, immunoprecipitated with SETDB1, and localized at the agRNA-targeted promoter. Agreeing with the presence of SETDB1, trimethyl-H3K9 was enriched in the AR promoter. Both EZH2 and trimethyl-H3K27 were also present in the targeted locus; accordingly, EZH2 immunoprecipitated with SETDB1. DNA methylation level was not significantly changed, suggesting the absence of de novo methylating activity in agRNA-induced AR promoter. Our results demonstrate that SETDB1, together with AGO2, plays an essential role in TGS through recruiting chromatin remodeler and/or other modifiers, consequently creating a repressive chromatin milieu at the targeted promoter.

Keywords: Antigene RNA; Argonaute-2 (AGO2); AR promoter; DNA methylation; Transcriptional gene silencing (TGS)

PMID: 25183519

TM4SF4 overexpression in radiation-resistant lung carcinoma cells activates IGF1R via elevation of IGF1


Choi SI, Kim SY, Lee J, Cho EW*, Kim IG. *Co-corresponding: Eun Wie Cho(ewcho@kribb.re.kr)

Transmembrane 4 L six family member 4 (TM4SF4) is a member of the tetraspanin L6 domain family. Other members of this family, TM4SF1 (also known as L6-Ag) and TM4SF5, have been shown to be upregulated in multiple tumors and involved in epithelial-to-mesenchymal transition and cell migration. However, unlike its homologs, little is known about TM4SF4. Here, we show that TM4SF4 was highly expressed in radiation-resistant lung adenocarcinoma cells, such as A549 and Calu-3 cells, and its expression activated cell growth, migration, and invasion. Overexpression of TM4SF4 in A549 cells increased the activation of PI3K, AKT, and NF-kappaB and the expression of PTEN. IGF1R was clearly activated by overexpression of TM4SF4, although EGFR was also slightly activated. TM4SF4 expression was correlated with the increased expression of IGF1, consequently resulting in IGF1R activation. Tumorogenic activity of TM4SF4 in lung adenocarcinoma cells was also demonstrated by xenograft assay; however, this activity was almost completely suppressed by treatment with anti-TM4SF4 antibody. Our results suggest that TM4SF4 overexpression in lung carcinoma cells results in resistance to radiotherapy via IGF1-induced IGF1R activation and blocking the activity of TM4SF4 using specific antibody can be a promising therapeutics against TM4SF4-overexpressing lung adenocarcinoma.

Keywords: IGF1; IGF1R activation; Lung adenocarcinoma; Lung carcinoma; Radiotherapy; TM4SF4; Tumorogenic activity

PMID: 25344917
Whole-exome sequencing identifies a novel genotype-phenotype correlation in the entactin domain of the known deafness gene TECTA


*Co-corresponding: Namshin Kim(deepreds@kribb.re.kr)

Postlingual progressive hearing loss, affecting primarily the high frequencies, is the clinical finding in most cases of autosomal dominant nonsyndromic hearing loss (ADNSHL). The molecular genetic etiology of ADNSHL is extremely heterogeneous. We applied whole-exome sequencing to reveal the genetic etiology of high-frequency hearing loss in a mid-sized Korean family without any prior linkage data. Whole-exome sequencing of four family members (two affected and two unaffected), together with our filtering strategy based on comprehensive bioinformatics analyses, identified 21 potential pathogenic candidates. Sanger validation of an additional five family members excluded 20 variants, leaving only one novel variant, TECTA c.710C>T (p.T237I), as the strongest candidate. This variant resides in the entactin (ENT) domain and co-segregated perfectly with non-progressive high-frequency hearing loss in the family. It was absent among 700 ethnically matched control chromosomes, and the T237 residue is conserved among species, which supports its pathogenicity. Interestingly, this finding contrasted with a previously proposed genotype-phenotype correlation in which variants of the ENT domain of TECTA were associated with mid-frequency hearing loss. Based upon what we observed, we propose a novel "genotype to phenotype" correlation in the ENT domain of TECTA. Our results shed light on another important application of whole-exome sequencing: the establishment of a novel genotype-phenotype in the molecular genetic diagnosis of autosomal dominant hearing loss.

**Keywords**: ADNSHL; Bioinformatics analyses; ENT domain; Genetic etiology; Genotype-phenotype; TECTA; Whole-exome sequencing

PMID: 24816743

The family-wide structure and function of human dual-specificity protein phosphatases


*Co-corresponding: Seung Jun Kim(ksj@kribb.re.kr)

Dual-specificity protein phosphatases (DUSPs), which dephosphorylate both phosphoserine/threonine and phosphotyrosine, play vital roles in immune activation, brain function and cell-growth signalling. A family-wide structural library of human DUSPs was constructed based on experimental structure determination supplemented with homology modelling. The catalytic domain of each individual DUSP has characteristic features in the active site and in surface-charge distribution, indicating substrate-interaction specificity. The active-site loop-to-strand switch occurs in a subtype-specific manner, indicating that the switch process is necessary for characteristic substrate interactions in the corresponding DUSPs. A comprehensive analysis of the activity-inhibition profile and active-site geometry of DUSPs revealed a novel role of the active-pocket structure in the substrate specificity of DUSPs. A structure-based analysis of redox responses indicated that the additional cysteine residues are important for the protection of enzyme activity. The family-wide structures of DUSPs form a basis for the understanding of phosphorylation-mediated signal transduction and the development of therapeutics.

**Keywords**: Cysteine residues; Comprehensive analysis; Dual-specificity protein phosphatases; DUSPs; Enzyme activity; Protein tyrosine phosphatases

PMID: 24531476
A novel maltose-forming α-amylase (PSMA) was recently found in the hyperthermophilic archaeon *Pyrococcus* sp. ST04. This enzyme shows <13% amino-acid sequence identity to other known α-amylases and displays a unique enzymatic property in that it hydrolyzes both α-1,4-glucosidic and α-1,6-glucosidic linkages of substrates, recognizing only maltose units, in an exo-type manner. Here, the crystal structure of PSMA at a resolution of 1.8 Å is reported, showing a tight ring-shaped tetramer with monomers composed of two domains: an N-domain (amino acids 1-341) with a typical GH57 family (βα7)-barrel fold and a C-domain (amino acids 342-597) composed of α-helical bundles. A small closed cavity observed in proximity to the catalytic residues Glu153 and Asp253 at the domain interface has the appropriate volume and geometry to bind a maltose unit, accounting for the selective exo-type maltose hydrolysis of the enzyme. A narrow gate at the putative subsite +1 formed by residue Phe218 and Phe452 is essential for specific cleavage of glucosidic bonds. The closed cavity at the active site is connected to a short substrate-binding channel that extends to the central hole of the tetramer, exhibiting a geometry that is significantly different from classical malto- and β-amylases. The structural features of this novel exo-type maltose-forming α-amylase provide a molecular basis for its unique enzymatic characteristics and for its potential use in industrial applications and protein engineering.

**Keywords**: *Pyrococcus* sp. ST04; Exo-type hydrolase; Glycoside hydrolase family 57; Maltose-forming α-amylase (PSMA)

PMID: 24914977

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**ER stress-inducible ATF3 suppresses BMP2-induced ALP expression and activation in MC3T3-E1 cells**


Co-corresponding: Jeong-Woong Lee(jwlee@kribb.re.kr)

Endoplasmic reticulum (ER) stress suppresses osteoblast differentiation. Activating transcription factor (ATF) 3, a member of the ATF/cAMP response element-binding protein family of transcription factors, is induced by various stimuli including cytokines, hormones, DNA damage, and ER stress. However, the role of ATF3 in osteoblast differentiation has not been elucidated. Treatment with tunicamycin (TM), an ER stress inducer, increased ATF3 expression in the preosteoblast cell line, MC3T3-E1. Overexpression of ATF3 inhibited bone morphogenetic protein 2-stimulated expression and activation of alkaline phosphatase (ALP), an osteogenic marker. In addition, suppression of ALP expression by TM treatment was rescued by silencing of ATF3 using shRNA. Taken together, these data indicate that ATF3 is a novel negative regulator of osteoblast differentiation by specifically suppressing ALP gene expression in preosteoblasts.

**Keywords**: Activating transcription factor 3 (ATF3); ALP; β-glycerophosphate; Bone morphogenetic protein 2 (BMP2); cAMP response; Endoplasmic reticulum; Osteoblast differentiation; Tunicamycin

PMID: 24315873
Targeting of p53 peptide analogues to anti-apoptotic Bcl-2 family proteins as revealed by NMR spectroscopy


Shin JS, Ha JH, Chi SW*. Corresponding: Seung-Wook Chi(swchi@kribb.re.kr)

Inhibition of the interaction between the p53 tumor suppressor and its negative regulator MDM2 is of great importance to cancer therapy. The anti-apoptotic Bcl-2 family proteins are also attractive anti-cancer molecular targets, as they are key regulators of apoptotic cell death. Previously, we reported the interactions between the p53 transactivation domain (p53TAD) and diverse members of the anti-apoptotic Bcl-2 family proteins. In this study, we investigated the binding of MDM2-inhibiting p53TAD peptide analogues, p53-MDM2/MDMX inhibitor (PMI) and pDI, with anti-apoptotic Bcl-2 family proteins, Bcl-X\textsubscript{L} and Bcl-2, by using NMR spectroscopy. The NMR chemical shift perturbation data demonstrated the direct binding of the p53 peptide analogues to Bcl-X\textsubscript{L} and Bcl-2 and showed that the PMI and pDI peptides bind to a conserved hydrophobic groove of the anti-apoptotic Bcl-2 family proteins. Furthermore, the structural model of the Bcl-X\textsubscript{L}/PMI peptide complex showed that the binding mode of the PMI peptide is highly similar to that of pro-apoptotic Bcl-2 homology 3 (BH3) peptides. Finally, our structural comparison provided a molecular basis for how the same PMI peptide can bind to two distinct anti-cancer target proteins Bcl-X\textsubscript{L} and MDM2, which may have potential applications for multi-targeting cancer therapy.

Keywords: Bcl-2 family protein; Cancer therapy; MDM2; Multi-targeting; NMR spectroscopy; PMI; p53 Peptide analogue; pDI

PMID: 24342622

Structural basis for the conserved binding mechanism of MDM2-inhibiting peptides and anti-apoptotic Bcl-2 family proteins


Lee MS, Ha JH, Yoon HS, Lee CK, Chi SW*. Corresponding: Seung-Wook Chi(swchi@kribb.re.kr)

The interaction between tumor suppressor p53 and the anti-apoptotic Bcl-2 family proteins serves a critical role in the transcription-independent apoptosis mechanism of p53. Our previous studies showed that an MDM2-inhibiting motif (residues 15-29) in the p53 transactivation domain (p53TAD) mediates the interaction with anti-apoptotic Bcl-2 family proteins. In this study, we provided structural models of the complexes between the MDM2-inhibiting p53TAD peptide and McI-1, Bcl-w, and Kaposi sarcoma-associated herpes virus (KSHV) Bcl-2 using NMR chemical shift perturbation data. The binding mode of the MDM2-inhibiting p53TAD peptide is highly conserved among the anti-apoptotic Bcl-2 family proteins despite their distinct specificities for pro-apoptotic Bcl-2 family proteins. We also identified the binding of a phage-display-derived MDM2-inhibiting peptide 12-1 to anti-apoptotic Bcl-X\textsubscript{L} protein by using NMR spectroscopy. The structural model of the Bcl-X\textsubscript{L}/12-1 peptide complex revealed that the conserved residues Phe4, Trp8, and Leu11 in the MDM2-inhibiting peptide fit into a hydrophobic cleft of Bcl-X\textsubscript{L} in a manner similar to that of pro-apoptotic Bcl-2 homology 3 (BH3) peptides. Our results shed light on the mechanism underlying dual-targeting of the FxxWxxL-based \alpha-helical motif to MDM2 and anti-apoptotic Bcl-2 family proteins for anticancer therapy.

Keywords: Bcl-2 family protein; Cancer therapy; Dual-targeting; MDM2-inhibiting peptide; NMR spectroscopy; p53 transactivation domain

PMID: 24491548
Association of bi-functional activity in the N-terminal domain of glycogen debranching enzyme


Lee MH, Song HN, Cho JE, Lan TP, Park S, Park JT, Woo EJ.
*Corresponding: Eui-Jeon Woo(ejwoo@kribb.re.kr)

Glycogen debranching enzyme (GDE) in mammals and yeast exhibits α-1,4-transferase and α-1,6-glucosidase activities within a single polypeptide chain and facilitates the breakdown of glycogen by a bi-functional mechanism. Each enzymatic activity of GDE is suggested to be associated with distinct domains; α-1,4-glycosyltransferase activity with the N-terminal domain and α-1,6-glucosidase activity with the C-terminal domain. Here, we present the biochemical features of the GDE from Saccharomyces cerevisiae using the substrate glucose(n)-β-cyclodextrin (Gn-β-CD). The bacterially expressed and purified GDE N-terminal domain (aa 1-644) showed α-1,4-transferase activity on maltotetraose (G4) and G4-β-CD, yielding various lengths of (Gn). Surprisingly, the N-terminal domain also exhibited α-1,6-glucosidase activity against G1-β-CD and G4-β-CD, producing G1 and β-CD. Mutational analysis showed that residues D535 and E564 in the N-terminal domain are essential for the transferase activity but not for the glucosidase activity. These results indicate that the N-terminal domain (1-644) alone has both α-1,4-transferase and the α-1,6-glucosidase activities and suggest that the bi-functional activity in the N-domain may occur via one active site, as observed in some archaeal debranching enzymes.

Keywords: α-1,4-Transferase activity; α-1,6-Glucosidase activity; Bi-functional; Glycogen debranching enzyme (GDE); Saccharomyces cerevisiae

PMID: 24491554

Structural insights into the transcription-independent apoptotic pathway of p53


Chi SW*.
*Corresponding: Seung-Wook Chi(swchi@kribb.re.kr)

Reactivating the p53 pathway in tumors is an important strategy for anticancer therapy. In response to diverse cellular stresses, the tumor suppressor p53 mediates apoptosis in a transcription-independent and transcription-dependent manner. Although extensive studies have focused on the transcription-dependent apoptotic pathway of p53, the transcription-independent apoptotic pathway of p53 has only recently been discovered. Molecular interactions between p53 and Bcl-2 family proteins in the mitochondria play an essential role in the transcription-independent apoptosis of p53. This review describes the structural basis for the transcription-independent apoptotic pathway of p53 and discusses its potential application to anticancer therapy.

Keywords: Anticancer therapy; Apoptosis; Bcl-2 family protein; p53; Structure

PMID: 24499665
SMILE inhibits BMP-2-induced expression of osteocalcin by suppressing the activity of the RUNX2 transcription factor in MC3T3E1 cells

Bone. 61:10-8.


Small heterodimer partner interacting leucine zipper protein (SMILE) is an orphan nuclear receptor and a member of the bZIP family of proteins. Several recent studies have suggested that SMILE is a novel co-repressor that is involved in nuclear receptor signaling; however, the role of SMILE in osteoblast differentiation has not yet been elucidated. This study demonstrates that SMILE inhibits osteoblast differentiation by regulating the activity of Runt-related transcription factor-2 (RUNX2). Tunicamycin, an inducer of endoplasmic reticulum stress, stimulated SMILE expression. Bone morphogenetic protein-2-induced expression of alkaline phosphatase and osteocalcin, both of which are osteogenic genes, was suppressed by SMILE. The molecular mechanism by which SMILE affects osteocalcin expression was also determined. An immunoprecipitation assay revealed a physical interaction between SMILE and RUNX2 that significantly impaired the RUNX2-dependent activation of the osteocalcin gene. A ChIP assay revealed that SMILE repressed the ability of RUNX2 to bind to the osteocalcin gene promoter. Taken together, these findings demonstrate that SMILE negatively regulates osteocalcin via a direct interaction with RUNX2.

Keywords: BMP2; Osteoblast; Osteocalcin; Runx2; SMILE
PMID: 24389415

Discovery of novel DUSP4 inhibitors through the virtual screening with docking simulations

Bull Kor Chem Soc. 35(9): 2655-9.

Park H, Jeon TJ, Chien PN, Park SY, Oh SM, Kim SJ*, Ryu SE. 'Co-corresponding: Seung Jun Kim(ksj@kribb.re.kr)

Dual specificity protein phosphatase 4 (DUSP4) has been considered a promising target for the development of therapeutics for various human cancers. Here, we report the first example for a successful application of the structure-based virtual screening to identify the novel small-molecule DUSP4 inhibitors. As a consequence of the virtual screening with the modified scoring function to include an effective molecular solvation free energy term, five micromolar DUSP4 inhibitors are found with the associated IC₅₀ values ranging from 3.5 to 10.8 μM. Because these newly identified inhibitors were also screened for having desirable physicochemical properties as a drug candidate, they may serve as a starting point of the structure-activity relationship study to optimize the medical efficacy. Structural features relevant to the stabilization of the new inhibitors in the active site of DUSP4 are discussed in detail.

Keywords: Cancer; Docking simulation; Drug design; Dual specificity protein phosphatase 4 (DUSP4); Inhibitor; Virtual screening
Human ChlR1 stimulates endonuclease activity of hFen1 independently of ATPase activity


Kim DH, Kim JH, Park BC, Lee DH, Cho S, Park SG. 'Co-corresponding: Sung Goo Park(sgpark@kribb.re.kr)

Human ChlR1 protein (hChlR1), a member of the cohesion establishment factor family, plays an important role in the segregation of sister chromatids for maintenance of genome integrity. We previously reported that hChlR1 interacts with hFen1 and stimulates its nuclease activity on the flap-structured DNA substrate covered with RPA. To elucidate the relationship between hChlR1 and Okazaki fragment processing, the effect of hChlR1 on in vitro nuclease activities of hFen1 and hDna2 was examined. Independent of ATPase activity, hChlR1 stimulated endonuclease activity of hFen1 but not that of hDna2. Our findings suggest that the acceleration of Okazaki fragment processing near cohesions may aid in reducing the size of the replication machinery, thereby facilitating its entry through the cohesin ring.

Keywords: ChlR1 protein; Dna2; Endonuclease activity; Fen1; hChlR1; hDna2; hFen1; Okazaki fragment processing

Refolded scFv antibody fragment against myoglobin shows rapid reaction kinetics


Song HN, Jang JH, Kim YW, Kim DH, Park SG, Lee MK, Paek SH, Woo EF. 'Co-corresponding: Eui-Jeon Woo(ejwoo@kribb.re.kr)

Myoglobin is one of the early biomarkers for acute myocardial infarction. Recently, we have screened an antibody with unique rapid reaction kinetics toward human myoglobin antigen. Antibodies with rapid reaction kinetics are thought to be an early IgG form produced during early stage of in vivo immunization. We produced a recombinant scFv fragment for the premature antibody from Escherichia coli using refolding technology. The scFv gene was constructed by connection of the V(H)-V(L) sequence with a (Gly4Ser)₃ linker. The scFv fragment without the pelB leader sequence was expressed at a high level, but the solubility was extremely low. A high concentration of 8 M urea was used for denaturation. The dilution refolding process in the presence of arginine and the redox reagents GSH and GSSG successfully produced a soluble scFv protein. The resultant refolded scFv protein showed association and dissociation values of 9.32 × 10⁻⁴ M⁻¹s⁻¹ and 6.29 × 10⁻³ s⁻¹, respectively, with an affinity value exceeding 10⁻⁷ M⁻¹ (k(on)/k(off)), maintaining the original rapid reaction kinetics of the premature antibody. The refolded scFv could provide a platform for protein engineering for the clinical application for diagnosis of heart disease and the development of a continuous biosensor.

Keywords: Acute myocardial infarction; Biomarker; Human myoglobin antigen; Premature antibody; Single-chain variable fragment (scFv)

PMID: 25530617
Comparative proteomic analyses of synovial fluids and serums from rheumatoid arthritis patients


*Co-corresponding: Byoung Chul Park(parkbc@kribb.re.kr), Jeong-Hoon Kim(jhoonkim@kribb.re.kr)

Rheumatoid arthritis (RA) is a chronic and systemic inflammatory disorder that primarily affects the flexible joints and may also affect a number of tissues and organs. The progression of RA involves an inflammatory response of the capsule around the joint, swelling of synovial cells with excess synovial fluid (SF), and the development of fibrous tissue in the synovium. Since the progressive pathology of the disease often leads to the irreversible destruction of articular cartilage and ankylosis of the joint, early diagnosis of RA is essential. Thus, we undertook a comparative proteomic approach to investigate novel biomarkers for early diagnosis using SFs and serums from RA patients. As a result, we identified 32 differentially expressed spots in SFs and 34 spots in serums. The differential expression of the STEAP4 and ZNF 658 proteins were validated using immunoblotting of the SFs and serums, respectively. These data suggest that differentially expressed proteins in SFs and serums could be used as RA-specific biomarkers for the diagnosis and monitoring of RA. Furthermore, these findings advance our understanding of the molecular etiopathogenesis of RA.

**Keywords**: Biomarker; Inflammatory disease; Proteomic analysis; Rheumatoid arthritis (RA); Serum; Synovial fluid (SF)

PMID: 24105271

Identification of novel binding partners for caspase-6 using a proteomic approach


Jung JY, Lee SR, Kim S, Chi SW, Bae KH, Park BC, Kim JH*, Park SG'.

*Co-corresponding: Sung Goo Park(sgpark@kribb.re.kr), Jeong-Hoon Kim(jhoonkim@kribb.re.kr)

Apoptosis is the process of programmed cell death executed by specific proteases, the caspases, which mediate the cleavage of various vital proteins. Elucidating the consequences of this endoproteolytic cleavage is crucial to understanding cell death and other related biological processes. Although a number of possible roles for caspase-6 have been proposed, the identities and functions of proteins that interact with caspase-6 remain uncertain. In this study, we established a cell line expressing tandem affinity purification (TAP)-tagged caspase-6 and then used LC-MS/MS proteomic analysis to analyze the caspase-6 interactome. Eight candidate caspase-6-interacting proteins were identified. Of these, five proteins (hnRNP-M, DHX38, ASPP2, MTA2, and UACA) were subsequently examined by co-immunoprecipitation for interactions with caspase-6. Thus, we identified two novel members of the caspase-6 interactome: hnRNP-M and MTA2.

**Keywords**: Apoptosis; Caspase-6; hnRNP-M; Interactome; MTA2; Proteomic analysis; Tandem affinity purification (TAP)

PMID: 24572280
Expression of the pro-domain-deleted active form of caspase-6 in *Escherichia coli*


Lee PY, Cho JH, Chi SW, Bae KH, Cho S, Park BC, Kim JH*, Park SG'.
'Co-corresponding: Sung Goo Park(sgpark@kribb.re.kr), Jeong-Hoon Kim(jhoonkim@kribb.re.kr)

Caspases are a family of cysteine proteases that play an important role in the apoptotic pathway. Caspase-6 is an apoptosis effector that cleaves a variety of cellular substrates. The active form of the enzyme is required for use in research. However, it has been difficult to obtain sufficient quantities of active caspase-6 from *Escherichia coli*. In the present study, we constructed a caspase-6 with a 23-amino-acid deletion in the pro-domain. This engineered enzyme was expressed as a soluble protein in *E. coli* and was purified using affinity resin. *In vitro* enzyme assay and cleavage analysis revealed that the engineered active caspase-6 protein had characteristics similar to those of wild-type caspase-6. This novel method can be a valuable tool for obtaining active caspase-6 that can be used for screening caspase-6-specific substrates, which in turn can be used to elucidate the function of caspase-6 in apoptosis.

**Keywords**: Active form; Apoptosis; Caspase-6 protein; *E. coli*; Enzyme assay

PMID: 24572277

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Discovery of novel protein tyrosine phosphatase sigma inhibitors through the virtual screening with modified scoring function


Park H, Lee HS, Ku B, Kim SJ'.
'Co-corresponding: Seung Jun Kim(ksj@kribb.re.kr)

Protein tyrosine phosphatase sigma (PTPσ) is a promising target for the development of therapeutics for the neurological diseases caused by the impaired recovery from neural injury. Based on the virtual screening with the scoring function involving a new accurate solvation energy term and *in vitro* enzyme assay, we identified seven competitive PTPσ inhibitors with the associated IC₅₀ values ranging from 5 to 11 μM. These inhibitors are structurally diverse and expected to have desirable physicochemical properties as a drug candidate. Therefore, they deserve consideration for further development by structure-activity relationship studies to optimize the inhibitory activities against the neurological diseases. Structural features relevant to the stabilization of the newly identified inhibitors in the active site of PTPσ are discussed in detail.

**Keywords**: Docking; Inhibitor; Neurological disease; Protein tyrosine phosphatase sigma (PTPσ); Solvation; Virtual screening
Proteomic analysis of the effect of retinoic acids on the human breast cancer cell line MCF-7


*Co-corresponding: Kwang-Hee Bae (khabae@kribb.re.kr), Sang Chul Lee (lesach@kribb.re.kr)

Breast cancer is the most common type of cancer in women in many areas and is increasing found in developing countries, where the majority of cases are diagnosed in late stages. Retinoic acids, through their associated nuclear receptors, exert intoxicating effects on cell growth, differentiation and apoptosis, and hold significant promise in relation to cancer therapy and chemoprevention. To enhance our understanding of the molecular mechanisms associated with retinoic acids in the breast cancer cell line MCF-7 in a time-dependent manner, we conducted a proteomic analysis of MCF-7 cells using the 2-DE couple with high-throughput mass spectrometry and bioinformatics tools. In the 2-DE patterns of MCF-7 cells treated with retinoic acid in a time-dependent manner, 35 protein spots were found to be differentially expressed. These were 17 increased, 4 decreased, and 14 unevenly expressed protein spots, all of which were analyzed using LTQ-FTICR mass spectrometry. Furthermore, five candidate proteins, up-regulated, were validated by western blotting. These were nucleoredoxin, latexitin, aminomethyltransferase, translationally controlled one tumor protein, and rab GDP dissociation inhibitor β. These observations represent novel findings leading to new insight into the exact mechanism behind the effect of retinoic acids in MCF-7 cells while also identifying possible therapeutic targets for breast cancer diagnosis and novel drug development paths for the treatment of this disease.

Keywords: Breast cancer; MCF-7 cell; Proteomic analysis; Retinoic acid; Therapeutic target

A conserved mechanism for binding of p53 DNA-binding domain and anti-apoptotic Bcl-2 family proteins


*Co-corresponding: Seung-Wook Chi (swchi@kribb.re.kr)

The molecular interaction between tumor suppressor p53 and the anti-apoptotic Bcl-2 family proteins plays an essential role in the transcription-independent apoptotic pathway of p53. In this study, we investigated the binding of p53 DNA-binding domain (p53DBD) with the anti-apoptotic Bcl-2 family proteins, Bcl-w, Mcl-1, and Bcl-2, using GST pull-down assay and NMR spectroscopy. The GST pull-down assays and NMR experiments demonstrated the direct binding of the p53DBD with Bcl-w, Mcl-1, and Bcl-2. Further, NMR chemical shift perturbation data showed that Bcl-w and Mcl-1 bind to the positively charged DNA-binding surface of p53DBD. Noticeably, the refined structural models of the complexes between p53DBD and Bcl-w, Mcl-1, and Bcl-2 showed that the binding mode of p53DBD is highly conserved among the anti-apoptotic Bcl-2 family proteins. Furthermore, the chemical shift perturbations on Bcl-w, Mcl-1, and Bcl-2 induced by p53DBD binding occurred not only at the p53DBD-binding acidic region but also at the BH3 peptide-binding pocket, which suggests an allosteric conformational change similar to that observed in Bcl-XL. Taken altogether, our results revealed a structural basis for a conserved binding mechanism between p53DBD and the anti-apoptotic Bcl-2 family proteins, which shed light on to the molecular understanding of the transcription-independent apoptosis pathway of p53.

Keywords: Apoptosis; Bcl-2 family protein; Binding mechanism; DNA-binding domain; p53; p53DBD

PMID: 24646834
Identification of DNA aptamers toward epithelial cell adhesion molecule via cell-SELEX


*Co-corresponding: Kwang-Hee Bae(khbae@kribb.re.kr), Sang Chul Lee(lesach@kribb.re.kr)

The epithelial cell adhesion molecule (EpCAM, also known as CD326) is a transmembrane glycoprotein that is specifically detected in most adenocarcinomas and cancer stem cells. In this study, we performed a Cell systematic evolution of ligands by exponential enrichment (SELEX) experiment to isolate the aptamers against EpCAM. After seven round of Cell SELEX, we identified several aptamer candidates. Among the selected aptamers, EP166 specifically binds to cells expressing EpCAM with an equilibrium dissociation constant (Kd) in a micromolar range. On the other hand, it did not bind to negative control cells. Moreover, EP166 binds to J1ES cells, a mouse embryonic stem cell line. Therefore, the isolated aptamers against EpCAM could be used as a stem cell marker or in other applications in both stem cell and cancer studies.

**Keywords**: Aptamer; Cancer; CD326; EpCAM; SELEX; Stem cell marker; Transmembrane glycoprotein

PMID: 25266702

Molecular basis for unidirectional scaffold switching of human Plk4 in centriole biogenesis


*Co-corresponding: Seung Jun Kim(ksj@kribb.re.kr)

Polo-like kinase 4 (Plk4) is a key regulator of centriole duplication, an event critical for the maintenance of genomic integrity. We show that Plk4 relocates from the inner Cep192 ring to the outer Cep152 ring as newly recruited Cep152 assembles around the Cep192-encircled daughter centriole. Crystal-structure analyses revealed that Cep192- and Cep152-derived peptides bind the cryptic polo box (CPB) of Plk4 in opposite orientations and in a mutually exclusive manner. The Cep152 peptide bound to the CPB markedly better than did the Cep192 peptide and effectively 'snatched' the CPB away from a preformed CPB-Cep192 peptide complex. A cancer-associated Cep152 mutation impairing the Plk4 interaction induced defects in procentriole assembly and chromosome segregation. Thus, Plk4 is intricately regulated in time and space through ordered interactions with two distinct scaffolds, Cep192 and Cep152, and a failure in this process may lead to human cancer.

**Keywords**: Cancer; Centriole duplication; Cep152 peptide; Cryptic polo box (CPB); Polo-like kinase 4 (Plk4)

PMID: 24997597
**HAX1 regulates E3 ubiquitin ligase activity of cIAPs by promoting their dimerization**


'Co-corresponding: Sung Goo Park(sgpark@kribb.re.kr), Jeong-Hoon Kim(jhoonkim@kribb.re.kr)

HS-1-associated protein X-1 (HAX1) is a multi-functional protein which was first identified as a Hematopoietic cell specific Lyn Substrate 1 (HS1)-binding protein. Although the roles of HAX1 in apoptosis have been unraveled and HAX1 has been proposed to be involved in several diseases, additional roles of HAX1 are still being identified. Here, we demonstrated that HAX1 directly interacted with cellular Inhibitor of Apoptosis Proteins (cIAPs), ubiquitin E3 ligases which regulate the abundance of cellular proteins, via ubiquitin-dependent proteasomal degradation. We showed that HAX1 promotes auto-ubiquitination and degradation of cIAPs by facilitating the intermolecular homodimerization of RING finger domain. Moreover, HAX1 regulates the non-canonical Nuclear Factor-κB (NF-κB) signaling pathway by modulating the stability of NF-κB-Inducing Kinase (NIK), which is one of the substrates of cIAPs. Taken together, these results unveil a novel role of HAX1 in the non-canonical NF-κB pathway, and provide an important clue that HAX1 is a potential therapeutic target for the treatment of cancer.

**Keywords**: Apoptosis; cIAPs; Dimerization; E3 ubiquitin ligase; HS1-binding protein; HS-1-associated protein X-1 (HAX1); NF-κB

PMID: 25275296

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**STAT3 inhibition suppresses proliferation of retinoblastoma through down-regulation of positive feedback loop of STAT3/miR-17-92 clusters**


'Co-corresponding: Jeong-Ki Min(jekmin@kribb.re.kr)

Retinoblastoma, the most common intraocular malignant tumor in children, is characterized by the loss of both functional alleles of RB1 gene, which however alone cannot maintain malignant characteristics of retinoblastoma cells. Nevertheless, the investigation of other molecular aberrations such as matrix metalloproteinases (MMPs) and miRNAs is still lacking. In this study, we demonstrate that STAT3 is activated in retinoblastoma cells, Ki67-positive areas of in vivo orthotopic tumors in BALB/c nude mice, and human retinoblastoma tissues of the advanced stage. Furthermore, target genes of STAT3 including BCL2, BCL2L1, BIRC5, and MPP9 are up-regulated in retinoblastoma cells compared to other retinal constituent cells. Interestingly, STAT3 inhibition by targeted siRNA suppresses the proliferation of retinoblastoma cells and the formation of in vivo orthotopic tumors. In line with these results, STAT3 siRNA effectively induces down-regulation of target genes of STAT3. In addition, miRNA microarray analysis and further real-time PCR experiments with STAT3 siRNA treatment show that STAT3 activation is related to the up-regulation of miR-17-92 clusters in retinoblastoma cells via positive feedback loop between them. In conclusion, we suggest that STAT3 inhibition could be a potential therapeutic approach in retinoblastoma through the suppression of tumor proliferation.

**Keywords**: Malignant tumor; miR-17-92; Retinoblastoma cell; STAT3 siRNA; Transcription factor

PMID: 25359779
Suppression of colorectal cancer liver metastasis by apolipoprotein(a) kringle V in a nude mouse model through the induction of apoptosis in tumor-associated endothelial cells


Ahn JH, Yu HK, Lee HJ, Hong SW, Kim SJ, Kim JS*. (Co-corresponding: Jang-Seong Kim@krrib.re.kr)

The formation of liver metastases in colorectal cancer patients is the primary cause of patient death. Current therapies directed at liver metastasis from colorectal cancer have had minimal impact on patient outcomes. Therefore, the development of alternative treatment strategies for liver metastasis is needed. In the present study, we demonstrated that recombinant human apolipoprotein(a) kringle V, also known as rhLK8, induced the apoptotic turnover of endothelial cells in vitro through the mitochondrial apoptosis pathway. The interaction of rhLK8 with glucose-regulated protein 78 (GRP78) may be involved in the induction of apoptosis because the inhibition of GRP78 by GRP78-specific antibodies or siRNA knockdown inhibited the rhLK8-mediated apoptosis of human umbilical vein endothelial cells in vitro. Next, to evaluate the effects of rhLK8 on angiogenesis and metastasis, an experimental model of liver metastasis was established by injecting a human colorectal cancer cell line, LS174T, into the spleens of BALB/c nude mice. The systemic administration of rhLK8 significantly suppressed liver metastasis from human colorectal cancer cells and improved host survival compared with controls. The combination of rhLK8 and 5-fluorouracil substantially increased these survival benefits compared with either therapy alone. Histological observation showed significant induction of apoptosis among tumor-associated endothelial cells in liver metastases from rhLK8-treated mice compared with control mice. Collectively, these results suggest that the combination of rhLK8 with conventional chemotherapy may be a promising approach for the treatment of patients with life-threatening colorectal cancer liver metastases.

Keywords: Apolipoproteins A; Apoptosis; Colorectal cancer; Conventional chemotherapy; Endothelial cell; GRP78; Liver metastasis; rhLK8

PMID: 24699568

Selection of aptamers for mature white adipocytes by cell SELEX using flow cytometry


Kim EY, Kim JW, Kim WK, Han BS, Park SG, Chung BH, Lee SC*, Bae KH*. (Co-corresponding: Kwang-Hee Bae(khbac@krrib.re.kr), Sang Chul Lee(lesach@krrib.re.kr)

BACKGROUND: Adipose tissue, mainly composed of adipocytes, plays an important role in metabolism by regulating energy homeostasis. Obesity is primarily caused by an abundance of adipose tissue. Therefore, specific targeting of adipose tissue is critical during the treatment of obesity, and plays a major role in overcoming it. However, the knowledge of cell-surface markers specific to adipocytes is limited.

METHODS AND RESULTS: We applied the CELL SELEX (Systematic Evolution of Ligands by EXponential enrichment) method using flow cytometry to isolate molecular probes for specific recognition of adipocytes. The aptamer library, a mixture of FITC-tagged single-stranded random DNAs, is used as a source for acquiring molecular probes. With the increasing number of selection cycles, there was a steady increase in the fluorescence intensity toward mature adipocytes. Through 12 rounds of SELEX, enriched aptamers showing specific recognition toward mature 3T3-L1 adipocyte cells were isolated. Among these, two aptamers (MA-33 and 91) were able to selectively bind to mature adipocytes with an equilibrium dissociation constant (Kd) in the nanomolar range. These aptamers did not bind to preadipocytes or other cell lines (such as HeLa, HEK-293, or C2C12 cells). Additionally, it was confirmed that MA-33 and 91 can distinguish between mature primary white and primary brown adipocytes.

CONCLUSIONS: These selected aptamers have the potential to be applied as markers for detecting mature white adipocytes and monitoring adipogenesis, and could emerge as an important tool in the treatment of obesity.

Keywords: Adipose tissue; Aptamer; Cell SELEX; Cell-surface marker; Obesity

PMID: 24844710
Antiangiogenic therapy with human apolipoprotein(a) kringle V and paclitaxel in a human ovarian cancer mouse model

Transl Oncol. 7(3):368-76.

Yu HK, Lee HJ, Yun SJ, Lee SJ, Langley RR, Yoon Y, Yi LS, Bae DS, Kim JS*, Kim SJ.
*Co-corresponding: Jang-Seong Kim(jangskim@kribb.re.kr)

INTRODUCTION: The present study compared the effect of combination therapy using human apolipoprotein(a) kringle V (rhLK8) to conventional chemotherapy with paclitaxel for human ovarian carcinoma producing high or low levels of vascular endothelial growth factor (VEGF). MATERIALS AND METHODS: Human ovarian carcinoma cells producing high (SKOV3ip1) or low (HeyA8) levels of VEGF were implanted into the peritoneal cavity of female nude mice. Seven days later, mice were randomized into four groups: control (vehicle), paclitaxel [5 mg/kg, weekly intraperitoneal (i.p.) injection], rhLK8 (50 mg/kg, daily i.p. injection), or the combination of paclitaxel and rhLK8. Mice were treated for 4 weeks and examined by necropsy.

RESULTS: In mice implanted with SKOV3ip1 cells, rhLK8 treatment had no significant effect on tumor incidence or the volume of ascites but induced a significant decrease in tumor weight compared with control mice. Paclitaxel significantly reduced tumor weight and ascites volume, and combination treatment with paclitaxel and rhLK8 had an additive therapeutic effect. Similarly, in HeyA8 mice, the effect of combination treatment on tumor weight and tumor incidence was statistically significantly greater than that of paclitaxel or rhLK8 alone. Immunohistochemical analysis showed a significant decrease in microvessel density and a marked increase of apoptosis in tumor and tumor-associated endothelial cells in response to combination treatment with paclitaxel and rhLK8.

CONCLUSION: Collectively, these results suggest that antiangiogenic therapy with rhLK8 in combination with taxane-based conventional chemotherapy could be effective for the treatment of ovarian carcinomas, regardless of VEGF status.

Keywords: Antiangiogenic therapy; Apolipoproteins A; Combination therapy; Ovarian carcinoma; Paclitaxel; rhLK8; VEGF

PMID: 25180060

Distinction of white, beige and brown adipocytes derived from mesenchymal stem cells

World J Stem Cells. 6(1):33-42.

Park A, Kim WK, Bae KH*.
*Corresponding: Kwang-Hee Bae(khbae@kribb.re.kr)

Adipose tissue is a major metabolic organ, and it has been traditionally classified as either white adipose tissue (WAT) or brown adipose tissue (BAT). WAT and BAT are characterized by different anatomical locations, morphological structures, functions, and regulations. WAT and BAT are both involved in energy balance. WAT is mainly involved in the storage and mobilization of energy in the form of triglycerides, whereas BAT specializes in dissipating energy as heat during cold- or diet-induced thermogenesis. Recently, brown-like adipocytes were discovered in WAT. These brown-like adipocytes that appear in WAT are called beige or brite adipocytes. Interestingly, these beige/brite cells resemble white fat cells in the basal state, but they respond to thermogenic stimuli with increased levels of thermogenic genes and increased respiration rates. In addition, beige/brite cells have a gene expression pattern distinct from that of either white or brown fat cells. The current epidemic of obesity has increased the interest in studying adipocyte formation (adipogenesis), especially in beige/brite cells. This review summarizes the developmental process of adipose tissues that originate from the mesenchymal stem cells and the features of these three different types of adipocytes.

Keywords: Adipogenesis; Beige/brite adipocytes; Brown adipocytes; Browning; Mesenchymal stem cells; Thermogenesis; White adipocytes

PMID: 24567786
2014 KRIBB Article Abstracts:
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PHLPP1 regulates contact inhibition by dephosphorylating Mst1 at the inhibitory site


Jung S, Kang JG, Lee JH, Song KJ, Ko JH, Kim YS*. 'Corresponding: Yong-Sam Kim(omsys1@kribb.re.kr)

Contact inhibition has been largely elusive despite that a loss of contact inhibition is a critical event for cancer development and progression. Here, we report that PHLPP1 is a binding protein for Mst1 and it modulates the Hippo pathway by dephosphorylating Mst1 at the inhibitory Thr(387) of Mst1. Yap1 was localized predominantly in the nucleus but marginally in the cytoplasm in HeLa cells under sparse conditions, whereas the functional protein was more directed to sequestration in the cytoplasm under dense environments. Furthermore, loss of PHLPP1 resulted in a failure of the apoptotic control. It is interesting that down-regulated expression of PHLPP1 appears to mimic the loss of contact inhibition, a hallmark of cancer.

Keywords: Apoptotic control; Cancer; Contact inhibition; Dephosphorylating Mst1; Hippo pathway; PHLPP1
PMID: 24393845

Aging. 67(7):524-44.

Kim JY, Park YK, Lee KP, Lee SM, Kang TW, Kim HJ, Dho SH, Kim SY*, Kwon KS*. 'Co-corresponding: Ki-Sun Kwon(kwonks@kribb.re.kr), Seon-Young Kim(kimsy@kribb.re.kr)

Skeletal muscle degenerates progressively, losing mass (sarcopenia) over time, which leads to reduced physical ability and often results in secondary diseases such as diabetes and obesity. The regulation of gene expression by microRNAs is a key event in muscle development and disease. To understand genome-wide changes in microRNAs and mRNAs during muscle aging, we sequenced microRNAs and mRNAs from mouse gastrocnemius muscles at two different ages (6 and 24 months). Thirty-four microRNAs (15 upregulated and 19 downregulated) were differentially expressed with age, including the microRNAs miR-206 and -434, which were differentially expressed in aged muscle in previous studies. Interestingly, eight microRNAs in a microRNA cluster at the imprinted Dlk1-Dio3 locus on chromosome 12 were coordinately downregulated. In addition, sixteen novel microRNAs were identified. Integrative analysis of microRNA and mRNA expression revealed that microRNAs may contribute to muscle aging through the positive regulation of transcription, metabolic processes, and kinase activity. Many of the age-related microRNAs have been implicated in human muscular diseases. We suggest that genome-wide microRNA profiling will expand our knowledge of microRNA function in the muscle aging process.

Keywords: Aging; Imprinted Dlk1-Dio3; Integrative analysis; microRNA cluster; microRNA profiling; Skeletal muscle
PMID: 25063768
Astrocytic phospholipase A2 contributes to neuronal glutamate toxicity

*Brain Res.* 1590:97-106.

Ha JS, Dho SH, Youm TH, Kwon KS, Park SS'.

Corresponding: Sung Sup Park(sspark@kribb.re.kr)

The role of astrocytes in glutamate toxicity has been controversial. Here, we show that astrocytes in neuron-astrocyte co-cultures increased neuronal sensitivity to chronic glutamate exposure but not to acute exposure. Enhanced neuronal toxicity by chronic exposure was dependent on astrocyte cell numbers. A reduced generation of extracellular H$_2$O$_2$ induced by glutamate was observed in co-cultures. Further, neuronal glutamate toxicity was not suppressed by NADPH oxidase (Nox) inhibitors, catalase or Nox4 knockdown in co-cultures, whereas these compounds effectively reduced the toxicity in pure neuron cultures. Instead, the intracellular scavenger of reactive oxygen species, N-acetylcysteine (NAC), reduced neuronal cytotoxicity in co-cultures, whereas catalase worked in pure neuron cultures. Lipoxigenase (LOX) inhibitors attenuated neuronal glutamate toxicity in co-cultures but not in pure neuron cultures. Neuronal 5-LOX activity was increased only in co-cultures, whereas 12-LOX activity was increased in both types of cultures. The cyclooxygenase (COX) inhibitors, indomethacin and NS-398, and the phospholipase A2 (PLA2) inhibitors, LY311727 and MAFP, more effectively reduced neuronal glutamate toxicity in co-cultures than in pure neuron cultures. However, in co-cultures, pre-treating neurons and astrocytes with the same inhibitors generated opposite results. COX inhibitors suppressed neuronal glutamate toxicity in pre-treated neurons rather than astrocytes, whereas PLA2 inhibitors reduced the toxicity in pre-treated astrocytes rather than neurons. Gene-specific knockdown of PLA2 confirmed these results. Knockdown of sPLA2-α and/or sPLA2-V in astrocytes rather than in neurons more effectively reduced glutamate toxicity in co-cultures. These findings suggest that astrocytic PLA2 activity increases neuronal sensitivity to chronic glutamate exposure in neuron-astrocyte co-cultures.

Keywords: Chronic glutamate toxicity; Cyclooxygenase (COX); Neuron-astrocyte co-culture; Phospholipase A2 (PLA2); Pure neuron culture

PMID: 25451090

API5 confers tumoral immune escape through FGF2-dependent cell survival pathway


Co-first: Seok-Ho Kim(kims@kribb.re.kr)

Identifying immune escape mechanisms used by tumors may define strategies to sensitize them to immunotherapies to which they are otherwise resistant. In this study, we show that the antiapoptotic gene API5 acts as an immune escape gene in tumors by rendering them resistant to apoptosis triggered by tumor antigen-specific T cells. Its RNAi-mediated silencing in tumor cells expressing high levels of API5 restored antigen-specific immune sensitivity. Conversely, introducing API5 into API5(low) cells conferred immune resistance. Mechanistic investigations revealed that API5 mediated resistance by upregulating FGF2 signaling through a FGFR1/PKCδ/ERK effector pathway that triggered degradation of the proapoptotic molecule BIM. Blockade of FGF2, PKCδ, or ERK phenocopied the effect of API5 silencing in tumor cells expressing high levels of API5 to either murine or human antigen-specific T cells. Our results identify a novel mechanism of immune escape that can be inhibited to potentiate the efficacy of targeted active immunotherapies.

Keywords: Antiapoptotic gene API5; Apoptosis; FGF2 signaling; Immune escape; Immunotherapy

PMID: 24769442
Effect of exposure to interleukin-21 at various time points on human natural killer cell culture

Cytotherapy. 16(10):1419-30.

*Co-first: Seok-Ho Kim(kims@kribb.re.kr)

BACKGROUND AIMS: Interleukin-21 (IL-21) can enhance the effector function of natural killer (NK) cells but also limits their proliferation when continuously combined with IL-2/IL-15. Paradoxically, membrane-bound (mb)-IL-21 has been shown to improve human NK cell proliferation when cultured with IL-2/mb-IL-15. To clarify the role of IL-21, we investigated the effect of the timing of IL-21 addition to NK cell culture.

METHODS: IL-2/IL-15-activated NK cells were additionally treated with IL-21 according to the following schedules; (i) control (without IL-21); (ii) first week (day 0 to day 7); (iii) intermittent (the first 3 days of each week for 7 weeks); (iv) after 1 week (day 8 to day 14); and (v) continuous (day 0 to day 49). The expression of NK receptors, granzyme B, perforin, CD107a, interferon-γ, telomere length and NK cell death were measured by flow cytometry.

RESULTS: Compared with the control (2004.2-fold; n = 10 healthy donors) and intermittent groups (2063.9-fold), a strong proliferative response of the NK cells on day 42 was identified in the "first week" group (3743.8-fold) (P < 0.05). NK cells treated with IL-21 in the "first week" group showed cytotoxicity similar to that in control cells. On day 28, there was a significant increase in cytotoxicity of "first week" NK cells that received IL-21 treatment for an additional 2 days compared with the "first week" NK cells (P < 0.05).

CONCLUSIONS: These data suggest that controlling temporal exposure of IL-21 during NK cell proliferation can be a critical consideration to improve the yields and cytotoxicity of NK cells.

Keywords: Cytotoxicity; IL-2; IL-15; IL-21; Natural killer (NK) cell; NK cell proliferation
PMID: 24950680

Peroxisiredoxin 3 has a crucial role in the contractile function of skeletal muscle by regulating mitochondrial homeostasis


*Corresponding: Ki-Sun Kwon(kwonks@kribb.re.kr)

Antioxidant systems against reactive oxygen species (ROS) are important factors in regulating homeostasis in various cells, tissues, and organs. Although ROS are known to cause to muscular disorders, the effects of mitochondrial ROS in muscle physiology have not been fully understood. Here, we investigated the effects of ROS on muscle mass and function using mice deficient in peroxiredoxin 3 (Prx3), which is a mitochondrial antioxidant protein. Ablation of Prx3 deregulated the mitochondrial network and membrane potential of myotubes, in which ROS levels were increased. We showed that the DNA content of mitochondria and ATP production were also reduced in Prx3-KO muscle. Of note, the mitofusin 1 and 2 protein levels decreased in Prx3-KO muscle, a biochemical evidence of impaired mitochondrial fusion. Contractile dysfunction was examined by measuring isometric forces of isolated extensor digitorum longus (EDL) and soleus muscles. Maximum absolute forces in both the EDL and the soleus muscles were not significantly affected in Prx3-KO mice. However, fatigue trials revealed that the decrease in relative force was greater and more rapid in soleus from Prx3-KO compared to wild-type mice. Taken together, these results suggest that Prx3 plays a crucial role in mitochondrial homeostasis and thereby controls the contractile functions of skeletal muscle.

Keywords: Fatigue; Free radical; Mitochondria; Mitofusin; Myoblast; Myogenesis; Oxidative stress; Peroxisiredoxin 3 (Prx3); Reactive oxygen species (ROS); Skeletal muscle
PMID: 25224038
Systematic targeted gene deletion using the gene-synthesis method in fission yeast


*Co-corresponding: Dong-Uk Kim(kimdongu@kribb.re.kr)

Genome-wide targeted gene deletion, a systematic method to study gene function by replacing target genes with deletion cassettes, using serial-PCR or block-PCR requires elaborate skill. We developed a novel gene-synthesis method to systematically prepare deletion cassettes on a 96-well basis in fission yeast. We designed the 2129-bp deletion cassette as three modules: a central 1397-bp KanMX4 selection marker module and two flanking 366-bp gene-specific artificial linker modules. The central KanMX4 module can be used in multiple deletion cassettes in combination with different sets of flanking modules. The deletion cassettes consisted of 147 oligonucleotides (93 for the central module+25 for each of the flanking modules+4 for the joints) and the oligonucleotides were designed as ~29mers using an in-house program. Oligonucleotides were synthesized on a 96-well basis and ligated into deletion cassettes without gaps by ligase chain reaction, which was followed by two rounds of nested PCR to amplify trace amounts of the ligated cassettes. After the artificial linkers were removed from the deletion cassettes, the cassettes were transformed into wild-type diploid fission yeast strain SP286. We validated the transformed colonies via check PCR and subjected them to tetrad analysis to confirm functional integrity. Using this method, we systematically deleted 563 genes in the fission yeast Schizosaccharomyces pombe with a >90% success rate and a point-mutation rate of ~0.4 mutations per kb. Our method can be used to create systematic gene deletions in a variety of yeasts especially when it included a bar-code system for parallel analyses.

**Keywords**: Artificial sequence linker; Deletion cassette; Fission yeast; Gene deletion; Gene synthesis; Ligase chain reaction; Schizosaccharomyces pombe

PMID: 25150109

Transcriptional regulation of Caenorhabditis elegans FOXO/DAF-16 modulates lifespan

Longev. Healthspan. 3:5.


*Co-first: Eun-Soo Kwon(eunsoo.kwon@kribb.re.kr)

BACKGROUND: Insulin/IGF-1 signaling plays a central role in longevity across phylogeny. In C. elegans, the forkhead box O (FOXO) transcription factor, DAF-16, is the primary target of insulin/IGF-1 signaling, and multiple isoforms of DAF-16 (a, b, and d/f) modulate lifespan, metabolism, dauer formation, and stress resistance. Thus far, across phylogeny modulation of mammalian FOXOs and DAF-16 have focused on post-translational regulation with little focus on transcriptional regulation. In C. elegans, we have previously shown that DAF-16/d/f cooperates with DAF-16a to promote longevity. In this study, we generated transgenic strains expressing near-endogenous levels of either daf-16a or daf-16d/f, and examined temporal expression of the isoforms to further define how these isoforms contribute to lifespan regulation.

RESULTS: Here, we show that DAF-16a is sensitive both to changes in gene dosage and to alterations in the level of insulin/IGF-1 signaling. Interestingly, we find that as worms age, the intestinal expression of daf-16d/f but not daf-16a is dramatically upregulated at the level of transcription. Preventing this transcriptional upregulation shortens lifespan, indicating that transcriptional regulation of daf-16d/f promotes longevity. In an RNAi screen of transcriptional regulators, we identify elt-2 (GATA transcription factor) and swm-1 (core subunit of SWI/SNF complex) as key modulators of daf-16d/f gene expression. ELT-2 and another GATA factor, ELT-4, promote longevity via both DAF-16a and DAF-16d/f while the components of SWI/SNF complex promote longevity specifically via DAF-16d/f.

CONCLUSIONS: Our findings indicate that transcriptional control of C. elegans FOXO/DAF-16 is an essential regulatory event. Considering the conservation of FOXO across species, our findings identify a new layer of FOXO regulation as a potential determinant of mammalian longevity and age-related diseases such as cancer and diabetes.

**Keywords**: Aging; C. elegans; DAF-16/FOXO; Insulin/IGF-1; Isoform; Longevity; Transcription

PMID: 24834345
Quantitative proteome analysis of age-related changes in mouse gastrocnemius muscle using mTRAQ


"Co-corresponding: Ki-Sun Kwon(kwonks@kribb.re.kr)"

Aging is associated with a progressive loss of skeletal muscular function that often leads to progressive disability and loss of independence. Although muscle aging is well documented, the molecular mechanisms of this condition still remain unclear. To gain greater insight into the changes associated with aging of skeletal muscle, we performed quantitative proteomic analyses on young (6 months) and aged (27 months) mouse gastrocnemius muscles using mTRAQ stable isotope mass tags. We identified and quantified a total of 4585 peptides corresponding to 236 proteins (protein probability >0.9). Among them, 33 proteins were more than 1.5-fold upregulated and 20 proteins were more than 1.5-fold downregulated in aged muscle compared with young muscle. An ontological analysis revealed that differentially expressed proteins belonged to distinct functional groups, including ion homeostasis, energy metabolism, protein turnover, and Ca(2+) signaling. Identified proteins included aralar1, β-enolase, fatty acid-binding protein 3, 3-hydroxyacyl-CoA dehydrogenase (Hadh), F-box protein 22, F-box, and leucine-rich repeat protein 18, voltage-dependent L-type calcium channel subunit beta-1, ryanoide receptor (RyR), and calsequestrin. Ectopic expression of calsequestrin in C2C12 myoblast resulted in decreased activity of nuclear factor of activated T-cells and increased levels of atrogin-1 and MuRF1 E3 ligase, suggesting that these differentially expressed proteins are involved in muscle aging.

**Keywords**: Aging; Animal proteomics; Biomarker; Proteomic analysis; Skeletal muscle; mTRAQ

PMID: 24243720

MLK3 is part of a feedback mechanism that regulates different cellular responses to reactive oxygen species

Sci Signal. 7(328):ra52.

Lee HS, Hwang CY, Shin SY, Kwon KS, Cho KH.  
"Co-corresponding: Ki-Sun Kwon(kwonks@kribb.re.kr)"

Reactive oxygen species (ROS) influence diverse cellular processes, including proliferation and apoptosis. Both endogenous and exogenous ROS activate signaling through mitogen-activated proteins kinase (MAPK) pathways, including those involving extracellular signal-regulated kinases (ERKs) or c-Jun N-terminal kinases (JNKs). Whereas low concentrations of ROS generally stimulate proliferation, high concentrations result in cell death. We found that low concentrations of ROS induced activating phosphorylation of ERKs, whereas high concentrations of ROS induced activating phosphorylation of JNKS. Mixed lineage kinase 3 (MLK3, also known as MAP3K11) directly phosphorylates JNKS and may control activation of ERKs. Mathematical modeling of MAPK networks revealed a positive feedback loop involving MLK3 that determined the relative phosphorylation of ERKs and JNKS by ROS. Cells exposed to an MLK3 inhibitor or cells in which MLK3 was knocked down showed increased activation of ERKs and decreased activation of JNKS and were resistant to cell death when exposed to high concentrations of ROS. Thus, the data indicated that MLK3 is a critical factor controlling the activity of kinase networks that control the cellular responses to different concentrations of ROS.

**Keywords**: ERKs; JNK; Mitogen-activated proteins kinase (MAPK); Mixed lineage kinase 3 (MLK3, MAP3K11); Reactive oxygen species (ROS)

PMID: 24894995
Dominant role of peroxiredoxin/JNK axis in stemness regulation during neurogenesis from embryonic stem cells

Stem Cells. 32(4):998-1011.


Redox balance has been suggested as an important determinant of "stemness" in embryonic stem cells (ESCs). In this study, we demonstrate that peroxiredoxin (Prx) plays a pivotal role in maintenance of ESC stemness during neurogenesis through suppression of reactive oxygen species (ROS)-sensitive signaling. During neurogenesis, Prx I and Oct4 are expressed in a mutually dependent manner and their expression is abruptly downregulated by an excess of ROS. Thus, in Prx I or Prx II ESCs, rapid loss of stemness can occur due to spontaneous ROS overload, leading to their active commitment into neurons; however, stemness is restored by the addition of an antioxidant or an inhibitor of c-Jun N-terminal kinase (JNK). In addition, Prx I and Prx II appear to have a tight association with the mechanism underlying the protection of ESC stemness in developing teratomas. These results suggest that Prx functions as a protector of ESC stemness by opposing ROS/JNK cascades during neurogenesis. Therefore, our findings have important implications for understanding of maintenance of ESC stemness through involvement of antioxidant enzymes and may lead to development of an alternative stem cell-based therapeutic strategy for production of high-quality neurons in large quantity.

Keywords: Antioxidant enzyme; Embryonic stem cell; ESC stemness; JNK; Neurogenesis; Peroxiredoxin (Prx); Reactive oxygen species (ROS)

PMID: 24715692

Methylosulfionylmethanethane suppresses hepatic tumor development through activation of apoptosis

World J Hepatol. 6(2):98-106.


’Corresponding: Dae-Yeul Yu@kribb.re.kr)

AIM: To investigate the effect of methylosulfionylmethanethane (MSM), recently reported to have anti-cancer effects, in liver cancer cells and transgenic mice.

METHODS: Three liver cancer cell lines, HepG2, Huh7-Mock and Huh7-H-rasG12V, were used. Cell growth was measured by Cell Counting Kit-8 and soft agar assay. Western blot analysis was used to detect caspases, poly(ADP-ribose) polymerase (PARP), and B-cell lymphoma 2 (Bcl-2) expressions. For in vivo study, we administered MSM to H-rasG12V transgenic mice for 3 mo.

RESULTS: MSM decreased the growth of HepG2, Huh7-Mock and Huh7-H-rasG12V cells in a dose-dependent manner. That was correlated with significantly increased apoptosis and reduced cell numbers in MSM treated cells. Cleaved caspase-8, cleaved caspase-3 and cleaved PARP were remarkably increased in the liver cancer cells treated with 500 mmol/L of MSM; however, Bcl-2 was slightly decreased in 500 mmol/L. Liver tumor development was greatly inhibited in the H-rasG12V transgenic mice treated with MSM, compared to control, by showing reduced tumor size and number. Cleaved PARP was significantly increased in non-tumor treated with MSM compared to control.

CONCLUSION: Liver injury was also significantly attenuated in the mice treated with MSM. Taken together, all the results suggest that MSM has anti-cancer effects through inducing apoptosis in liver cancer.

Keywords: Anti-cancer effect; Apoptosis; Hepatic tumorigenesis; Liver cancer; Methylosulfionylmethanethane (MSM); Transgenic mice

PMID: 24575169
Short-term differential adaptation to anaerobic stress via genomic mutations by Escherichia coli strains K-12 and B lacking alcohol dehydrogenase

Front Microbiol. 5:476.

Kim HJ, Jeong H, Hwang S, Lee MS, Lee YJ, Lee DW, Lee SJ’.

*Co-corresponding: Sang Jun Lee(keesj@kribb.re.kr)

Microbial adaptations often occur via genomic mutations under adverse environmental conditions. This study used Escherichia coli ΔadhE cells as a model system to investigate adaptation to anaerobic conditions, which we then compared with the adaptive mechanisms of two closely related E. coli strains, K-12 and B. In contrast to K-12 ΔadhE cells, the E. coli B ΔadhE cells exhibited significantly delayed adaptive growth under anaerobic conditions. Adaptation by the K-12 and B strains mainly employed anaerobic lactate fermentation to restore cellular growth. Several mutations were identified in the pta or pflB genes of adapted K-12 cells, but mostly in the pta gene of the B strains. However, the types of mutation in the adapted K-12 and B strains were similar. Cellular viability was affected directly by severe redox imbalance in B ΔadhE cells, which also impaired their ability to adapt to anaerobic conditions. This study demonstrates that closely related microorganisms may undergo different adaptations under the same set of adverse conditions, which might be associated with the specific metabolic characteristics of each strain. This study provides new insights into short-term microbial adaptation to stressful conditions, which may reflect dynamic microbial population changes in nature.

Keywords: Alcohol dehydrogenase; Anaerobic condition; Genomic mutation; Microbial adaptation; pflB gene; pta gene; Redox balance

PMID: 25250024

Oral administration of poly-γ-glutamate ameliorates atopic dermatitis in Nc/Nga mice by suppressing Th2-biased immune response and production of IL-17A


Lee TY, Kim DJ, Won JN, Lee IH, Sung MH, Poo H’.

*Corresponding: Haryoung Poo(haryoung@kribb.re.kr)

Atopic dermatitis (AD) is a chronic inflammatory skin disease that is closely related to dysregulation of the T helper type 1 and 2 (Th1/Th2) balance. A previous study showed that high molecular mass poly-γ-glutamate (γ-PGA) isolated from Bacillus subtilis sp. Chungkookjang induces the production of IL-12 from dendritic cells (DCs). Here, we investigated the effect of γ-PGA on AD-like skin disease using an Nc/Nga mouse model. In vitro, γ-PGA activated DCs and induced IL-12 production in mice. In vivo, oral administration of γ-PGA markedly reduced the AD symptoms, similar to the response seen in the dexamethasone (Dex)-treated group. Treatment with γ-PGA also decreased the serum levels of IgG1, the skin levels of Th2 cytokines, the extent of skin inflammation, and the accumulation of mast cells. Furthermore, γ-PGA was effective against established AD, significantly decreasing serum IgE and Th2 cytokines in the inflamed tissue. Interestingly, the production of IL-17A in splenocytes was also suppressed by γ-PGA, indicating that it inhibits both Th2 and Th17 immune responses. Collectively, these results suggest that oral administration of γ-PGA could be a therapeutic strategy for treating AD via the modulation of Th2-biased immune responses in an Nc/Nga mouse model.

Keywords: Atopic dermatitis (AD); Chronic inflammatory; IL-12; IL-17A; Immune response; Poly-γ-glutamate (γ-PGA); Skin inflammation

PMID: 24025551
Metabolite changes signal genetic regulatory mechanisms for robust cell behavior


Lee SJ*, Trostel A, Adhya S.
*First: Sang Jun Lee(leesj@kribb.re.kr)

Exploiting mechanisms of utilizing the sugar d-galactose in Escherichia coli as a model system, we explored the consequences of accumulation of critical intermediates of the d-galactose metabolic pathways by monitoring cell growth, metabolites, and transcript profiles. These studies revealed both metabolic network changes far from the d-galactose pathway and changes in the global gene regulatory network. The concentration change of a critical intermediate disturbs the equilibrium state, generating a ripple effect through several metabolic pathways that ends up signaling up- or downregulation of specific sets of genes in a programmed manner to cope with the imbalance. Such long-range effects on metabolites and genetic regulatory mechanisms not only may be a common feature in bacteria but very likely operate during cellular development and differentiation in higher organisms as well as in disease cells, like cancer cells.

IMPORTANCE: Metabolite accumulation can create adverse intracellular conditions that are relieved by compensatory immediate changes of metabolite pools and later changes of transcript levels. It has been known that gene expression is normally regulated by added catabolic substrates (induction) or anabolic end products (repression). It is becoming apparent now that change in the concentration of metabolic intermediates also plays a critical role in genetic regulatory networks for metabolic homeostasis. Our study provides new insight into how metabolite pool changes transduce signals to global gene regulatory networks.

Keywords: Adverse intracellular condition; d-galactose pathway; Exploiting mechanism; Genetic regulatory; Metabolic network

PMID: 24473130

The structural basis for the negative regulation of thioredoxin by thioredoxin-interacting protein

Nat Commun. 5:2958.

*Corresponding: Myung Hee Kim(mhk8n@kribb.re.kr)

The redox-dependent inhibition of thioredoxin (TRX) by thioredoxin-interacting protein (TXNIP) plays a pivotal role in various cancers and metabolic syndromes. However, the molecular mechanism of this regulation is largely unknown. Here, we present the crystal structure of the TRX-TXNIP complex and demonstrate that the inhibition of TRX by TXNIP is mediated by an intermolecular disulphide interaction resulting from a novel disulphide bond-switching mechanism. Upon binding to TRX, TXNIP undergoes a structural rearrangement that involves switching of a head-to-tail interprotomer Cys63-Cys247 disulphide between TXNIP molecules to an interdomain Cys63-Cys190 disulphide, and the formation of a de novo intermolecular TXNIP Cys247-TRX Cys32 disulphide. This disulphide-switching event unexpectedly results in a domain arrangement of TXNIP that is entirely different from those of other arrestin family proteins. We further show that the intermolecular disulphide bond between TRX and TXNIP dissociates in the presence of high concentrations of reactive oxygen species. This study provides insight into TRX and TXNIP-dependent cellular regulation.

Keywords: Cellular regulation; Disulphide-switching; Intermolecular disulphide; Thioredoxin (TRX); Thioredoxin-interacting protein (TXNIP); TRX-TXNIP

PMID: 24389582
TMPRSS4 induces cancer cell invasion through pro-uPA processing


Min HJ, Lee MK, Lee JW, Kim S. 

"Co-corresponding: Semi Kim(semikim@kribb.re.kr)

TMPRSS4 is a novel type II transmembrane serine protease that is highly expressed on the cell surface in pancreatic, thyroid, colon, and other cancer tissues. Previously, we demonstrated that TMPRSS4 mediates cancer cell invasion, epithelial-mesenchymal transition, and metastasis and that increased TMPRSS4 expression correlates with colorectal cancer progression. We also demonstrated that TMPRSS4 upregulates urokinase-type plasminogen activator (uPA) gene expression to induce cancer cell invasion. However, it remains unknown how proteolytic activity of TMPRSS4 contributes to invasion. In this study, we report that TMPRSS4 directly converted inactive pro-uPA into the active form through its proteolytic activity. Analysis of conditioned medium from cells expressing TMPRSS4 demonstrated that the active TMPRSS4 protease domain is released from the cells and is associated with the plasma membrane. Furthermore, TMPRSS4 could increase pro-uPA-mediated invasion in a serine proteolytic activity-dependent manner. These observations suggest that TMPRSS4 is an upstream regulator of pro-uPA activation. This study provides valuable insights into the proteolytic function of TMPRSS4 as well as mechanisms for the control of invasion.

**Keywords**: Cancer cell invasion; Processing; Proteolytic function; TMPRSS4; Transmembrane serine protease; Urokinase-type plasminogen activator (uPA)

PMID: 24434139

A PAUF-neutralizing antibody targets both carcinoma and endothelial cells to impede pancreatic tumor progression and metastasis


Pancreatic adenocarcinoma up-regulated factor (PAUF) is expressed in pancreatic ductal adenocarcinoma (PDAC) and plays an important role in tumor progression and metastasis. Here we evaluate the anti-tumor efficacy of a human monoclonal antibody against PAUF, PMAb83, to provide a therapeutic intervention to treat the disease. PMAb83 reduced tumor growth and distant metastasis in orthotopically xenografted mice of human PDAC cells. PMAb83 treatment retarded proliferation along with weakened aggressiveness traits of the carcinoma cells. AKT/β-catenin signaling played a role in the carcinoma cell proliferation and the treated xenograft tumors exhibited reduced levels of β-catenin and cyclin D1. Moreover PMAb83 abrogated the PAUF-induced angiogenic responses of endothelial cells, reducing the density of CD31+ vessels in the treated tumors. In combination with gemcitabine, PMAb83 conferred enhanced survival of xenografted mice by about twofold compared to gemcitabine alone. Taken together, our findings show that PMAb83 treatment decreases the aggressiveness of carcinoma cells and suppresses tumor vascularization, which culminates in mitigated tumor growth and metastasis with improved survival in PDAC mouse models.

**Keywords**: Anti-tumor efficacy; Metastasis; Monoclonal antibody; Pancreatic adenocarcinoma up-regulated factor (PAUF); Pancreatic cancer; Pancreatic ductal adenocarcinoma (PDAC); PMAb83

PMID: 25450371
Donor-derived natural killer cells infused after human leukocyte antigen-haploidentical hematopoietic cell transplantation: a dose-escalation study


‘Co-corresponding: Inpyo Choi(ipchoi@kribb.re.kr)

The doses of donor-derived natural killer (NK) cells that can be given safely after human leukocyte antigen (HLA)-haploidentical hematopoietic cell transplantation (HCT) remain to be defined. Forty-one patients (ages 17 to 75 years) with hematologic malignancy underwent HLA-haploidentical HCT after reduced-intensity conditioning containing busulfan, fludarabine, and antithymocyte globulin. Cell donors (ages 7 to 62 years) underwent growth factor-mobilized leukapheresis for 3 to 4 days. Cells collected on the first 2 to 3 days were used for HCT, whereas those collected on the last day were CD3-depleted and cultured into NK cells using human interleukins-15 and -21. These NK cells were then infused into patients twice at 2 and 3 weeks after HCT at an escalating doses of .2 × 10(8) cells/kg of body weight (3 patients), .5 × 10(8) cells/kg (3 patients), 1.0 × 10(8) cells/kg (8 patients), and ≥ 1.0 × 10(8) cells/kg or available cells (27 patients). At all dose levels, no acute toxicity was observed after NK cell infusion. After HLA-haploidentical HCT and subsequent donor NK cell infusion, when referenced to 31 historical patients who had undergone HLA-haploidentical HCT after the same conditioning regimen but without high-dose NK cell infusion, there was no significant difference in the cumulative incidences of major HCT outcomes, including engraftment (absolute neutrophil count ≥ 500/μL, 85% versus 87%), grade 2 to 4 acute graft-versus-host disease (GVHD, 17% versus 16%), moderate to severe chronic GVHD (15% versus 10%), and transplantation-related mortality (27% versus 19%). There was, however, a significant reduction in leukemia progression (74% to 46%), with post-transplantation NK cell infusion being an independent predictor for less leukemia progression (hazard ratio, .527). Our findings showed that, when given 2 to 3 weeks after HLA-haploidentical HCT, donor-derived NK cells were well tolerated at a median total dose of 2.0 × 10(8) cells/kg. In addition, they may decrease post-transplantation progression of acute leukemia.

Keywords: Donor natural killer cell; Hematopoietic cell transplantation (HCT); Human leukocyte antigen (HLA); Leukemia progression; NK cell infusion

PMID: 24525278

ZEB2-Sp1 cooperation induces invasion by upregulating cadherin-11 and integrin α5 expression

Carcinogenesis. 35(2):302-14.

Nam EH, Lee Y, Zhao XF, Park YK, Lee JW, Kim S’. Corresponding: Semi Kim(semikim@kribb.re.kr)

Epithelial-mesenchymal transition (EMT) is a process implicated in invasion and metastasis. EMT is characterized by repression of epithelial markers and induction of mesenchymal markers. ZEB2 is a transcriptional repressor of E-cadherin, leading to EMT. Previously, we have shown that ZEB2 directly upregulates integrin α5 transcription by cooperating with the transcription factor Sp1. In this study, we investigated the precise mechanism by which ZEB2 modulates invasion and EMT events and the role of Sp1 in ZEB2-induced invasion. We found that ZEB2 directly induced cadherin-11 transcription in an Sp1-dependent, but Smad- and E-box-independent, manner and repressed E-cadherin expression in an Sp1- and Smad-independent manner, leading to cadherin switch. Furthermore, ZEB2 upregulated Sp1 by enhancing Sp1 protein stability, and Sp1 was found to be critical for ZEB2-induced cancer cell invasion, mainly through induction of cadherin-11 and integrin α5. Expression levels of cadherin-11 and integrin α5 were interdependent and both modulated c-Jun N-terminal kinase-signaling activity and invasion. Immunofluorescence analysis showed that nuclear expression of ZEB2 was positively correlated with Sp1 expression in human colorectal cancers. Together, these findings demonstrate a previously unrecognized interplay between ZEB2, Sp1, cadherin-11 and integrin α5 that is, probably, significant in tumor progression and metastasis.

Keywords: Colorectal cancer; Epithelial-mesenchymal transition (EMT); Metastasis; Sp1 protein stability; Tumor progression; ZEB2-induced invasion

PMID: 24130169
TMPRSS4 upregulates uPA gene expression through JNK signaling activation to induce cancer cell invasion

Cell Signal. 26(2):398-408.

Min HJ, Lee Y, Zhao XF, Park YK, Lee MK, Lee JW, Kim S'.
'Corresponding: Semi Kim(semikim@kribb.re.kr)

TMPRSS4 is a novel type II transmembrane serine protease that is highly expressed in pancreatic, thyroid, colon, and other cancer tissues. Previously, we demonstrated that TMPRSS4 mediates tumor cell invasion, migration, and metastasis. However, the mechanisms by which TMPRSS4 contributes to invasion are not fully understood. Here, we demonstrated that TMPRSS4-induced transcription of the urokinase-type plasminogen activator (uPA) gene through activating the transcription factors Sp1, Sp3, and AP-1 in a JNK-dependent manner and that the induction of uPA was required for TMPRSS4-mediated cancer cell invasion and signaling events. In addition, the uPA receptor was involved in TMPRSS4-induced signaling activation and subsequent uPA expression probably through its association with TMPRSS4 on the cell surface. Immunohistochemical analysis showed that uPA expression was significantly correlated with TMPRSS4 expression in human lung and prostate cancers. These observations suggest that TMPRSS4 is an important regulator of uPA gene expression; the upregulation of uPA by TMPRSS4 contributes to invasion and may represent a novel mechanism for the control of invasion.

Keywords: Cancer cell invasion; TMPRSS4; Transcription factor; Transmembrane serine protease; Tumor; Urokinase-type plasminogen activator (uPA)

PMID: 23978400

Thioredoxin-interacting protein, hematopoietic stem cells, and hematopoiesis


Jung H, Choi I’.
'Corresponding: Inpyo Choi(ipchoi@kribb.re.kr)

PURPOSE OF REVIEW: Reactive oxygen species (ROS) can regulate diverse signaling pathways and functions in hematopoietic cells. Thioredoxin-interacting protein (TXNIP) plays an important role in mammalian cells by inhibiting thioredoxin (TRX) under oxidative stress conditions. TXNIP is expressed in hematopoietic stem cells (HSCs), and its expression decreases as HSCs differentiate into precursor cells. However, this reduction in expression does not sufficiently explain the function of TXNIP in hematopoietic cells under oxidative stress conditions. Here, we review how ROS can regulate hematopoiesis by focusing on the function of TXNIP in hematopoietic cells under oxidative stress conditions.

RECENT FINDINGS: Studies of Txnip−/− mice have demonstrated an antioxidant function of TXNIP in hematopoietic cells or immune cells. This antioxidant function differs from the conventional pro-oxidant activity of TXNIP observed in other cell types under oxidative stress. The data suggest a context-dependent function of TXNIP under oxidative stress conditions and, in particular, a differential function of TXNIP in hematopoietic cells via its direct interaction with other redox regulatory proteins.

SUMMARY: The regulation of ROS is important in determining cellular fate decisions. TXNIP acts as a negative regulator of TRX via direct interaction, and it increases the levels of ROS under oxidative stress. However, TXNIP has an antioxidant function in hematopoietic cells or immune cells, as ROS levels are elevated and induce apoptosis in Txnip−/− hematopoietic cells. These results suggest that the amount of TXNIP is inversely associated with ROS levels, and the loss of TXNIP can increase ROS levels in immune cells or hematopoietic cells.

Keywords: Hematopoiesis; Hematopoietic stem cells (HSCs); Oxidative stress; Reactive oxygen species (ROS); Thioredoxin (TRX); Thioredoxin-interacting protein (TXNIP)

PMID: 24686462
Membrane proteins involved in epithelial-mesenchymal transition and tumor invasion: studies on TMPRSS4 and TM4SF5


Kim S', Lee JW.
*Co-corresponding: Semki Kim(semikim@kribb.re.kr)

The epithelial-mesenchymal transition (EMT) is one mechanism by which cells with mesenchymal features can be generated and is a fundamental event in morphogenesis. Recently, invasion and metastasis of cancer cells from the primary tumor are now thought to be initiated by the developmental process termed the EMT, whereby epithelial cells lose cell polarity and cell-cell interactions, and gain mesenchymal phenotypes with increased migratory and invasive properties. The EMT is believed to be an important step in metastasis and is implicated in cancer progression, although the influence of the EMT in clinical specimens has been debated. This review presents the recent results of two cell surface proteins, the functions and underlying mechanisms of which have recently begun to be demonstrated, as novel regulators of the molecular networks that induce the EMT and cancer progression.

**Keywords**: Cancer progression; Epithelial-mesenchymal transition (EMT); Invasion; Membrane protein; Metastasis; TM4SF5; TMPRSS4

PMID: 24748857

An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression

Hum Reprod. 29(10):2176-89.

*Corresponding: Suk Ran Yoon(sryoon@kribb.re.kr)

STUDY QUESTION: Is the decreased natural killer (NK) cell cytolytic activity in the peritoneal fluid (PF) of endometriosis patients due to primary cytokine activity? SUMMARY ANSWER: An increased level of interleukin-6 (IL-6) in the PF of patients with endometriosis suppresses NK cell cytolytic activity by down-regulating cytolytic granule components, such as granzyme B and perforin, through the modulation of Src homology region 2-containing protein tyrosine phosphatase-2 (SHP-2) expression.

WHAT IS ALREADY KNOWN: Endometriosis is known to be related to a defect in NK cell cytolytic activity. Additionally, the levels of inflammatory cytokines are elevated in the PF of women with endometriosis.

STUDY DESIGN, SIZE, DURATION: The effects of PF on the differentiation and functional activity of NK cells were investigated in patients with or without endometriosis, and cytokines that reduce NK cell cytolytic activity in endometriosis patients were examined. The study included women who underwent laparoscopic examination for the diagnosis of endometriosis from August 2012 to July 2013 (33 women with, and 15 women without, endometriosis).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women of reproductive age (20-40 years old) who underwent laparoscopic examination for endometriosis were included. Cytokines present in the PF were identified by enzyme-linked immunosorbent assay. The cytolytic activity of NK cells in the PF was also analyzed using a calcein-acetoxy methyl ester (AM) release assay.

MAIN RESULTS AND THE ROLE OF CHANCE: PF from patients with endometriosis suppressed the differentiation and cytoltoxicity of NK cells compared with PF from controls (P < 0.05). Increased levels of IL-6 were also found in the PF of patients with endometriosis (P < 0.01), and IL-6 levels were negatively correlated with the cytolytic activity of NK cells (r = -0.558, P = 0.03). Furthermore, IL-6 reduced the cytolytic activity of NK cells, concomitantly with the down-regulation of granzyme B and perforin (P < 0.05), by modulating SHP-2. Importantly, the addition of anti-IL-6 to the PF of endometriosis patients restored the activity of NK cells (P < 0.01), suggesting that IL-6 plays a crucial role in the reduction of NK cell activity in the PF of patients with endometriosis.

LIMITATIONS, REASONS FOR CAUTION: PF contains various inflammatory cytokines in addition to IL-6 and so it is possible that other cytokines may affect the differentiation and activity of NK cells.

WIDER IMPLICATIONS OF THE FINDINGS: Our results imply that the suppression of IL-6 using an anti-IL-6 antibody or soluble IL-6 receptor could rescue the impairment of NK cell activity in patients with endometriosis.

**Keywords**: Cytotoxic activity; Cytokotoxicity; Endometriosis; Interleukin-6 (IL-6); Natural killer (NK) cell; SHP-2

PMID: 25035432

2014 KRIBB Article Abstracts | 39 |
MicroRNA-150 regulates the cytotoxicity of natural killers by targeting perforin-1


'Co-corresponding: Inpyo Choi(ipchoi@kribb.re.kr), Taedon Kim(tdkim@kribb.re.kr)

BACKGROUND: Perforin-1 (Prf1) is the predominant cytolytic protein secreted by natural killer (NK) cells. For a rapid immune response, resting NK cells contain high Prf1 mRNA concentrations while exhibiting minimal cytotoxicity caused by a blockage of Prf1 protein synthesis, implying that an unknown posttranscriptional regulatory mechanism exists.

OBJECTIVE: We sought to determine whether microRNA-150 (miR-150) posttranscriptionally regulates Prf1 translation in both mouse and human NK cells at rest and at various time points after activation.

METHODS: Mouse NK cells with a targeted deletion of miR-150 (miR-150−/− NK cells), primary human NK cells, and NK92 MI cells were used to investigate the role of miR-150 in NK cells. NK cell cytotoxicity assays and Western blotting proved that activated miR-150−/− NK cells expressed upregulated Prf1, augmenting NK cell cytotoxicity. When immunodeficient mice were injected with miR-150−/− NK cells, there was a significant reduction in tumor growth and metastasis of B16F10 melanoma.

RESULTS: We report that miR-150 binds to 3′ untranslated regions of mouse and human Prf1, posttranscriptionally downregulating its expression. Mouse wild-type NK cells displayed downregulated miR-150 expression in response to IL-15, which led to corresponding repression and induction of Prf1 during rest and after IL-15 activation, respectively.

CONCLUSION: Our results indicate that miR-150 is a common posttranscriptional regulator for Prf1 in mouse and human NK cells that represses NK cell lytic activity. Thus the therapeutic control of miR-150 in NK cells could enhance NK cell-based immunotherapy against cancer, providing a better clinical outcome.

**Keywords**: Immunotherapy; Metastasis; miR-150; NK cell cytotoxicity; Perforin-1 (Prf1); Post-transcriptional regulation; Tumor growth.

PMID: 24698324

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Integrated mRNA-microRNA profiling of human NK cell differentiation identifies MiR-583 as a negative regulator of IL2Rγ expression


'Co-corresponding: Inpyo Choi(ipchoi@kribb.re.kr), Taedon Kim(tdkim@kribb.re.kr)

Natural killer (NK) cells are innate immune effector cells that protect against cancer and some viral infections. Until recently, most studies have investigated the molecular signatures of human or mouse NK cells to identify genes that are specifically expressed during NK cell development. However, the mechanism regulating NK cell development remains unclear. Here, we report a regulatory network of potential interactions during in vitro differentiation of human NK cells, identified using genome-wide mRNA and miRNA databases through hierarchical clustering analysis, gene ontology analysis and a miRNA target prediction program. The microRNA (miR)-583, which demonstrated the largest ratio change in mature NK cells, was highly correlated with IL2 receptor gamma (IL2Rγ) expression. The overexpression of miR-583 had an inhibitory effect on NK cell differentiation. In a reporter assay, the suppressive effect of miR-583 was ablated by mutating the putative miR-583 binding site of the IL2Rγ 3′ UTR. Therefore, we show that miR-583 acts as a negative regulator of NK cell differentiation by silencing IL2Rγ. Additionally, we provide a comprehensive database of genome-wide mRNA and miRNA expression during human NK cell differentiation, offering a better understanding of basic human NK cell biology for the application of human NK cells in immunotherapy.

**Keywords**: IL2 receptor gamma (IL2Rγ) expression; Immunotherapy; miR-583; Natural killer (NK) cell; Regulatory network.

PMID: 25313504
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Toward a generalized and high-throughput enzyme screening system based on artificial genetic circuits


Choi SL, Rha E, Lee SJ, Kim H, Kwon K, Jeong YS, Rhee YH, Song JJ, Kim HS, Lee SG.

Corresponding: Seung Goo Lee(sglee@kribb.re.kr)

Large-scale screening of enzyme libraries is essential for the development of cost-effective biological processes, which will be indispensable for the production of sustainable biobased chemicals. Here, we introduce a genetic circuit termed the Genetic Enzyme Screening System that is highly useful for high-throughput enzyme screening from diverse microbial metagenomes. The circuit consists of two AND logics. The first AND logic, the two inputs of which are the target enzyme and its substrate, is responsible for the accumulation of a phenol compound in cell. Then, the phenol compound and its inducible transcription factor, whose activation turns on the expression of a reporter gene, interact in the other logic gate. We confirmed that an individual cell harboring this genetic circuit can present approximately a 100-fold higher cellular fluorescence than the negative control and can be easily quantified by flow cytometry depending on the amounts of phenolic derivatives. The high sensitivity of the genetic circuit enables the rapid discovery of novel enzymes from metagenomic libraries, even for genes that show marginal activities in a host system. The crucial feature of this approach is that this single system can be used to screen a variety of enzymes that produce a phenol compound from respective synthetic phenyl-substrates, including cellulase, lipase, alkaline phosphatase, tyrosine phenol-lyase, and methyl parathion hydrolyase. Consequently, the highly sensitive and quantitative nature of this genetic circuit along with flow cytometry techniques could provide a widely applicable toolkit for discovering and engineering novel enzymes at a single cell level.

Keywords: Enzyme library; Enzyme screening; Flow cytometry; Genetic circuit; Microbial metagenome

PMID: 24295047

Molecular cloning and characterization of two novel fructose-specific transporters from the osmotolerant and fructophilic yeast Candida magnoliae JH110

Appl Microbiol Biotechnol. 98(8):3569-78.

Lee DH*, Kim SJ, Seo JH.

*First: Dae-Hee Lee(dhlee@kribb.re.kr)

Sugar transport is very critical in developing an efficient and rapid conversion process of a mixture of sugars by engineered microorganisms. By using expressed sequence tag data generated for the fructophilic yeast Candida magnoliae JH110, we identified two fructose-specific transporters, CmFsy1 and CmFFZ1, which show high homology with known fructose transporters of other yeasts. The CmFsy1 and CmFFZ1 genes harbor no introns and encode proteins of 574 and 582 amino acids, respectively. Heterologous expression of the two fructose-specific transporter genes in a Saccharomyces cerevisiae, which is unable to utilize hexoses, revealed that both transporters are functionally expressed and specifically transport fructose. These results were further corroborated by kinetic analysis of the fructose transport that showed that CmFsy1p is a high-affinity fructose-proton symporter with low capacity (K_M = 0.13 ± 0.01 mM, V_max = 2.1 ± 0.3 mmol h⁻¹ [gdw]⁻¹) and that CmFFZ1p is a low-affinity fructose-specific facilitator with high capacity (K_M = 105 ± 12 mM, V_max = 8.6 ± 0.7 mmol h⁻¹ [gdw]⁻¹). These fructose-specific transporters can be used for improving fructose transport in engineered microorganisms for the production of biofuels and chemicals from fructose-containing feedstock.

Keywords: Biofuel; Candida magnoliae JH110; Fructose transporter; Fructose-rich feedstock

PMID: 24048639
Characterization of putative glycosylphosphatidylinositol-anchoring motifs for surface display in the methylotrophic yeast *Hansenula polymorpha*


Cheon SA, Jung J, Choo JH, Oh DB*, Kang HA.
*Corresponding: Doo-Byoung Oh(dboh@kribb.re.kr)*

Bioinformatic analysis of the genome of the methylotrophic yeast *Hansenula polymorpha* revealed 39 putative glycosylphosphatidylinositol-anchored proteins (GPI-proteins). Notably, dibasic motifs in the proximal site, that has been reported as a plasma membrane retention signal in *Saccharomyces cerevisiae* GPI-proteins, were not found in any of the predicted GPI-proteins of *H. polymorpha*. To evaluate the *in silico* prediction, C-terminal peptides of 40 amino acids derived from ten *H. polymorpha* GPI-proteins were fused to the *Aspergillus satoi* α-1,2-mannosidase (msdS). Cell wall fraction analysis showed that nine of the ten msdS-GPI fusion proteins were mostly localized at the cell wall. Surface expression of functional msdS was further confirmed by *in vitro* enzyme activity assay and by glycan structure analysis of cell wall mannoproteins. The recombinant *H. polymorpha* strains expressing surface-displayed msdS have the potential as useful hosts to produce glycoproteins with decreased mannosylation.

**Keywords**: Cell wall; Glycan trimming; Glycoprotein; Glycosylphosphatidylinositol-anchored; *Hansenula polymorpha*; Yeast surface display

PMID: 24930114

Modelling and analysis of gene regulatory networks based on the G-network


Kim H*.
*Corresponding: Haseong Kim(haseong@kribb.re.kr)*

G-networks are a class of stochastic models that have had a broad range of applications ranging from the performance analysis of computer systems and networks to the modelling of gene regulatory networks. Gene regulatory networks consist of thousands of genes and proteins which are dynamically interacting with each other. Once these regulatory structures are revealed, it is necessary to understand their dynamical behaviours since pathway activities could be changed by their given conditions. This review mainly focuses on a stochastic GRN modelling techniques based on G-networks which provide the analytical steady-state solution of a system for efficient GRN dynamics modelling. Three applications of the G-network model to GRNs show that this novel approach can serve to detect abnormalities from protein expression data, and that they can help to explicit the behaviour of complicated GRN models by dividing the gene regulatory processes into DNA and protein layers.

**Keywords**: Abnormality detection; DNA layer; G-network; Gene regulatory network; GRNs; Protein expression data; Protein layer; Stochastic modelling
Characterization of a lichenase isolated from soil metagenome


Kim SY, Oh DB, Kwon O.
*Corresponding: Ohsuk Kwon(oskwon@kribb.re.kr)

A lichenase gene (*mt-lic*) was identified for the first time through function-based screening of a soil metagenomic library. Its deduced amino acid sequence exhibited a high degree of homology with endo-β-1,3-1,4-glucanase (having both lichenase and chitosanase activities), encoded by the *bgc* gene of *Bacillus circulans* WL-12. The recombinant lichenase overexpressed and purified from *Escherichia coli* was able to efficiently hydrolyze both barley β-glucan and lichenan. The enzyme showed maximal activity at a pH of 6.0 at 50°C, with Azo-barley-glucan as the substrate. The metal ions Mn³⁺, Mg²⁺, Ca²⁺, and Fe³⁺ enhanced the enzymatic activity, whereas the Cu²⁺ and Zn²⁺ ions inhibited the enzymatic activity. The *Kₘ* and *Vₘₐₓ* values of the purified lichenase were determined to be 0.45 mg/ml and 24.83 U/min/mg of protein, respectively.

**Keywords**: Endo-β-1,3-1,4-glucanase; Function-based screening; Glycosyl hydrolase family 8; Lichenase; Recombinant protein; Soil metagenome

PMID: 25152058

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Controlled localization of functionally active proteins to inclusion bodies using leucine zippers


Choi SL, Lee SJ, Yeom SJ, Kim HJ, Rhee YH, Jung HC, Lee SG.
*Corresponding: Seung-Goo Lee(sglee@kribb.re.kr)

Inclusion bodies (IBs) are typically non-functional particles of aggregated proteins. However, some proteins in fusion with amyloid-like peptides, viral coat proteins, and cellulose binding domains (CBDs) generate IB particles retaining the original functions in cells. Here, we attempted to generate CBD IBs displaying functional leucine zipper proteins (LZs) as bait for localizing cytosolic proteins in *E. coli*. When a red fluorescent protein was tested as a target protein, microscopic observations showed that the IBs red-fluoresced strongly. When different LZ pairs with *KₐB* of 8-1,000 µM were tested as the bait and prey, the localization of the red fluorescence appeared to change following the affinities between the LZs, as observed by fluorescence imaging and flow cytometry. This result proposed that LZ-tagged CBD IBs can be applied as an *in vivo* matrix to entrap cytosolic proteins in *E. coli* while maintaining their original activities. In addition, easy detection of localization to IBs provides a unique platform for the engineering and analyses of protein-protein interactions in *E. coli*.

**Keywords**: CBD IBs; Cellulose binding domains (CBDs); Cytosolic protein; Flow cytometry; Fluorescence imaging; Inclusion bodies (IBs); Protein-protein interaction; Zipper proteins (LZs)

PMID: 24897378
Mechanism of the pH-induced conformational change in the sensor domain of the DraK histidine kinase via the E83, E105, and E107 residues

PLoS One. 9(9):e107168.

*Co-corresponding: Ohshak Kwon(oskwon@kribb.re.kr)

The DraR/DraK two-component system was found to be involved in the differential regulation of antibiotic biosynthesis in a medium-dependent manner; however, its function and signaling and sensing mechanisms remain unclear. Here, we describe the solution structure of the extracellular sensor domain of DraK and suggest a mechanism for the pH-dependent conformational change of the protein. The structure contains a mixed alpha-beta fold, adopting a fold similar to the ubiquitous sensor domain of histidine kinase. A biophysical study demonstrates that the E83, E105, and E107 residues have abnormally high pKa values and that they drive the pH-dependent conformational change for the extracellular sensor domain of DraK. We found that a triple mutant (E83L/E105L/E107A) is pH independent and mimics the low pH structure. An in vivo study showed that DraK is essential for the recovery of the pH of Streptomyces coelicolor growth medium after acid shock. Our findings suggest that the DraR/DraK two-component system plays an important role in the pH regulation of S. coelicolor growth medium. This study provides a foundation for the regulation and the production of secondary metabolites in Streptomyces.

**Keywords**: Antibiotic biosynthesis; DraR/DraK; pH regulation; Secondary metabolite; Sensor domain; Streptomyces coelicolor

PMID: 25203403

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Highly improved specificity for hybridization-based microRNA detection by controlled surface dissociation


Yoon HR, Lee JM, Jung J, Lee CS, Chung BH*, Jung Y.
*Co-corresponding: Bong Hyun Chung(chungbh@kribb.re.kr)

Poor specificity has been a lingering problem in many microRNA profiling methods, particularly surface hybridization-based methods such as microarrays. Here, we carefully investigated surface hybridization and dissociation processes of a number of sequentially similar microRNAs against nucleic acid capture probes. Single-base mismatched microRNAs were similarly hybridized to a complementary DNA capture probe and thereby poorly discriminated during conventional stringent hybridization. Interestingly, however, mismatched microRNAs showed significantly faster dissociation from the probe than the perfectly matched microRNA. Systematic analysis of various washing conditions clearly demonstrated that extremely high specificity can be obtained by releasing non-specific microRNAs from assay surfaces during a stringent and controlled dissociation step. For instance, compared with stringent hybridization, surface dissociation control provided up to 6-fold better specificity for Let-7a detection than for other Let-7 family microRNAs. In addition, a synthetically introduced single-base mismatch on miR206 was almost completely discriminated by optimized surface dissociation of captured microRNAs, while this mismatch was barely distinguished from target miR206 during stringent hybridization. Furthermore, a single dissociation condition was successfully used to simultaneously measure four different microRNAs with extremely high specificity using melting temperature-equalized capture probes. The present study on selective dissociation of surface bound microRNAs can be easily applied to various hybridization-based detection methods for improved specificity.

**Keywords**: Dissociation processes; microRNA profiling; miR-206; Surface hybridization; Systematic analysis

PMID: 24205510
**Article 85**

**A signal-on fluorescent assay for DNA methyltransferase activity using a methylation-resistant endonuclease**


Quach QH, Chung BH.  
*Corresponding: Bong Hyun Chung(chungbh@kribb.re.kr)*

A simple, rapid, and signal-on fluorescent assay was developed for activity analysis of DNA methyltransferase and for screening of its inhibitors based on a methylation-resistant endonuclease and SYBR Green I.

**Keywords**: Dam MTase activity; DNA methyltransferase (MTase); Fluorescent assay; Inhibitor; Initial screening; Label-free assay; Methylation-resistant endonuclease

**PMID**: 24714961

**Article 86**

**Detection of UV-induced mutagenic thymine dimer using graphene oxide**


Chung CH, Kim JH, Chung BH.  
*Corresponding: Bong Hyun Chung(chungbh@kribb.re.kr)*

In this paper, we report for the first time that graphene oxide (GO) can interact with mutagenic DNA but not intact DNA. After UV-irradiated fluorophore-linked DNA containing thymine repeats was mixed with GO, a decrease in fluorescence was observed in a time-dependent manner. In contrast, no fluorescence change was observed with intact DNA, indicating that UV irradiation of DNA resulted in the formation of mutagenic bases. Because GO is known to act as a fluorescence quencher, the decreased fluorescence implies adsorption of the UV-irradiated DNA onto GO. It appears that the decreased fluorescence might result from the greater accessibility of hydrophobic methyl groups and phenyl rings of thymine dimers to GO and from deformed DNA structures with less effective charge shielding under salt-containing conditions. Using this affinity of GO for mutagenic DNA, we could detect UV-irradiated DNA at concentrations as low as 100 pM. We were also able to analyze the ability of phototoxic drugs to catalyze the formation of mutagens under UV irradiation with GO. Because our method is highly sensitive and feasible and does not require the pretreatment of DNA, we propose that it could accelerate the screening of potential phototoxic drug candidates that would be able to sensitize mutagenic dsDNA.

**Keywords**: Fluorescence change; Graphene oxide (GO); Mutagenic DNA; Phototoxic drug; Screening; UV irradiation

**PMID**: 25375800
Live imaging of cellular dynamics using a multi-imaging vector in single cells


Park K, Jeong J, Chung BH*.  
*Corresponding: Bong Hyun Chung(chungbh@kribb.re.kr)

Real-time monitoring of cellular dynamics in living organisms is highly challenging. We developed a multi-imaging vector based on 2A peptides. Live imaging of subcellular compartments can be performed following the transfection of cells with another vector, the multi-labeling vector, which contains localization signals and various fluorescent protein variants.

**Keywords**: Cellular dynamics; Fluorescent protein variant; Monitoring; Multi-imaging vector; Live imaging

PMID: 25087700

RNA-guided genome editing in *Drosophila* with the purified Cas9 protein


*Co-corresponding: Kweon Yu(kweonyu@kribb.re.kr)

We report a method for generating *Drosophila* germline mutants effectively via injection of the complex of the purified Cas9 protein, tracrRNA, and gene-specific crRNAs, which may reduce delayed mutations because of the transient activity of the Cas9 protein, combined with the simple mutation detection in GO founders by the T7E1 assay.

**Keywords**: Cas9 protein; Delayed mutation; *Drosophila* germline mutant; Genome editing; RGEN

PMID: 24875628
Applications of animal biosensors


*Corresponding: Moonil Kim(kimm@kribb.re.kr)

Odorous compounds perceived by humans or animal species produce a response in chemical- or electronic-based analytical detection systems (chemical sensors, electronic noses, gas chromatography, mass spectrometers, and so on). Animal noses can also produce a recognizable behavioral response in the animal, when exposed to those compounds. Recently, much attention has been paid to the use of animals for scent detection based on their behavioral responses, referred to as animal biosensors. So far, behavioral odor detection by animals has been applicable in some fields, such as forensic sciences, homeland security, or, more recently, cancer diagnostics. The major advantage of animal biosensors is that the animals can be conditioned rapidly and cost-effectively, offering benefits in terms of noninvasive detection and early diagnosis. Here, we review the applications of living biosensors as whole animal biosensors and discuss the main issues, approaches, and challenges.

**Keywords** : Animal biosensor; Behavioral response; Living biosensor; Odor discrimination; Olfactory sensor; Scent detection

Discovery of coumarin derivatives as fluorescence acceptors for intrinsic fluorescence resonance energy transfer of proteins

Mol Biosyst. 10(1):30-3.

Kim JH, Sumranjit J, Kang HJ, Chung SJ*.
*Corresponding: Sang J. Chung

Coumarin analogues were synthesised and evaluated as acceptors for the intrinsic fluorescence resonance energy transfer (iFRET) of tryptophan residues in target proteins. The fluorescence properties such as quantum yields, iFRET efficiencies, and Förster distances of the prepared coumarin analogs were determined in a model system, by their conjugation to biotin, utilizing streptavidin (SAV) as the iFRET donor. The coumarin derivatives reported here represent the most efficient iFRET acceptors for tryptophan, known to date.

**Keywords** : Coumarin analogue; Coumarin derivative; Fluorescence acceptor; Intrinsic fluorescence resonance energy transfer (iFRET); Streptavidin (SAV)

PMID: 24172686
Gadolinium-based nanoparticles for highly efficient T1-weighted magnetic resonance imaging

Nanotechnology. 25(24):245103.


We developed Pyrene-Gadolinium (Py-Gd) nanoparticles as pH-sensitive magnetic resonance imaging (MRI) contrast agents capable of showing a high-Mr signal in cancer-specific environments, such as acidic conditions. Py-Gd nanoparticles were prepared by coating Py-Gd, which is a complex of gadolinium with pyrenyl molecules, with pyrenyl polyethyleneglycol (PEG) using a nano-emulsion method. These particles show better longitudinal relaxation time (T1) MR signals in acidic conditions than they do in neutral conditions. Furthermore, the particles exhibit biocompatibility and MR contrast effects in both in vitro and in vivo studies. From these results, we confirm that Py-Gd nanoparticles have the potential to be applied for accurate cancer diagnosis and therapy.

**Keywords**: Cancer diagnosis; Gadolinium; High sensitivity; Imaging agent; Magnetic resonance image; Py-Gd nanoparticle

PMID: 24872113

Photophysical properties and singlet oxygen generation efficiencies of water-soluble fullerene nanoparticles


Stasheuski AS, Galievsky VA, Stupak AP, Dzhagarov BM, Choi MJ, Chung BH, Jeong JY*. *Corresponding: Jin Young Jeong(jyjeong@kribb.re.kr)

As various fullerene derivatives have been developed, it is necessary to explore their photophysical properties for potential use in photoelectronics and medicine. Here, we address the photophysical properties of newly synthesized water-soluble fullerene-based nanoparticles and polyhydroxylated fullerene as a representative water-soluble fullerene derivative. They show broad emission band arising from a wide-range of excitation energies. It is attributed to the optical transitions from disorder-induced states, which decay in the nanosecond time range. We determine the kinetic properties of the singlet oxygen (1O2) luminescence generated by the fullerene nanoparticles and polyhydroxylated fullerene to consider the potential as photodynamic agents. Triplet state decay of the nanoparticles was longer than 1O2 lifetime in water. Singlet oxygen quantum yield of a series of the fullerene nanoparticles is comparably higher ranging from 0.15 to 0.2 than that of polyhydroxylated fullerene, which is about 0.06.

**Keywords**: Fullerene nanoparticle; Optical transition; Photophysical property; Polyhydroxylated fullerene; Water-soluble fullerene derivative

PMID: 24893622
Ultra-specific zeptomole microRNA detection by plasmonic nanowire interstice sensor with bi-temperature hybridization

Small. 10(20):4200-6.

*Co-corresponding: Bong Hyun Chung(chungbh@kribb.re.kr)

MicroRNAs (miRNAs) are emerging new biomarkers for many human diseases. To fully employ miRNAs as biomarkers for clinical diagnosis, it is most desirable to accurately determine the expression patterns of miRNAs. The optimum miRNA profiling method would feature 1) highest sensitivity with a wide dynamic range for accurate expression patterns, 2) supreme specificity to discriminate single nucleotide polymorphisms (SNPs), and 3) simple sensing processes to minimize measurement variation. Here, an ultra-specific detection method of miRNAs with zeptomole sensitivity is reported by applying bi-temperature hybridizations on single-crystalline plasmonic nanowire interstice (PNI) sensors. This method shows near-perfect accuracy of SNPs and a very low detection limit of 100 am (50 zeptomole) without any amplification or labeling steps. Furthermore, multiplex sensing capability and wide dynamic ranges (100 am-100 pm) of this method allows reliable observation of the expression patterns of miRNAs extracted from human tissues. The PNI sensor offers combination of ultra-specificity and zeptomole sensitivity while requiring two steps of hybridization between short oligonucleotides, which could present the best set of features for optimum miRNA sensing method.

Keywords: Bi-temperature hybridization; Biomarker; microRNA; miRNA profiling; Plasmonic nanowire interstice (PNI) sensor; Ultra-specific zeptomole

PMID: 24975681

Understanding cross-communication between aboveground and belowground tissues via transcriptome analysis of a sucking insect whitefly-infested pepper plants


Park YS, Ryu CM*.
*Corresponding: Choong-Min Ryu(cmryu@kribb.re.kr)

Plants have developed defensive machinery to protect themselves against herbivore and pathogen attacks. We previously reported that aboveground whitefly (Bemisia tabaci Gemm.) infestation elicited induced resistance in leaves and roots and influenced the modification of the rhizosphere microflora. In this study, to obtain molecular evidence supporting these plant fitness strategies against whitefly infestation, we performed a 300 K pepper microarray analysis using leaf and root tissues of pepper (Capsicum annuum L.) applied with whitefly, benzo-(1,2,3)-thiadiazole-7-carboxylic acid S-methyl ester (BTH), and the combination of BTH+whitefly. We defined differentially expressed genes (DEGs) as genes exhibiting more than 2-fold change (1.0 based on log2 values) in expression in leaves and roots in response to each treatment compared to the control. We identified a total of 16,188 DEGs in leaves and roots. Of these, 6685, 6752, and 4045 DEGs from leaf tissue and 6768, 7705, and 7667 DEGs from root tissue were identified in the BTH, BTH+whitefly, and whitefly treatment groups, respectively. The total number of DEGs was approximately two-times higher in roots than in whitefly-infested leaves subjected to whitefly infestation. Among DEGs, whitefly feeding induced salicylic acid and jasmonic acid/ethylene-dependent signaling pathways in leaves and roots. Several transporters and auxin-responsive genes were upregulated in roots, which can explain why biomass increase is facilitated. Using transcriptome analysis, our study provides new insights into the molecular basis of whitefly-mediated intercommunication between aboveground and belowground plant tissues and provides molecular evidence that may explain the alteration of rhizosphere microflora and root biomass by whitefly infestation.

Keywords: Auxin; BTH; Capsicum annuum L.; Root biomass; Transcriptome; Transporter; Whitefly

PMID: 24309116
The Lactone form of stachybotrydial: a new inhibitor of dihydrofolate reductase from *Stachybotrys* sp. FN298

*Kwon YJ, Sohn MJ, Kim HJ, Kim WG*.  
*Corresponding: Won-Gon Kim(wgkim@kribb.re.kr)

Dihydrofolate reductase (DHFR) has been confirmed to be a novel target for antibacterial drug development. In this study, we determined that a fungal metabolite from *Stachybotrys* sp. FN298 can inhibit the DHFR of *Staphylococcus aureus*. Its structure was identified as a lactone form of stachybotrydial using mass spectrometry and nuclear magnetic resonance analysis. This compound inhibited *S. aureus* DHFR with a half-maximal inhibitory concentration of 41 µM. It also prevented the growth of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) with a minimum inhibitory concentration of 32 µg·mL⁻¹. To our knowledge, this is the first description of a DHFR inhibitor of microbial origin. The inhibitory function of the lactone form of stachybotrydial highlights its potential for development into a new broad-spectrum antibacterial agent and as an agent against MRSA.

**Keywords**: Antibacterial drug; Dihydrofolate reductase (DHFR); Stachybotrydial; *Stachybotrys* sp. FN298; *Staphylococcus aureus*

PMID: 25087962

Verrulactone C with an unprecedented dispiro skeleton, a new inhibitor of *Staphylococcus aureus* enoyl-ACP reductase, from *Penicillium verruculosum* F375

*Corresponding: Won-Gon Kim(wgkim@kribb.re.kr)

An highly quaternary and unprecedented dispiro compound, verrulactone C, with the known compound, altenuisol, were isolated from a culture broth of the fungal strain *Penicillium verruculosum* F375 and their structures were established by various spectral analysis. Verrulactone C and altenuisol showed FabI-selective inhibition. Especially altenuisol had the high correlation between FabI-inhibition and whole cell antibacterial activity against *Staphylococcus aureus* and MRSA with MICs of 8-32 µg/mL.

**Keywords**: Antibacterial activity; Dispiro skeleton; FabI-selective inhibition; *Penicillium verruculosum* F375; *Staphylococcus aureus*; Verrulactone C

PMID: 24332629
In principle, protein display is enabled by fusing target proteins to naturally secreted, surface-anchored protein motifs. In this work, we developed a method of native protein display on the Bacillus spore surface that obviates the need to construct fusion proteins to display a motif. Spore coat proteins are expressed in the mother cell compartment and are subsequently assembled and deposited on the surface of spores. Therefore, target proteins overexpressed in the mother cell compartment during the late sporulation phase were expected to be targeted and displayed on the spore surface. As a proof of principle, we demonstrated the display of carboxymethylcellulase (CMCase) in its native form on the spore surface. The target protein, CMCase, was expressed under the control of the crylAa promoter, which is controlled by $\sigma^d$ and $\sigma^\beta$ and is expressed in the mother cell compartment. The correct display was confirmed using enzyme activity assays, flow cytometry, and immunogold electron microscopy. In addition, we demonstrated the display of a $\beta$-galactosidase tetramer and confirmed its correct display using enzyme activity assays and protein characterization.

This native protein display system, combined with the robust nature of Bacillus spores, will broaden the range of displayable target proteins. Consequently, the applications of display technology will be expanded, including high-throughput screening, vaccines, biosensors, biocatalysis, bioremediation, and other innovative bioprocesses.

**Keywords:** Bacillus subtilis; $\beta$-galactosidase; Carboxymethylcellulase (CMCase); Native protein display; Spore surface

**PMID:** 25168353

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**Display of native proteins on Bacillus subtilis spores**


Pan JG', Choi SK, Jung HC, Kim EJ.

'Corresponding: Jae-Gu Pan(jgpan@kriib.re.kr)

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**Field evaluation of the bacterial volatile derivative 3-pentanol in priming for induced resistance in pepper**


Choi HK, Song GC, Yi HS, Ryu CM'.

'Corresponding: Choong-Min Ryu(cmryu@kriib.re.kr)

Plants are defended from attack by emission of volatile organic compounds (VOCs) that can act directly against pathogens and herbivores or indirectly by recruiting natural enemies of herbivores. However, microbial VOC have been less investigated as potential triggers of plant systemic defense responses against pathogens in the field. *Bacillus amyloliquefaciens* strain IN937a, a plant growth-promoting rhizobacterium that colonizes plant tissues, stimulates induced systemic resistance (ISR) via its emission of VOCs. We investigated the ISR capacity of VOCs and derivatives collected from strain IN937a against bacterial spot disease caused by *Xanthomonas axonopodis* pv. vesicatoria in pepper. Of 15 bacterial VOCs and their derivatives, 3-pentanol, which is a C8 amyl alcohol reported to be a component of sex pheromones in insects, was selected for further investigation. Pathogens were infiltrated into pepper leaves 10, 20, 30, and 40 days after treatment and transplantation to the field. Disease severity was assessed 7 days after transplantation. Treatment with 3-pentanol significantly reduced disease severity caused by *X. axonopodis* and naturally occurring *Cucumber mosaic virus* in field trials over 2 years. We used quantitative real-time polymerase chain analysis to examine Pathogenesis-Related genes associated with salicylic acid (SA), jasmonic acid (JA), and ethylene defense signaling. The expression of *Capsicum annum* Pathogenesis-Related protein 1 (CaPR1), CaPR2, and Ca protease inhibitor2 (CaPIN2) increased in field-grown pepper plants treated with 3-pentanol. Taken together, our results show that 3-pentanol triggers induced resistance by priming SA and JA signaling in pepper under field conditions.

**Keywords:** 3-Pentanol; Defense priming; ISR; Pepper; PGPR; Systemic acquired resistance; Volatile organic compound

**PMID:** 25149655
Inactivation of the phosphoglucomutase gene pgm in Paenibacillus polymyxa leads to overproduction of fusaricidin


Kim HR, Park SY, Kim SB, Jeong H, Choi SK, Park SH*. *Corresponding: Seung-Hwan Park(shpark@kribb.re.kr)

Fusaricidin, a lipodepsipeptide isolated from Paenibacillus polymyxa, has high antimicrobial activity against fungi and Gram-positive bacteria. Through mutagenesis, we obtained two mutant strains, N1U7 and N17U7, which produce 6.2- to 7.9-fold more fusaricidin than their parent strain. Causal mutations were identified by whole-genome sequencing, and the two strains each contained at least eleven point mutations, including four common mutations. A mutation in the PPE04441 gene (pgm), encoding an α-phosphoglucomutase, was found to be an important factor in fusaricidin overproduction by complementation experiments. Null mutation of pgm in the parental strain increased fusaricidin production by 5.2-fold. Increased growth and cell viability in stationary phase, reduced exopolysaccharide production, and increased fusA expression were observed in the pgm mutant strains, which might be related to fusaricidin overproduction. This is the first report revealing that PGM deficiency leads to an overproduction of fusaricidin.

Keywords: Antimicrobial activity; Fusaricidin; Mutagenesis; Paenibacillus polymyxa; pgm mutation; Phosphoglucomutase

PMID: 24939175

Genome sequence and comparative genome analysis of Pseudomonas syringae pv. syringae type strain ATCC 19310


Park YS1, Jeong H, Sim YM, Yi HS, Ryu CM*. *Corresponding: Choong-Min Ryu(cmryu@kribb.re.kr)

Pseudomonas syringae pv. syringae (Psy) is a major bacterial pathogen of many economically important plant species. Despite the severity of its impact, the genome sequence of the type strain has not been reported. Here, we present the draft genome sequence of Psy ATCC 19310. Comparative genomic analysis revealed that Psy ATCC 19310 is closely related to Psy B728a. However, only a few type III effectors, which are key virulence factors, are shared by the two strains, indicating the possibility of host-pathogen specificity and genome dynamics, even under the pathovar level.

Keywords: Comparative genomics; Pseudomonas syringae pv. syringae (Psy); Psy ATCC 19310; Type III effector; Type strain;

PMID: 24444998
Stabilization of homoserine-O-succinyltransferase (MetA) decreases the frequency of persisters in Escherichia coli under stressful conditions


Mordukhova EA, Pan JG*

Corresponding: Jae-Gu Pan(jgpan@kribb.re.kr)

Bacterial persisters are a small subpopulation of cells that exhibit multi-drug tolerance without genetic changes. Generally, persistence is associated with a dormant state in which the microbial cells are metabolically inactive. The bacterial response to unfavorable environmental conditions (heat, oxidative, acidic stress) induces the accumulation of aggregated proteins and enhances formation of persister cells in Escherichia coli cultures. We have found that methionine supplementation reduced the frequency of persisters at mild (37°C) and elevated (42°C) temperatures, as well as in the presence of acetate. Homoserine-o-succinyltransferase (MetA), the first enzyme in the methionine biosynthetic pathway, is prone to aggregation under many stress conditions, resulting in a methionine limitation in E. coli growth. Overexpression of MetA induced the greatest number of persisters at 42°C, which is correlated to an increased level of aggregated MetA. Substitution of the native metA gene on the E. coli K-12 WE chromosome by a mutant gene encoding the stabilized MetA led to reduction in persisters at the elevated temperature and in the presence of acetate, as well as lower aggregation of the mutated MetA. Decreased persister formation at 42°C was confirmed also in E. coli K-12 W3110 and a fast-growing WEph+ mutant harboring the stabilized MetA. Thus, this is the first study to demonstrate manipulation of persister frequency under stressful conditions by stabilization of a single aggregation-prone protein, MetA.

Keywords: Bacterial persister; Dormant state; Homoserine-o-succinyltransferase (MetA); Microbial cell; Multi-drug tolerance; Stressful condition

PMID: 25329174

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Genome information of Methylobacterium oryzae, a plant-probiotic methylotroph in the phyllosphere

PLoS One. 9(9):e106704.

Kwak MJ, Jeong H*, Madhaiyan M, Lee Y, Sa TM, Oh TK, Kim JF.

*Co-first: Haeyoung Jeong(hyjeong@kribb.re.kr)

Pink-pigmented facultative methylotrophs in the Rhizobiales are widespread in the environment, and many Methylobacterium species associated with plants produce plant growth-promoting substances. To gain insights into the life style at the phyllosphere and the genetic bases of plant growth promotion, we determined and analyzed the complete genome sequence of Methylobacterium oryzae CBMB20, a strain isolated from rice stem. The genome consists of a 6.29-Mb chromosome and four plasmids, designated as pMOC1 to pMOC4. Among the 6,274 coding sequences in the chromosome, the bacterium has, besides most of the genes for the central metabolism, all of the essential genes for the assimilation and dissimilation of methanol that are either located in methylotrophy islands or dispersed. M. oryzae is equipped with several kinds of genes for adaptation to plant surfaces such as defense against UV radiation, oxidative stress, desiccation, or nutrient deficiency, as well as high proportion of genes related to motility and signaling. Moreover, it has a array of genes involved in metabolic pathways that may contribute to promotion of plant growth; they include auxin biosynthesis, cytokine biosynthesis, vitamin B₁₂ biosynthesis, urea metabolism, biosorption of heavy metals or decrease of metal toxicity, pyrrolquinoline quinone biosynthesis, 1-aminoacyclopropene-1-carboxylate deamination, phosphate solubilization, and thiosulfate oxidation. Through the genome analysis of M. oryzae, we provide information on the full gene complement of M. oryzae that resides in the aerial parts of plants and enhances plant growth. The plant-associated lifestyle of M. oryzae pertaining to methylotrophy and plant growth promotion, and its potential as a candidate for a bioinoculant targeted to the phyllosphere and focused on phytostimulation are illuminated.

Keywords: Bioinoculant target; Genome analysis; Metabolic pathway; Methylobacterium oryzae; Phyllosphere; Phytostimulation; Plant growth

PMID: 25211235
Future Biotechnology Research Division

- Biomedical Translational Research Center
- Plant Systems Engineering Research Center
- Industrial Bio-materials Research Center
Highly stable colorimetric aptamer sensors for detection of ochratoxin A through optimizing the sequence with the covalent conjugation of hemin

Analyst. 139(7):1622-7.

*aCorresponding: Tai Hwan Ha(taihwan@kribb.re.kr)

Optimization of hairpin DNA is introduced to detect ochratoxin A (OTA) by chemically conjugating its cofactor, hemin, toward the 5’-end. The newly designed OTA aptasensor showed enhanced stability and sensitivity, thereby lowering the detection limit to an ~1 nM level. Furthermore, an optimal spacer for hemin conjugation was investigated for stable responses toward very diluted OTA solutions.

**Keywords**: Hairpin DNA; Hemin conjugation; Ochratoxin A (OTA); Optimal spacer; OTA aptasensor

**Biomedical Translational Research Center**

**Article 104**

**Contribution of proline to the pre-structuring tendency of transient helical secondary structure elements in intrinsically disordered proteins**


*aCorresponding: Kyou-Hoon Han(khhnan600@kribb.re.kr)

**BACKGROUND**: IDPs function without relying on three-dimensional structures. No clear rationale for such a behavior is available yet. PreSMos are transient secondary structures observed in the target-free IDPs and serve as the target-binding “active” motifs in IDPs. Prolines are frequently found in the flanking regions of PreSMos. Contribution of prolines to the conformational stability of the helical PreSMos in IDPs is investigated.

**METHODS**: MD simulations are performed for several IDP segments containing a helical PreSMo and the flanking prolines. To measure the influence of flanking-prolines on the structural content of a helical PreSMo, calculations were done for wild type as well as for mutant segments with Pro→Asp, His, Lys, or Ala. The change in the helicity due to removal of a proline was measured both for the PreSMo region and for the flanking regions.

**RESULTS**: The α-helical content in ~70% of the helical PreSMos at the early stage of simulation decreases due to replacement of an N-terminal flanking proline by other residues whereas the helix content in nearly all PreSMos increases when the same replacements occur at the C-terminal flanking region. The helix destabilizing/terminating role of the C-terminal flanking prolines is more pronounced than the helix promoting effect of the N-terminal flanking prolines.

**GENERAL SIGNIFICANCE**: This work represents a novel example demonstrating that a proline is encoded in an IDP with a defined purpose. The helical PreSMos presage their target-bound conformations. As they most likely mediate IDP-target binding via conformational selection their helical content can be an important feature for IDP function.

**Keywords**: Flanking proline; Intrinsically disordered protein (IDP); MD simulation; Molecular dynamics simulation; PreSMo (Pre-Structured Motif)

**PMID**: 24211251
Label-free measurement of cell viability via counting cells attached on affinity substrates

Ahn J, Park J, Kim YG, Lee EG, Kim MG, Shin YB. *Corresponding: Yong-Beom Shin(ybshin@kribb.re.kr)

The commonly used trypan blue dye exclusion method and other modified cell viability methods, such as fluorescein dye and tetrazolium dye exclusion, artificially introduce toxic chemicals to cells and, thus, alter cellular organelles when measuring cell viability. Therefore, cell viability could be affected by the processes currently used to observe viability. In this study, the cell viability of Chinese hamster ovary (CHO) cells was measured by simply counting attached cells after the cultured CHO cells were attached on a Concanavalin A (Con A) substrate. The efficiency of cell attachment to Con A surfaces was different for live and dead cells allowing the cell viability of CHO cells to be measured without any chemical modifications to the cells.

**Keywords**: Affinity binding; Cell attachment; Cell viability; CHO cell; Concanavalin A (Con A); Label-free measurement

Unprecedented lower critical solution temperature behavior of polyimides in organic media

Kim SD, Kim SY, Chung IS. *Co-corresponding: Im Sik Chung(cis123@kribb.re.kr)

Polymers with a lower critical solution temperature (LCST) undergo an entropy-driven phase transition. Owing to thermoresponsiveness, polymers exhibiting LCST behavior have found a diverse range of applications. Poly(N-isopropyl acrylamide) is a typical substance which displays LCST behavior. Aromatic polyimides (PIs) are typical rigid polymers and high-performance polymeric materials. The PIs showed LCST behavior in organic media. This LCST behavior of the rigid polymers in organic solvents was induced by the variation of the weak interactions of the polymer chains with the solvents. Thermoresponsive rigid polymers which work even in organic media by introducing CF₃ groups onto rigid PI chains. This unprecedented LCST phenomenon of rigid polymers may result from a change in the interaction strength in the vicinity of CF₃ between the polymer and the acetyl-containing solvent. The potential for developing various thermoresponsive polymers by locating CF₃ groups on polymer structures.

**Keywords**: Aromatic polyimides (PIs); LCST behavior; Lower critical solution temperature (LCST); Thermoresponsive rigid polymer
Genkwadaphnin induces IFN-γ via PKD1/NF-κB/STAT1 dependent pathway in NK-92 cells


Kang HB, Ahn KS, Oh SR, Kim JW.*
*Corresponding: Jae Wha Kim(wjkim@kribb.re.kr)

The flower buds of Daphne genkwa Sieb. et Zucc. have been used as a traditional Chinese medicine although their functional mechanisms have not been discovered yet. We have studied the potential effects of the plant extracts on natural killer (NK) cell activation, and isolated an active fraction. Genkwadaphnin (GD-1) displayed a potent efficacy to induce IFN-γ transcription in NK cells with concentration- and time-dependent manners. GD-1 treatment triggered the phosphorylation of PKD1, a member of PKC family, MEK and ERK, resulting in IKK activation to induce IkB degradation, and the nuclear localization of p65, an NF-κB subunit, which regulates IFN-γ transcription. GD-1 effect on IFN-γ production was blocked by the addition of Rottlerin, a PKC inhibitor, CID 755673, a PKD inhibitor, or Bay11-7082, an IKKα inhibitor. The nuclear localization of p65 was also inhibited by the kinase inhibitors. Secreted IFN-γ activates STAT1 phosphorylation as autocinze-loops to sustain its secretion. GD-1 induced the phosphorylation of STAT1 probably through the increase of IFN-γ. STAT1 inhibitor also abrogated the sustained IFN-γ secretion. These results suggest that GD-1 is involved in the activation of PKD1 and/or ERK pathway, which activate NK-κB triggering IFN-γ production. As positive feedback loops, secreted IFN-γ activates STAT1 and elongates its production in NK-92 cells.

**Keywords**: Daphne genkwa; Genkwadaphnin (GD-1); Natural killer (NK) cell; NK-92 cell; PKD1; STAT1 phosphorylation

PMID: 25517939

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Nanoplasmonic biosensor for cancer diagnosis: design and fabrication

Proceedings of SPIE. 9129:91292M.

Shin YB*, Cho NR, Lee KJ.
*First: Yong-Beom Shin(ybshin@kribb.re.kr)

In this study, gold nanoplasmonic biosensors using localized surface plasmon resonance (LSPR) were fabricated for the diagnosis of cancer. We optimized the structures of the gold nanodot array (GNA) via the experiments for the optical characteristics. In addition, the nanoimprint lithography was employed for realizing nanoplasmonic structures, which is a more efficient technique for mass production than nanolithography such as electron beam lithography (EBL) or focused ion beam (FIB) lithography that is a quite intricate, time-consuming and expensive process. After the UV nanoimprinting process using a film stamp and metal films were deposited using an electron-beam evaporator, followed by the lift-off step. Consequently, the nanoplasmonic MNA was realized on 5-inch glass wafer and the pitch, diameter and height of MNA were 300nm, 150 nm and 20 nm, respectively. The wavelength of nanoplasmonic resonance peak represented from the MNA sensors was about 740nm under aqueous ambient. The capture antibodies of the lung and the pancreas cancer marker, respectively, were immobilized on the surfaces of MNA sensor. Using a compact fiber-optic spectrometer and a reflection optical probe, we were able to confirm the binding of cancer markers with their antibodies due to the immunoreactions between each cancer marker and its corresponding antibody on the sensor surfaces. The amount of the cancer markers in serum were analyzed through the observation of nanoplasmonic resonance wavelength-shift on the reflection spectra. To amplify a sensitivity of detection demonstrated by the nanoplasmonic resonance peak shift, we applied enzyme-precipitation reaction on the surface of MNA biosensor. The enzyme-catalyzed precipitation method in the GNA biosensor could be extended to detect other clinical biomarkers at extremely low concentrations in actual clinical samples.

**Keywords**: Cancer diagnosis; Gold nanodot array (GNA); Localized surface plasmon resonance (LSPR); Nanoimprint
Label-free CRP detection using optical biosensor with one-step immobilization of antibody on nitrocellulose membrane


Kim BB, Im WJ, Byun JY, Kim HM, Kim MG, Shin YB. *Co-corresponding: Yong-Beom Shin(ybshin@kribb.re.kr)

To assess the potential of using the MCLW sensor as a label-free optical biosensor system for clinical diagnosis, we evaluated the potential of detecting C-reactive protein (CRP) in a diluted real serum sample by using nitrocellulose as an adlayer. Nitrocellulose was coated on the MCLW sensor chip via spin coating and the CRP antibody was directly immobilized on the sensor surface by hydrophobic physical adsorption. Biosensing using the nitrocellulose surface is highly convenient and does not require any additional treatment for the immobilization of ligand. We optimized the condition for the deposition of nitrocellulose on the MCLW sensor chip with the goal of generating the largest signal difference of the sample against the control signal. In addition, label-free immunosensing of CRP was achieved using the nitrocellulose-coated MCLW sensor in real time. As a result, we were able to quantitatively detect CRP in a human serum within a concentration range of 0.1-10 μg/mL.

**Keywords**: C-reactive protein (CRP); Label-free; Metal clad waveguide; Nitrocellulose; Optical biosensor

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**Plant Systems Engineering Research Center**

SIPMEI, a pollen-specific gene in tomato


Kim WB, Lim CJ, Jang HA, Yi SY, Oh SK, Lee HY, Kim HA, Park YI, Kwon SY. *Corresponding: Suk-Yoon Kwon(sykwon@kribb.re.kr)

Pectin is one of the main components of plant cell walls, and its biosynthesis is controlled by pectin methylsterase (PME). Pectin methylsterase inhibitors (PMEIs) are key regulators of PME. We report here the cloning and characterization of a novel Solanum lycopersicum L. PME gene, SIPMEI. RT-PCR studies of leaf, seed, fruit, flower, and flower organs confirmed that SIPMEI is expressed specifically in pollen. Promoter analysis of SIPMEI revealed pollen-specific cis-acting elements (pollen lat52 and g10). In addition, SIPMEI is expressed independently of abiotic stress, pathogen exposure, and growth stage in tomato, and a histochemical analysis of promoter activity revealed pollen-specific expression in both Arabidopsis and tomato. Under the microscope, we observed pollen-specific GUS expression in the stamen of transgenic tomato plant. These results indicate that the promoter of SIPMEI has strong pollen-specific activity, and could therefore be useful for development of industrially and agronomically important transgenic plants.

**Keywords**: Arabidopsis; PMEI; Pollen-specific gene; SIPMEI promoter; Stress; Tomato

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2014 KRIBB Article Abstracts | 59 |
Expression of cucumber LOX genes in response to powdery mildew and defense-related signal molecules


Oh SK, Jang HA, Kim J, Choi D, Park YI, Kwon SY*. ’Corresponding: Suk-Yoon Kwon(sykwon@kribb.re.kr)

The cucumber genome contains 23 lipoxygenase (LOX) genes. The expression of seven type-I and six type-II LOX genes was induced when cucumber leaves were challenged with Sphaerotheca fuliginea and treated with salicylic acid, methyl jasmonate, and abscisic acid. These 13 CsLOX genes were differentially regulated during biotic and abiotic stresses.

Keywords: Abiotic stress; Biotic stress; Cucumber; Lipoxygenase (LOX); Powdery mildew

Identification of a pollen-specific gene, SICRK1 (RFK2) in tomato


Kim WB, Yi SY, Oh SK, Lim CJ, Kim HA, Jang HA, Lee HY, Park YI, Kwon SY*. ’Corresponding: Suk-Yoon Kwon(sykwon@kribb.re.kr)

Plant receptor-like kinases (RLKs) are proteins that are involved in the regulation of development, hormone signaling, abiotic, and biotic stress responses. It has been suggested that cysteine-rich receptor-like kinases (CRKs), which are one of the largest RLK groups, is significant in pathogen defense and programmed cell death. The CRK1 gene is isolated and characterized from tomato (Solanum lycopersicum L.). The SICRK1 has two C-X8-C-X2-C motifs: a trans-membrane region and a kinase domain similar to other CRKs. The semi-quantitative RT-PCR exhibits the specific expression of SICRK1 in the flower, but not in the root, leaf, seed, and fruit of the tomato. In addition, SICRK1 exhibits pollen-specific expression in the floral organ. SICRK1 has pollen-specific cis-acting elements in the promoter region, and its promoter has pollen-specific activity in the homozygous transgenic plants of tomato and Arabidopsis as confirmed through histochemical GUS assays. Moreover, the expression of SICRK1 is not detected via stress treatment or hormone treatment. In this study, SICRK1 from tomato is characterized and its promoter can be useful in developing transgenic plants with foreign genes that should be expressed in pollens.

Keywords: Arabidopsis; Pollen; Promoter; SICRK1; Tissue-specific gene; Tomato
Responses of sweet potato peroxidases to sodium nitroprusside-mediated nitric oxide


Kim YH, Jeong JC, Lee HS, Kwak SS*.
*Corresponding: Sang-Soo Kwak(sskwak@kribb.re.kr)

To ascertain the response of sweetpotato peroxidases (PODs) to nitric oxide (NO), we treated the leaves of sweet potato with the NO generator sodium nitroprusside (SNP) and the NO scavenger carboxyl-PTIO (cPTIO). Exogenous application of more than 5 mM SNP caused damage to sweetpotato leaves at 24 h after treatment. The accumulation of NO in leaves was positively correlated with the SNP dose. The specific activity of PODs in sweet potato leaves was markedly increased by treatment with greater than 1 mM SNP for 24 h, whereas POD activity and accumulated NO content decreased to low levels by treatment with cPTIO. Expression analysis of POD genes in response to treatment with SNP and cPTIO revealed that major stress-inducible acidic genes, such as swpa1, swpa2, swpa3, and swpa4, were specifically regulated. These results indicate that increased NO levels in sweet potato leaves are closely linked to an improved defense capability mediated by stress-inducible PODs.

**Keywords**: Carboxyl-PTIO; Nitric oxide (NO); Peroxidase; Sodium nitroprusside; Sweetpotato

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Cucumber Pti1-L is a cytoplasmic protein kinase involved in defense responses and salt tolerance


Oh SK, Jang HA, Lee SS, Cho HS, Lee DH, Choi D, Kwon SY*.
*Corresponding: Suk-Yoon Kwon(sykwon@kribb.re.kr)

Homologs of the cytoplasmic protein kinase Pti1 are found in diverse plant species. A clear role of Pti1 in plant defense response has not been established. We identified a Pti1 homolog in cucumber (CsPti1-L). CsPti1-L expression was induced when cucumber plants were challenged with the fungal pathogen Sphaerotheca fuliginea or with salt treatment. CsPti1-L expression in cucumber leaves also was induced by methyl jasmonate, salicylic acid, and abscisic acid. CsPti1-L exhibited autophosphorylation activity and was targeted to the cytoplasm. Transgenic Nicotiana benthamiana expressing CsPti1-L exhibited greater cell death and increased ion leakage in response to the bacterial pathogen Pseudomonas syringae pv. tomato DC3000, resistance to Botrytis cinerea infection, and higher tolerance to salt stress. RT-PCR analysis of transgenic N. benthamiana overexpressing CsPti1-L revealed constitutive upregulation of multiple genes involved in plant-defense and osmotic-stress responses. Our results suggest a functional role for CsPti1-L as a positive regulator of pathogen-defense and salt-stress responses.

**Keywords**: Cell death; CsPti1-L; Cytoplasmic protein kinase; Defense responses; Salt tolerance

PMID: 24877673
Synechocystis PCC6803 and PCC6906 dnaK2 expression confers salt and oxidative stress tolerance in Arabidopsis via reduction of hydrogen peroxide accumulation


Kim J, Ahn MS, Park YM, Kim SW, Min SR, Jeong WJ, Liu JR.  *Corresponding: Jang Ryol Liu(jrliu@kribb.re.kr)*

Abiotic stress slows plant growth and development. Because salt stress, particularly from NaCl, acts as an important limiting factor in agricultural productivity, the identification and manipulation of genes related to salt tolerance could improve crop productivity. Prokaryotic, heat shock protein (Hsp), DnaK from the ubiquitous Hsp70 family is upregulated in cells that are under abiotic stress. *Synechocystis* spp. cyanobacteria encode at least three potential DnaK proteins in their genome. Here, expressions of dnaK1s and dnaK2s from two *Synechocystis* spp. PCC6803 and PCC6906 (Sy6803), enhanced salt tolerance in a dnaK-defective *Escherichia coli* strain. In contrast, dnaK3s in both strains were ineffective, indicating that dnaK3 is functionally different from dnaK1 and dnaK2 in *Synechocystis* spp. under salt stress. Ectopic expression of dnaK2s from Sy6803 and Sy6906 conferred salt tolerance in transgenic *Arabidopsis* plants, which exhibited greater root length, chlorophyll content, fresh weight, and survival rate than wild type plants, all in the presence of NaCl. In transgenic plants, hydrogen peroxide (H$_2$O$_2$) accumulation was reduced under NaCl stress and loss of chlorophyll content was reduced under H$_2$O$_2$ stress. Overall results suggest that dnaK2s from Sy6803 and Sy6906 confer salt and oxidative tolerance in transgenic plants by reduction of H$_2$O$_2$ accumulation.

**Keywords**: *Arabidopsis thaliana*; Hydrogen peroxide; Salt stress tolerance; *Synechocystis*; Transgenic plant

PMID: 24415294

Differential responses of three sweetpotato metallothionein genes to abiotic stress and heavy metals


Kim SH, Jeong JC, Ahn YO, Lee HS*, Kwak SS*.  *Co-corresponding: Sang-Soo Kwak(sskwak@kribb.re.kr), Haeng-Soon Lee(hslee@kribb.re.kr)*

Metallothioneins (MTs) are cysteine-rich, low molecular weight, metal-binding proteins that are widely distributed in living organisms. Plants produce metal-chelating proteins such as MTs to overcome the toxic effects of heavy metals. We cloned three *MT* genes from sweetpotato leaves [Ipomoea batatas (L.) Lam.]. The three *IbMT* genes were classified according to their cysteine residue alignment into type 1 (*IbMT1*), type 2 (*IbMT2*), and type 3 (*IbMT3*). *IbMT1* was the most abundantly transcribed MT. It was predominantly expressed in leaves, roots, and callus. *IbMT2* transcript was detected only in stems and fibrous roots, whereas *IbMT3* was strongly expressed in leaves and stems. The *IbMT* expression profiles were investigated in plants exposed to heavy metals and abiotic stresses. The levels of *IbMT1* expression were strongly elevated in response to Cd and Fe, and moderately higher in response to Cu. The *IbMT3* expression pattern in response to heavy metals was similar to that of *IbMT1*. Exposure to abiotic stresses such as methyl viologen (MV; pararquat), NaCl, polyethylene glycol (PEG), and H$_2$O$_2$ up-regulated *IbMT* expression; *IbMT1* responded strongly to MV and NaCl, whereas *IbMT3* was induced by low temperature and PEG. Transgenic *Escherichia coli* overexpressing *IbMT1* protein exhibited results suggest that *IbMT* could be a useful tool for engineering plants with enhanced tolerance to environmental stresses and heavy metals.

**Keywords**: Abiotic stress; Heavy metal; Metallothionein; Phytoremediation; Reactive oxygen species (ROS); Sweetpotato

PMID: 25030835
Down-regulation of sweetpotato lycopene β-cyclase gene enhances tolerance to abiotic stress in transgenic calli


Kim SH, Jeong JC, Park S, Bae JY, Ahn MJ, Lee HS*, Kwak SS*, Co-corresponding: Sang-Soo Kwak(sskwak@kribb.re.kr), Haeng-Soon Lee(hslee@kribb.re.kr)

Lycopene β-cyclase (LCY-β) is a key enzyme involved in the synthesis of α- and β-branch carotenoids such as α-carotene and β-carotene through the cyclization of lycopene. *ibLCY-β* had a length of 1,506 bp and approximately 80% nucleotide sequence identity with that of tomato LCY-β. *ibLCY-β* was strongly expressed in leaves, and expression was enhanced by salt-stress and osmotic-stress conditions. To characterize the LCY-β gene (*ibLCY-β*) of sweetpotato (*Ipomoea batatas*), it was isolated and transformed into calli of white-fleshed sweetpotato using an *ibLCY-β*-RNAi vector. Transgenic *ibLCY-β*-RNAi calli had yellow to orange color and higher antioxidant activity compared to that of white, nontransgenic (NT) calli. Transgenic cells had significantly higher contents of total carotenoids, although lycopene was not detected in transgenic or NT cells. All transgenic calli had strongly activated expression of carotenoid biosynthetic genes such as β-carotene hydroxylases (*CHY-β*), cytochrome P450 monoxygenases (*P450*), and carotenoid cleavage dioxigenase 1 (*CCD1*). Transgenic cells exhibited less salt-induced oxidative-stress damage compared to that of NT cells, and also had greater tolerance for polyethylene glycol (PEG)-mediated drought compared to that of NT cells, due to the higher water content and reduced malondialdehyde (MDA) content. The abscisic acid content was also higher in transgenic cells. These results show that a study of *ibLCY-β* can facilitate understanding of the carotenoid biosynthetic pathway in sweetpotato. *IbLCY-β* could be useful for developing transgenic sweetpotato enriched with nutritional carotenoids and with greater tolerance to abiotic stresses.

**Keywords**: Carotenoid; Drought stress; Lycopene β-cyclase; Metabolic engineering; RNAi; Salt stress; Sweetpotato

PMID: 25213547

Development of the large-scale oligonucleotide chip for the diagnosis of plant viruses and its practical use


Nam M, Kim JS, Lim S, Park CY, Kim JG, Choi HS, Lim HS, Moon JS*, Lee SH, Co-corresponding: Jae Sun Moon(jsmoon@kribb.re.kr)

A large-scale oligonucleotide (LSN) chip was developed for the detection of the plant viruses with known genetic information. The LSN chip contains two sets of 3,978 probes for 538 species of targets including plant viruses, satellite RNAs and viroids. A hundred forty thousand probes, consisting of isolate-, species- and genus-specific probes respectively, are designed from 20,000 of independent nucleotide sequence of plant viruses. Based on the economic importance, the amount of genome information, and the number of strains and/or isolates, one to fifty-one probes for each target virus are selected and spotted on the chip. The standard and field samples for the analysis of the LSN chip have been prepared and tested by RT-PCR. The probe's specific and/or nonspecific reaction patterns by LSN chip allow us to diagnose the unidentified viruses. Thus, the LSN chip in this study could be highly useful for the detection of unexpected plant viruses, the monitoring of emerging viruses and the fluctuation of the population of major viruses in each plant.

**Keywords**: Large-scale oligonucleotide chip; LSON chip; Oligo chip; Plant virus; Virus diagnosis

PMID: 25288985
Characteristics of a Lettuce mosaic virus isolate infecting lettuce in Korea

Lim S, Zhao F, Yoo RH, Igori D, Lee SH, Lim HS, Moon JS.
'Corresponding: Jae Sun Moon(jsmoon@kribb.re.kr)

Lettuce mosaic virus (LMV) causes disease of plants in the family Asteraceae, especially lettuce crops. LMV isolates have previously been clustered in three main groups, LMV-Yar, LMV-Greek and LMVRoW. The first two groups, LMV-Yar and LMV-Greek, have similar characteristics such as no seed-borne transmission and non-resistance-breaking. The latter one, LMV-RoW, comprising a large percentage of the LMV isolates contains two large subgroups, LMV-Common and LMV-Most. To date, however, no Korean LMV isolate has been classified and characterized. In this study, LMV-Muju, the Korean LMV isolate, was isolated from lettuce showing pale green and mottle symptoms, and its complete genome sequence was determined. Classification method of LMV isolates based on nucleotide sequence divergence of the NbSGT1 junction showed that LMV-Muju was categorized as LMV-Common. LMV-Muju was more similar to LMV-O (LMV-Common subgroup) than to LMV-E (LMV-RoW group but not LMV-Common subgroup) even in the amino acid domains of HC-Pro associated with pathogenicity, and in the CI and VPg regions related to ability to overcome resistance. Taken together, LMV-Muju belongs to the LMV-Common subgroup, and is expected to be a seed-borne, non-resistance-breaking isolate. According to our analysis, all other LMV isolates not previously assigned to a subgroup were also included in the LMV-RoW group.

Keywords: Lettuce; LMV; Potyvirus; Resistance
PMID: 25289001

Rpi-blb2-mediated hypersensitive cell death caused by phytophthora infestans AVRblb2 requires SGT1, but not EDS1, NDR1, salicylic acid-, jasmonic acid-, or ethylene-mediated signaling

Oh SK, Kwon SY*, Choi D.
'Seconding: Suk-Yoon Kwon(sykwon@kribb.re.kr)

Potato Rpi-blb2 encodes a protein with a coiled-coil-nucleotide binding site and leucine-rich repeat (CC-NBS-LRR) motif that recognizes the Phytophthora infestans AVRblb2 effector and triggers hypersensitive cell death (HCD). To better understand the components required for Rpi-blb2-mediated HCD in plants, we used virus-induced gene silencing to repress candidate genes in Rpi-blb2-transgenic Nicotiana benthamiana plants and assayed the plants for AVRblb2 effector. Rpi-blb2 triggers HCD through NbSGT1-mediated pathways, but not NbEDS1- or NbNDR1-mediated pathways. In addition, the role of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) in Rpi-blb2-mediated HCD were analyzed by monitoring the responses of NbICS1-, NbCOI1-, or NbEIN2-silenced or Rpi-blb2::NahG-transgenic plants. Rpi-blb2-mediated HCD in response to AVRblb2 was not associated with SA accumulation. Thus, SA affects Rpi-blb2-mediated resistance against P. infestans, but not Rpi-blb2-mediated HCD in response to AVRblb2. Additionally, JA and ET signaling were not required for Rpi-blb2-mediated HCD in N. benthamiana. Taken together, these findings suggest that NbSGT1 is a unique positive regulator of Rpi-blb2-mediated HCD in response to AVRblb2, but EDS1, NDR1, SA, JA, and ET are not required.

Keywords: AVRblb2 effector; EDS1; Hypersensitive cell death; NDR1; Rpi-blb2; SGT1
PMID: 25289011
Transgenic alfalfa plants expressing *AtNDPK2* exhibit increased growth and tolerance to abiotic stresses

Plant Physiol Biochem. 84:67-77.

Wang Z, Li H, Ke Q, Jeong JC, Lee HS, Xu B, Deng XP, Lim YP, Kwak SS*. "Corresponding: Sang-Soo Kwak(sskwak@kribb.re.kr)

In this study, we generated and evaluated transgenic alfalfa plants (*Medicago sativa* L. cv. Xinjiang Daye) expressing the *Arabidopsis* nucleoside diphosphate kinase 2 (*AtNDPK2*) gene under the control of the oxidative stress-inducible *SWPA2* promoter (referred to as SN plants) to develop plants with enhanced tolerance to various abiotic stresses. We selected two SN plants (SN4 and SN7) according to the expression levels of *AtNDPK2* and the enzyme activity of NDKP in response to methyl viologen (MV)-mediated oxidative stress treatment using leaf discs for further characterization. SN plants showed enhanced tolerance to high temperature, NaCl, and drought stress on the whole-plant level. When the plants were subjected to high temperature treatment (42 °C for 24 h), the non-transgenic (NT) plants were severely wilted, whereas the SN plants were not affected because they maintained high relative water and chlorophyll contents. The SN plants also showed significantly higher tolerance to 250 mM NaCl and water stress treatment than the NT plants. In addition, the SN plants exhibited better plant growth through increased expression of auxin-related indole acetic acid (IAA) genes (*MsIAA3*, *MsIAA5*, *MsIAA6*, *MsIAA7*, and *MsIAA16*) under normal growth conditions compared to NT plants. The results suggest that induced overexpression of *AtNDPK2* in alfalfa will be useful for increasing biomass production under various abiotic stress conditions.

**Keywords**: Alfalfa; *AtNDPK2*; Biomass; Drought stress; Oxidative stress; Salt stress; *SWAP2* promoter

PMID: 25240265

Overexpression of *codA* gene confers enhanced tolerance to abiotic stresses in alfalfa

Plant Physiol Biochem. 85C:31-40.

Li H, Wang Z, Ke Q, Ji CY, Jeong JC, Lee HS, Lim YP, Xu B, Deng XP, Kwak SS*. "Corresponding: Sang-Soo Kwak(sskwak@kribb.re.kr)

We generated transgenic alfalfa plants (*Medicago sativa* L. cv. Xinjiang Daye) expressing a bacterial *codA* gene in chloroplasts under the control of the *SWPA2* promoter (referred to as SC plants) and evaluated the plants under various abiotic stress conditions. Three transgenic plants (SC7, SC8, and SC9) were selected for further characterization based on the strong expression levels of *codA* in response to methyl viologen (MV)-mediated oxidative stress. SC plants showed enhanced tolerance to NaCl and drought stress on the whole plant level due to induced expression of *codA*. When plants were subjected to 250 mM NaCl treatment for 2 weeks, SC7 and SC8 plants maintained higher chlorophyll contents and lower malondialdehyde levels than non-transgenic (NT) plants. Under drought stress conditions, all SC plants showed enhanced tolerance to drought stress through maintaining high relative water contents and increased levels of glycinebetaine and proline compared to NT plants. Under normal conditions, SC plants exhibited increased growth due to increased expression of auxin-related *IAA* genes compared to NT plants. These results suggest that the SC plants generated in this study will be useful for enhanced biomass production on global marginal lands, such as high salinity and arid lands, yielding a sustainable agricultural product.

**Keywords**: Alfalfa; Biomass; *codA*; Drought stress; Glycinebetaine; Oxidative stress; Salt stress; *SWAP2* promoter

PMID: 25394798
How does SA signaling link the Flg22 responses?

Plant Signal Behav. 9(11):e972806.

Yi SY, Kwon SY*. Corresponding: Suk-Yoon Kwon(sykwon@kribb.re.kr)

Salicylic acid (SA) has a central role in activating plant resistance to pathogens. SA levels increase in plant tissue following pathogen infection and exogenous SA enhances resistance to a broad range of pathogens. To study the relevance of the SA signaling in the flg22 response, we investigated the responses of SA-related mutants to flg22, a 22-amino acid peptide of the flagellin bacterial protein. We identified SA as an important component of the flg22-triggered oxidative burst, a very early event after flg22 detection, and gene induction, an early event. SA acted partially by enhancing accumulation of FLS2 mRNA. We also provide new evidence that NPR1 play a role in SA-induced priming event that enhances the flg22-triggered oxidative burst, which is correlated with enhancement of the flg22-induced callose deposition. Based on these observations, we conclude that SA signaling is required for early as well as late flg22 responses.

Keywords: Callose deposition; FLS2; FRK1; Flg22; FLS2-mediated priming; SID2; WRKY29
PMID: 25482762

The activated SA and JA signaling pathways have an influence on flg22-triggered oxidative burst and callose deposition


Yi SY, Shirasu K, Moon JS, Lee SG, Kwon SY*. Corresponding: Suk-Yoon Kwon(sykwon@kribb.re.kr)

The first line of defense in plants against pathogens is induced by the recognition of microbe-associated molecular patterns (MAMP). Perception of bacterial flagellin (flg22) by the pattern recognition receptor flagellin-sensing 2 (FLS2) is the best characterized MAMP response, although the underlying molecular mechanisms are not fully understood. Here we studied the relationship between salicylic acid (SA) or jasmonic acid (JA) signaling and FLS2-mediated signaling by monitoring flg22-triggered responses in known SA or JA related mutants of Arabidopsis thaliana (L.) Heynh. The sid2 mutant, impaired in SA biosynthesis, had less basal FLS2 mRNA accumulation than the wild type, which correlated with suppression of early flg22 responses such as ROS production and induction of marker genes, WRKY29 and FRK1. The JA-signaling mutants, jar1 and coi1, exhibited an enhanced flg22-triggered oxidative burst and more callose accumulation than the wild type, and pretreatment with SA or coronatine (COR), a structural mimic of JA-isoleucine, altered these flg22-induced responses. Nonexpressor of pathogenesis-related genes 1 (NPR1) acted downstream of SID2 and required SA-dependent priming for the enhanced flg22-triggered oxidative burst and callose deposition. Activation of JA signaling by COR pretreatment suppressed the flg22-triggered oxidative burst and callose accumulation in a coronatine insensitive 1 (COI1) dependent manner. COR had a negative effect on flg22 responses but only the flg22-triggered oxidative burst depended on SA-JA/COR signaling antagonism. Thus the activated SA and JA signaling pathways have an influence on flg22-triggered oxidative burst and callose deposition. These results may explain how SA and JA signaling are cross talked for regulation of flg22-triggered responses.

Keywords: Bacterial flagellin (flg22); Flg22-triggered response; Callose deposition; MAMP response; Oxidative burst; Signaling pathway
PMID: 24586453
**High-throughput sequencing and de novo assembly of Brassica oleracea var. Capitata L. for transcriptome analysis**


Kim HA, Lim CJ, Choe JK, Jo SH, Baek N, Kwon SY*.

*Corresponding: Suk-Yoon Kwon(sykwon@kribb.re.kr)

**BACKGROUND:** The cabbage, *Brassica oleracea* var. *capitata* L., has a distinguishable phenotype within the genus *Brassica*. Despite the economic and genetic importance of cabbage, there is little genomic data for cabbage, and most studies of *Brassica* are focused on other species or other *B. oleracea* subspecies. The lack of genomic data for cabbage, a non-model organism, hinders research on its molecular biology. Hence, the construction of reliable transcriptomic data based on high-throughput sequencing technologies is needed to enhance our understanding of cabbage and provide genomic information for future work.

**METHODOLOGY/PRINCIPAL FINDINGS:** We constructed cDNAs from total RNA isolated from the roots, leaves, flowers, seedlings, and calcium-limited seedling tissues of two cabbage genotypes: 102043 and 107140. We sequenced a total of six different samples using the Illumina HiSeq platform, producing 40.5 Gbp of sequence data comprising 401,454,986 short reads. We assembled 205,046 transcripts (≥ 200 bp) using the Velvet and Oases assembler and predicted 53,562 loci from the transcripts. We annotated 35,274 of the loci with 55,916 plant peptides in the Phytozone database. The average length of the annotated loci was 1,419 bp. We confirmed the reliability of the sequencing assembly using reverse-transcriptase PCR to identify tissue-specific gene candidates among the annotated loci.

**CONCLUSION:** Our study provides valuable transcriptome sequence data for *B. oleracea* var. *capitata* L., offering a new resource for studying *B. oleracea* and closely related species. Our transcriptomic sequences will enhance the quality of gene annotation and functional analysis of the cabbage genome and serve as a material basis for future genomic research on cabbage. The sequencing data from this study can be used to develop molecular markers and to identify the extreme differences among the phenotypes of different species in the genus *Brassica*.

**Keywords:** *Brassica*, Cabbage genome; Molecular marker; Sequencing assembly; Transcriptome analysis

PMID: 24682075

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**Biocatalytic properties and substrate-binding ability of a modular GH10 beta-1,4-xylanase from an insect-symbiotic bacterium, Streptomyces mexicanus HY-14**


*Corresponding: Ho-Yong Park(hypark@kribb.re.kr), Kwang-Hee Son(sonkh@kribb.re.kr)

The gene (1350 bp) encoding a modular β-1,4-xylanase (XylU), which consists of an N-terminal catalytic GH10 domain and a C-terminal carbohydrate-binding module 2 (CBM 2), from *Streptomyces mexicanus* HY-14 was cloned and functionally characterized. The purified His-tagged recombinant enzyme (rXylU, 44.0 kDa) was capable of efficiently hydrolyze diverse xylodiscic compounds, p-nitrophenyl-celllobioside, and p-nitrophenyl-xylapyranoside when incubated at pH 5.5 and 65°C. Specifically, the specific activities (649.8 U/mg and 587.0 U/mg, respectively) of rXylU toward oat spelt xylan and beechwood xylan were relatively higher than those (<500.0 U/mg) of many other GH10 homologs toward the same substrates. The results of enzymatic degradation of birchwood xylan and xyloooligosaccharides (xylotriose to xylohexaose) revealed that rXylU preferentially hydrolyzed the substrates to xylohexose (>75%) as the primary degradation product. Moreover, a small amount (4%<) of xylose was detected as the degradation product of the evaluated xylodiscic substrates, indicating that rXylU was a peculiar GH10 β -1,4-xylanase with substrate specificity, which was different from its retaining homologs. A significant reduction of the binding ability of rXylU caused by deletion of the C-terminal CBM 2 to various insoluble substrates strongly suggested that the additional domain might considerably contribute to the enzyme-substrate interaction.

**Keywords:** β-1,4-xylanase (XylU); Binding ability; GH10 domain; Modular enzyme; *Streptomyces mexicanus* HY-14

PMID: 25269606
Novel alkali-tolerant GH10 endo-β-1,4-xylanase with broad substrate specificity from Microbacterium trichothecenolyticum HY-17, a gut bacterium of the mole cricket Gryllotalpa orientalis


*Co-corresponding: Ho-Yong Park(hypark@kribb.re.kr), Kwang-Hee Son(sonkh@kribb.re.kr)*

The XylH gene (1,167-bp) encoding a novel hemicellulase (41,584 Da) was identified from the genome of Microbacterium trichothecenolyticum HY-17, a gastrointestinal bacterium of Gryllotalpa orientalis. The enzyme consisted of a single catalytic domain, which is 74% identical to that of an endo-β-1,4-xylanase (GH10) from Isoptericola variabilis 225. Unlike other endo-β-1,4-xylanases from invertebrate-symbiotic bacteria, rXylH was an alkali-tolerant multifunctional enzyme possessing endo-β-1,4-xylanase activity together with β-1,3/β-1,4-glucanase activity, which exhibited its highest xylanolytic activity at pH 9.0 and 60°C, and was relatively stable within a broad pH range of 5.0-10.0. The susceptibilities of different xylose-based polysaccharides to the XylH were assessed to be as follows: oat spelt xylan > beechwood xylan > birchwood xylan > wheat arabinoxylan. rXylH was also able to readily cleave p-nitrophenyl (pNP) cellobioside and pNP-xylolpyranoside, but did not hydrolyze other pNP-sugar derivatives, xylobiase, or hexose-based materials. Enzymatic hydrolysis of birchwood xylan resulted in the product composition of xylotriose (71.2%) and xylobiose (28.8%) as end products.

**Keywords**: Endo-β-1,4-xylanase; GH10 enzyme; Gut bacterium; Microbacterium trichothecenolyticum HY-17; Mole cricket

PMID: 24861346

Inhibition of the calcineurin pathway by two tannins, chebulagic acid and chebulanin, isolated from Harrisonia abyssinica Oliv


*Corresponding: Sung Uk Kim(kimsu@kribb.re.kr)*

In order to discover and develop novel signaling inhibitors from plants, a screening system was established targeting the two-component system of Cryptococcus neoformans by using the wild type and a calcineurin mutant of *C. neoformans*, based on the counter-regulatory action of high-osmolarity glycerol (Hog1) mitogen-activated protein kinase and the calcineurin pathways in *C. neoformans*. Among 10,000 plant extracts, that from Harrisonia abyssinica Oliv. exhibited the most potent inhibitory activity against *C. neoformans* var. grubii H99 with fludioxonil. Bioassay-guided fractionation was used to isolate two bioactive compounds from *H. abyssinica*, and these compounds were identified as chebulagic acid and chebulanin using spectroscopic methods. These compounds specifically inhibited the calcineurin pathway in *C. neoformans*. Moreover, they exhibited potent antifungal activities against various human pathogenic fungi with minimum inhibitory concentrations ranging from 0.25 to over 64 µg/ml.

**Keywords**: Antifungal activity; Calcineurin inhibitor; *Cryptococcus neoformans*; Signaling pathway; Two-component system

PMID: 25001554
Inhibitory effects of Rubi Fructus extracts on hepatic steatosis development in high-fat diet-induced obese mice


Nam MK, Choi HR, Cho JS, Cho SM, Ha KC, Kim TH, Ryu HY, Lee YI. *Corresponding: Young-Ik Lee(yilee@kribb.re.kr)

The present study was performed to investigate the potential effects of the unripened dried fruit of Rubus coreanus Miq., Rubi Fructus (RF), on hepatic steatosis and lipid metabolism in mice fed with a high-fat diet (HFD) known to induce obesity and hyperlipidaemia. Rubi Fructus extract (RFex) fed mice demonstrated a reduced body weight and adipose tissue weight. RFex fed mice also demonstrated decreased aminotransferase levels, lipid contents [triglyceride (TG), total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C)], leptin content and increased high-density lipoprotein-cholesterol (HDL-C) contents in the plasma. These effects were accompanied by a decreased expression of lipogenic genes, including sterol regulatory element binding protein-1c, liver X receptor, fatty acid synthase (FAS), acetyl-CoA carboxylase, cluster of differentiation 36, lipoprotein lipase and decreased lipogenic enzyme FAS and 3-hydroxy-3 methylglutamyl coenzyme reductase enzyme activities, while elevating carnitine palmitoyltransferase-1 activity. Based on these results, the present study hypothesized that the inhibitory effect on hepatic steatosis of RFex is the result of the suppression of lipid synthesis in mice fed with HFD, suggesting that RFex may be beneficial in preventing hepatic steatosis and liver lipotoxicity.

Keywords: Hepatic steatosis; High-fat diet (HFD); Lipid metabolism; Lipogenic enzyme FAS; Liver lipotoxicity; Rubi Fructus extract (RFex)

PMID: 25050832

Pterocarpan-enriched soy leaf extract ameliorates insulin sensitivity and pancreatic β-cell proliferation in type 2 diabetic mice


Kim LH, Yoon JH, Li H, Kang JH, Ji HS, Park KH, Shin DH, Park HY, Jeong TS. *Corresponding: Tae-Sook Jeong(tsjeong@kribb.re.kr)

In Korea, soy (Glycine max (L.) Merr.) leaves are eaten as a seasonal vegetable or pickled in soy sauce. Ethyl acetate extracts of soy leaves (EASL) are enriched in pterocarpan and have potent α-glucosidase inhibitory activity. This study investigated the molecular mechanisms underlying the anti-diabetic effect of EASL in C57BL/6J mice with high-fat diet (HFD)-induced type 2 diabetes. Mice were randomly divided into normal diet (ND), HFD (60 kcal% fat diet), EASL (HFD with 0.56% (wt/wt) EASL), and Pinitol (HFD with 0.15% (wt/wt) pinitol) groups. Weight gain and abdominal fat accumulation were significantly suppressed by EASL. Levels of plasma glucose, HbA1c, and insulin in the EASL group were significantly lower than those of the HFD group, and the pancreatic islet of the EASL group had greater size than those of the HFD group. EASL group up-regulated neurogenin 3 (Ngn3), paired box 4 (Pax4), and v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA), which are markers of pancreatic cell development, as well as insulin receptor substrate 1 (IRSI), IRS2, and glucose transporter 4 (GLUT4), which are related to insulin sensitivity. Furthermore, EASL suppressed genes involved in hepatic gluconeogenesis and steatosis. These results suggest that EASL improves plasma glucose and insulin levels in mice with HDF-induced type 2 diabetes by regulating β-cell proliferation and insulin sensitivity.

Keywords: Glycine max; Insulin sensitivity; Pancreas; Pterocarpans; Soy leaf; Type 2 diabetes

PMID: 25401395
Hepatoprotective effects of *Gardenia jasminoides ellis* extract in nonalcoholic fatty liver disease induced by a high fat diet in C57BL/6 mice


Nam MK, Choi HR, Cho JS, Cho SM, Lee YI. *Corresponding: Young-Ik Lee(yilec@kribb.re.kr)

This study was carried out to investigate the potential effects of *Gardenia jasminoides* (GJ) extracts, on hepatic steatosis and lipid metabolism in mice fed with high-fat diet (HFD). GJ extracts (100 mg/kg, × 10 weeks) fed mice showed reduced body weight, adipose tissue weight, reduced aminotransferase level in plasma and hepatic lipid (triglyceride, total cholesterol) content. These effects were accompanied by decreased expression of lipogenic genes, sterol regulatory element binding protein-1c (SREBP-1c), liver X receptor (LXR), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), cluster of differentiation 36 (CD36), lipoprotein lipase (LPL) and decreased lipogenic enzyme FAS and HMG-CoAR enzyme activities while increased carnitine palmitoyltransferase-1 (CPT) activity. Based on these results, we speculated that the inhibitory effect on hepatic steatosis of GJ extract containing geniposide is the result of suppression of lipid synthesis in mice fed with HFD, suggesting that GJ extract may be beneficial in preventing hepatic steatosis.

**Keywords**: Adipose tissue; *Gardenia jasminoides*; Hepatic steatosis; High-fat diet; Lipogenic gene

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9-O-butyl-13-(4-isopropylbenzyl)berberine, KR-72, is a potent antifungal agent that inhibits the growth of *Cryptococcus neoformans* by regulating gene expression


Bang S, Kwon H, Hwang HS, Park KD, Kim SU. *Corresponding: Sung Uk Kim(kimsu@kribb.re.kr)

In this study we explored the mode of action of KR-72, a 9-O-butyl-13-(4-isopropylbenzyl)berberine derivative previously shown to exhibit potent antifungal activity against a variety of human fungal pathogens. The DNA microarray data revealed that KR-72 treatment significantly changed the transcription profiles of *C. neoformans*, affecting the expression of more than 2,000 genes. Genes involved in translation and transcription were mostly upregulated, whereas those involved in the cytoskeleton, intracellular trafficking, and lipid metabolism were downregulated. KR-72 also exhibited a strong synergistic effect with the antifungal agent FK506. KR-72 treatment regulated the expression of several essential genes, including ECM16, NOP14, HSP10 and MGE1, which are required for *C. neoformans* growth. The KR-72-mediated induction of MGE1 also likely reduced the viability of *C. neoformans* by impairing cell cycle or the DNA repair system. In conclusion, KR-72 showed antifungal activity by modulating diverse biological processes through a mode of action distinct from those of clinically available antifungal drugs such as polyene and azole drugs.

**Keywords**: Antifungal agent; Antifungal drug; Biological process; *Cryptococcus neoformans*; Fungal pathogen; KR-72

PMID: 25302492
Division of Biological Infrastructure

› Microbial Resource Center

› Laboratory Animal Resource Center

› International Biological Material Research Center

› Human Derived Material Center

› Korea National Primate Research Center

› Bio-Evaluation Center

› ABS Research Support Department
A novel bacterial strain designated GJW-30 was isolated from soil of the lava forest, Gotjawal, located in Aewol, Jeju, Korea. Strain GJW-30 was found to be strictly aerobic, Gram-negative, and to form pleomorphic, non-motile rods and white colonies on R2A agar. The major fatty acids were identified as C₁₀:0 3c, C₁₀:0 3c, and C₁₇:0 Cyc, the predominant isoprenoid quinone as Q-10, the polar lipids as diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unidentified aminolipid and an unidentified lipid. The cell-wall sugar pattern of strain GJW-30 was found to be composed of glucose, ribose and rhamnose and meso-DAP as the diagnostic diamino acid in the cell-wall peptidoglycan. The DNA G+C content of strain GJW-30 is 62.2 mol%. Phylogenetic analysis, based on 16S rRNA gene sequence similarities, showed that strain GJW-30 forms a deep branch within the order Rhizobiales, sharing the highest level of sequence homology with Bradyrhizobium oligotrophicum LMG 10732TU (93.6%). On the basis of the phenotypic, chemotaxonomic and phylogenetic characteristics, strain GJW-30 is considered to represent a novel genus and species, for which the name Variibacter gotjawalensis gen. nov., sp. nov. (the type strain is GJW-30 = KCTC 32391 = CECT 8514 = LMG 28093TU) is proposed.

**Keywords:** Novel species; Proteobacteria; Soil; Taxonomy; Variibacter gotjawalensis

**PMID:** 24599521

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**Pseudoruegeria limi** sp. nov. isolated from mud flats in the Yellow Sea in Korea

**Antonie Van Leeuwenhoek.** 105(5):987-94.

Lee JB, Kim H, Park DS, Yang JH, Chun YY, Lee KH, Bae KS'.

'Co-corresponding: Kyung Sook Bae(ksbae@kribb.re.kr)

A Gram-negative, aerobic, non-motile and rod-shaped bacterial strain, D-17TU, was isolated from mud flats in the Yellow Sea in Korea. Phylogenetic trees based on the 16S rRNA gene sequence showed that strain D-17 belongs to the genus *Pseudoruegeria* and it shared 97.5% similarity with the type strain of *Pseudoruegeria haliotis* WM67. The sequence similarities with *Pseudoruegeria litimaris* HD-43 and *Pseudoruegeria aquimaris* SW-255 were 96.9 and 96.1%, respectively. Strain D-17TU was found to grow with 0.5-6% (w/v) NaCl, at 20-30 °C, and at pH 6.5-8.0. Strain D-17TU was determined to contain Q-10 as the predominant ubiquinone and summed feature 8 (C₁₀:0 3c and/or C₁₁:0 3c, as defined by the MIDI system) as the major fatty acids. The major polar lipids were identified as phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminolipid, an unidentified glycolipid, an unidentified lipid and four unidentified phospholipids. The DNA G+C content was determined to be 63.6 mol%. The DNA-DNA relatedness with *P. haliotis* WM67 was 32.5%. The differential phenotypic properties revealed that strain D-17TU can be separated from other *Pseudoruegeria* species. Based on the data presented in this study, strain D-17TU represents a novel species, for which the name *Pseudoruegeria limi* sp. nov. is proposed. The type strain is D-17TU (=KCTC 32460 = JCM 19487TU).

**Keywords:** Mud flat; Novel species; Proteobacteria; *Pseudoruegeria*; Taxonomy

**PMID:** 24664663
**Gordonibacter faecihominis** sp. nov., isolated from human faeces

**Antonie Van Leeuwenhoek.** 106(3):439-47.


*Corresponding: Jung-Sook Lee(jslee@kribb.re.kr)

A novel actinobacterial strain, designated CAT-2*, was isolated from human faeces as a bacterium capable of dehydroxylating (+)-catechin derivatives. Strain CAT-2* was found to be strictly anaerobic, Gram-positive, non-motile and non-spore-forming coccobacilli. The major fatty acids were identified as C16:0 DMA (dimethyl acetal), C16:0, C14:0 anteiso-C15:0 and iso-C14:0. The three predominant menaquinones were identified as MK-6 (menaquinone-6), MMK-6 (monomethylmenaquinone-6) and DMMK-6 (dimethylmenaquinone-6). The polar lipids were found to be phosphatidylglycerol, phosphatidylglycerol and four unidentified glycolipids. The DNA G+C content of strain CAT-2* was 68.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequence similarities showed that strain CAT-2* belongs to the genus *Gordonibacter*, sharing the highest level of sequence homology with *Gordonibacter pamelaeae* DSM 19378T (97.3%). Combined phenotypic, chemotaxonomic and phylogenetic characteristics support the conclusion that the strain CAT-2* represents a novel species, for which the name *Gordonibacter faecihominis* sp. nov. is proposed. The type strain is CAT-2* (=KCTC 15204T =JCM 16058T).

**Keywords**: Actinobacteria; *Gordonibacter faecihominis*; Human faeces; Novel species; Taxonomy

PMID: 24948086

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**Antarctobacter jejuensis** sp. nov., isolated from seawater in Jeju, Korea


*Co-corresponding: Doo-Sang Park(dspark@kribb.re.kr)

A novel bacterium, designated strain 13-2-B6*, was isolated from seawater adjacent to Songak Mountain on Jeju Island, South Korea. The novel strain was observed to be Gram-negative, aerobic, rod-shaped and motile with a single polar flagellum. On the basis of 16S rRNA gene sequence similarity, strain 13-2-B6* was determined to be phylogenetically closely related to the type strain of *Antarctobacter heliothermus*, currently the sole species of the genus *Antarctobacter* (family Rhodobacteraceae). Sequence similarity between the 16S rRNA genes of strain 13-2-B6* and *A. heliothermus* EL-219(T) is 96.9 %. Strain 13-2-B6* was found to grow optimally at 25-30 °C, pH 7.0-8.0 and 3 % (w/v) NaCl. The predominant isoprenoid quinone in strain 13-2-B6* was identified as ubiquinone Q-10 and the major fatty acids were identified as C15:0 3-Oc and/or C18:1 o6c. Phosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unknown aminolipids, two unknown phospholipids, an unknown glycolipid and an unknown lipid were found to be components of the polar lipid profile. The G+C content of strain 13-2-B6* was determined to be 62 mol %. On the basis of its phenotypic, chemotaxonomic and phylogenetic distinctiveness, strain 13-2-B6* is considered to represent a novel species of the genus *Antarctobacter*, for which the name *Antarctobacter jejuensis* sp. nov. is proposed. The type strain is 13-2-B6* (=KCTC 42009T =JCM 19898T).

**Keywords**: *Antarctobacter jejuensis*; Novel species; Rhodobacteraceae; Sea water bacteria; Taxonomy

PMID: 25195066
The importance of using realistic evolutionary models for retrodicting proteomes


Kim KM*, Nasir A, Caetano-Anollés G.
*First: Kyung Mo Kim(kmkim@kribb.re.kr)

The reconstruction of phylogenetic trees from molecular data requires selecting models of molecular evolution that adequately describe known processes of change. Operationally, these models optimize molecular changes along branches of the trees. The underlying processes must be realistic and must comply with well-supported biological assumptions. In a recent paper, a new model of proteome evolution that penalizes growth of the protein world provides an ‘upside down’ phylogeny and identifies a very complex ancestor of diversified life. Here we show that the model is phylogenetically self-inconsistent and at odds with considerable background knowledge, including the scale-free property of domain networks, genomic scaling laws, and the principle of continuity that supports the tenets of ideographic analysis and evolutionary thinking. While technical and conceptual limitations invalidate the main conclusions of the study, including the existence of bottlenecks in protein evolution caused by planetary cataclysms, we use the example to highlight the complexities and pitfalls of retrodiction in phylogenetic and phylogenomic analyses and reexamine the framework of ideographic exploration that is used in scientific inquiry.

**Keywords**: Evolution; Fold superfamilly; Phylogenetics; Superkingdom; Tree of life

PMID: 24316279

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Transcriptome analysis of mistletoe (Viscum album) haustorium development


Ko SM, Kwon YK, Kim JH, Song IJ, Lee HY, Choi DW, Liu JR, Kim SW*.
*Corresponding: Suk Weon Kim(kimsw@kribb.re.kr)

The haustorium is a specific organ for penetration of host tissue in parasitic plants. To better understand the molecular mechanisms of the establishment of host-parasite relationships using gene expression profiles, early stage haustorium germination from mature mistletoe seeds was examined. We have generated a cDNA library that is an excellent source of expressed sequence tags (ESTs) specifically related to haustorium development and host penetration. We analyzed 4,771 ESTs derived from a mistletoe (Viscum album) haustorium cDNA library. Cluster analysis identified 727 contigs (23.88%) and 2,317 singletons (76.12%), yielding a total of 3,044 unique genes. Among these genes, 1,760 clones (57.8%) showed significant similarity to known genes; the remaining 1,284 clones were novel. Annotation of genes with Gene Ontology indicated that the most abundant ESTs reveals that defense against biotic / abiotic stresses, primary metabolic processes, cell wall loosening and modification is critical for haustorium development and establishment of the host-parasite connection. Especially, it is likely that xyloglucan endotransglycosylases (XETs), glucanase, expansins and other cell wall hydrolases cooperate in cell wall modification during the stages of host-parasite connection. Functional expression of full-length forms corresponding to target ESTs was applied to characterize haustorium genes. This EST information will help gene discovery and characterization and provide promising targets for genetic engineering. The EST analysis presented here represents the first reported transcriptome data for V. album var. coloratum. Although the number of ESTs analyzed may not be sufficient to completely elucidate the mechanism of the host-parasite connection, the integrated approaches reported here represent an essential step toward understanding haustorium development in parasitic plants and provide a valuable resource for defining molecular mechanisms in the host-parasite connection.

**Keywords**: cDNA library; Expressed sequence tag; Gene ontology; Transcriptome; Viscum album
Glaciilhabitans tibetensis gen. nov., sp. nov., a psychrotolerant bacterium of the family Microbacteriaceae, isolated from glacier ice water


Li AH, Liu HC, Xin YH, Kim SG, Zhou YG. Co-corresponding: Song-Gun Kim(sgkim@kribb.re.kr)

A Gram-stain-positive, aerobic, non-spore-forming, short-rod-shaped bacterium, designated strain MP203 T, was isolated from ice water of Midui Glacier in Tibet Autonomous Region, China. The strain was psychrotolerant, growing at 0-25 °C. 16S rRNA gene sequence analysis showed that strain MP203 T was most similar to Frigrobacterium faeni NBRC 103066 T, Compositmonas suwensensis KACC 13354 T, Frigrobacterium mesophilum KCTC 19311 T, Marisediminicola antarctica CCTCC AB 209077 T and Alpinimonas psychrophila JCM 18951 T, with similarities of 97.4, 97.2, 97.2, 97.1 and 97.1 %, respectively. The maximum-likelihood phylogenetic tree indicated that strain MP203 T clustered with nine genera of the family Microbacteriaceae, namely Frigrobacterium, Compositmonas, Marisediminicola, Alpinimonas, Frondihabitans, Clavibacter, Subtercola, Klugiella and Agreia. However, bootstrap analysis showed that there was no significance in the branching pattern of the clades comprising strain MP203 T and any existing generic lineage of the family Microbacteriaceae. DNA-DNA hybridization results indicated levels of relatedness between strain MP203 T and Marisediminicola antarctica CCTCC AB 209077 T, Frigrobacterium faeni NBRC 103066 T, Frigrobacterium mesophilum KCTC 19311 T, Compositmonas suwensensis KACC 13354 T and Alpinimonas psychrophila JCM 18951 T were 25.8 ± 7.3, 29.6 ± 7.6, 19.7 ± 6.7, 16.0 ± 4.2 and 12.4 ± 5.1 % (mean ± SD), respectively. The G+C content of the genomic DNA was 64.1 mol%. Analysis of the cell-wall peptidoglycan revealed that the peptidoglycan structure of strain MP203 T was B10 type with Gly-[l-Hse]-D-Glu-D-DAB, containing 2, 4-diaminobutyric acid (DAB) as a diagnostic amino acid. The cell-wall sugars were rhamnose, ribose, mannose and glucose. The major fatty acids were anteiso-C15:0, iso-C16:0 and anteiso-A-C15:1. An unusual compound identified as anteiso-C15:0-DMA (1,1-dimethoxy-anteiso-pentadecane) was also present in strain MP203 T. The predominant menaquinone was MK-10. Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), one unknown glycolipid and four unknown lipids were detected in the polar lipid extracts. As strain MP203 T was distinguishable from phylogenetically related genera in the family Microbacteriaceae in terms of its physiological and chemotaxonomic characteristics and phylogenetic position, it was considered to represent a novel species of a new genus. Thus, the name Glaciilhabitans tibetensis gen. nov., sp. nov. is proposed. The type strain of Glaciilhabitans tibetensis is MP203 T (= CGMCC 1.12484 T = KCTC 29148 T).

**Keywords:** Glaciilhabitans tibetensis; Ice water; Microbacteriaceae; Novel species; Psychrotolerant bacterium

PMID: 24158943

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Luteimicrobium xylanilyticum sp. nov., isolated from the gut of a long-horned beetle, Massicus raddei


Kim H, Oh HW, Kim JA, Park DS, Park HM, Bae KS. Corresponding: Kyung Sook Baek(ksbae@kribb.re.kr)

A novel strain, designated W-15 T, was isolated from the gut of a long-horned beetle, Massicus raddei, collected in South Korea. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the strains belonged to the suborder Micrococccaceae. Strain W-15 T was most closely related to Luteimicrobium album R1148-L105 T (97.9 % similarity). Strain W-15 T was Gram-stain-positive, rod- and coccous-shaped and non-motile. Growth was observed at 15-37 °C, at pH 4.5-8.5 and in the presence of 0-5.0 % NaCl. The cell-wall peptidoglycan of the strain was A4u (L-Lys-d-ser-d-Asp). The major menaquinone present in this strain was MK-8 (H2) and the major cellular fatty acids were anteiso-C15:0, iso-C16:0, iso-C15:0 and anteiso-C17:0. The major polar lipids were diphasophatidylglycerol, phosphatidylglycerol, an unknown lipid, an unknown phospholipid and an unknown phosphoglycolipid. The G+C content of genomic DNA of the strain was 73.8 mol%. On the basis of evidence from our polyphasic taxonomic study, strain W-15 T is classified as representing a novel species in the suborder Micrococccaceae, for which the name Luteimicrobium xylanilyticum sp. nov. is proposed. The type strain of this species is strain W-15 T (= KCTC 19882 T = JCM 18090 T).

**Keywords:** Gut; Luteimicrobium xylanilyticum; Massicus raddei; Micrococccaceae; Novel species

PMID: 24491830
Bacillus solimangrovi sp. nov., isolated from mangrove soil


Lee GH, Rhee MS, Chang DH, Kwon KK, Bae KS, Yang SH, Kim BC.

"Corresponding: Byoung Chan Kim(bckim@kribb.re.kr)

Two novel bacterial strains, GH2-4\textsuperscript{T} and GH2-5, were isolated from mangrove soil near the seashore of Weno island in Chuuk state, Micronesia, and were characterized by a polyphasic approach. The two strains were strictly aerobic, Gram-staining-positive, motile, endospore-forming rods that were catalase- and oxidase-positive. Colonies were circular, convex, stringy and transparent yellowish (GH2-4\textsuperscript{T}) or opaque whitish (GH2-5). The 16S rRNA gene sequences of the two isolates were identical. The most closely related strains in terms of 16S rRNA gene sequence similarity were Bacillus kochii WCC 4582\textsuperscript{T}, B. horneckiae DSM 23495\textsuperscript{T}, B. azotoformans LMG 9581\textsuperscript{T}, B. cohii DSM 6307\textsuperscript{T} and B. halmapalus DSM 8723\textsuperscript{T} (95.6, 95.4, 95.4, 95.2 and 95.2% similarity, respectively). The partial groEL sequence of strain GH2-4\textsuperscript{T} was identical to that of strain GH2-5 and showed >85% similarity to those of the most closely related strains. The isolates grew at pH 5-12 (optimal growth at pH 9), at 10-40 °C (optimum 30-35 °C) and at 0-9% (w/v) NaCl (optimum 1-3% NaCl). The cell-wall peptidoglycan of strains GH2-4\textsuperscript{T} and GH2-5 contained meso-diaminopimelic acid and cell-wall hydrolysates contained ribose as a major sugar. The DNA G+C content was 36 mol% and DNA-DNA relatedness between the isolates and five related reference strains was 20-24%. Strain GH2-4\textsuperscript{T} exhibited 81% DNA-DNA relatedness with strain GH2-5. The major cellular fatty acids of both strains were iso-C\textsubscript{15:0}, iso-C\textsubscript{16:0}, iso-C\textsubscript{14:0} and anteiso-C\textsubscript{15:0} and the predominant menaquinone was MK-7. On the basis of the evidence from this polyphasic study, strains GH2-4\textsuperscript{T} and GH2-5 (=KCTC 33143=JCM 18995=DSM 27084) represent a novel species of the genus Bacillus, for which the name Bacillus solimangrovi sp. nov. is proposed; the type strain is GH2-4\textsuperscript{T} (=KCTC 33142=JCM 18994=DSM 27083\textsuperscript{T}).

Keywords: Bacillus solimangrovi; Novel species; Soil

PMID: 24488932

Roseivivax roseus sp. nov., an alphaproteobacterium isolated from a solar saltern soil sample


Zhang YQ, Lee JC, Park DJ, Lu XX, Mou XZ, Kim CJ.

"Corresponding: Chang-Jin Kim(changjm@kribb.re.kr)

A pink, Gram-stain-negative, motile, halotolerant bacterium with subpolar flagellum, designated strain BH87090\textsuperscript{T}, was isolated from a saline soil sample collected from the south-west coastal area of South Korea (125° 58’ 58.08’’ E 34° 45’ 37.32’’ N). The isolate formed opaque pink to red colonies on marine agar plates at 30 °C. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, sulfoquinovosyldiacylglycerol, phosphatidylcholine and one unidentified phospholipid. The sole respiratory quinone was ubiquinone-10 (Q-10). The major cellular fatty acids were C\textsubscript{18:1}ω7c, C19:0 cyclo ω8c, C\textsubscript{16:0} and 11-methyl C\textsubscript{18:1}ω7c. The genomic DNA G+C content was 61.8 mol%. These chemotaxonomic characteristics were all consistent with specific properties of the genus Roseivivax. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate affiliated to the cluster with members of the genus Roseivivax in the Roseobacter clade, which suggested that the strain belonged to the genus Roseivivax. However, the low 16S rRNA gene similarities (93.5-95.3%) of strain BH87090T with all the members of the genus Roseivivax indicated that it represented a novel species of the genus Roseivivax. On the basis of phenotypic and genotypic data, strain BH87090\textsuperscript{T} should be classified as a novel species of the genus Roseivivax. The name Roseivivax roseus sp. nov. is proposed, with strain BH87090\textsuperscript{T} (=DSM 23042=T=KCTC 22650\textsuperscript{T}) as the type strain.

Keywords: Alphaproteobacterium; Novel species; Roseivivax roseus; Soil

PMID: 24554641
A novel bacterial strain designated CB4 was isolated from soil from the Hallasan, Jeju, Korea. Strain CB4 was found to be strictly aerobic, Gram-stain-positive, rod-shaped, motile and formed creamy greyish colonies on nutrient agar. The major fatty acids were identified as iso-C_{15:0} and iso-C_{16:0}, and the predominant isoprenoid quinone as MK-7. The cell-wall peptidoglycan contained glycine and alanine as the diagnostic amino acids and phosphatidyl-di-acylglycerol as the polar lipids. The genomic DNA G+C content of strain CB4 was 46.5 mol%. Phylogenetic analysis, based on 16S rRNA gene sequence similarities, showed that strain CB4 forms a deep branch within the genus Aneurinibacillus, sharing the highest level of sequence homology with A. aneurinilyticus DSM 5562(T) (96.5%). On the basis of the phenotypic, chemotaxonomic and phylogenetic characteristics, strain CB4 is considered to represent a novel species within the genus Aneurinibacillus, for which the name Aneurinibacillus soli sp. nov. is proposed. The type strain is CB4 (=KCTC 33505^{T} =CECT 8566^{T}). An emended description of the genus Aneurinibacillus is also proposed.

**Keywords:** Aneurinibacillus soli; Novel species; Soil

**PMID:** 25142210

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**Article 144**

**Discrimination of cultivation ages and cultivars of ginseng leaves using Fourier transform infrared spectroscopy combined with multivariate analysis**


Kwon YK, Ahn MS, Park JS, Liu JR, In DS, Min BW, Kim SW^{’}.

*Corresponding: Suk Weon Kim(kimsw@kribb.re.kr)

To determine whether Fourier transform (FT)-IR spectral analysis combined with multivariate analysis of whole-cell extracts from ginseng leaves can be applied as a high-throughput discrimination system of cultivation ages and cultivars, a total of total 480 leaf samples belonging to 12 categories corresponding to four different cultivars (Yunpung, Kumpung, Chumpung, and an open-pollinated variety) and three different cultivation ages (1 yr, 2 yr, and 3 yr) were subjected to FT-IR. The spectral data were analyzed by principal component analysis and partial least squares-discriminant analysis. A dendrogram based on hierarchical clustering analysis of the FT-IR spectral data on ginseng leaves showed that leaf samples were initially segregated into three groups in a cultivation age-dependent manner. Then, within the same cultivation age group, leaf samples were clustered into four subgroups in a cultivar-dependent manner. The overall prediction accuracy for discrimination of cultivars and cultivation ages was 94.8% in a cross-validation test. These results clearly show that the FT-IR spectra combined with multivariate analysis from ginseng leaves can be applied as an alternative tool for discriminating of ginseng cultivars and cultivation ages. Therefore, we suggest that this result could be used as a rapid and reliable F1 hybrid seed-screening tool for accelerating the conventional breeding of ginseng.

**Keywords:** Fourier transform IR; Panax ginseng; Cultivation age; Ginseng cultivars; Ginseng leaves

**PMID:** 24558311
**Alicyclobacillus tengchongensis** sp. nov., a thermo-acidophilic bacterium isolated from hot spring soil


Kim MG1, Lee JC, Park DJ, Li WJ, Kim CJ.

*Corresponding: Chang-Jin Kim(changjin@kribb.re.kr)*

A thermo-acidophilic bacterium, designated strain ACK0067, was isolated from the soil of a hot spring at Tengchong in China. Cells were Gram-staining-positive, motile, catalase-positive and oxidase-negative, spore-forming rods. The isolate grew aerobically at 30-50°C (optimum at 45°C), pH 2.0-6.0 (optimum pH 3.2) and 0-5.0% (w/v) NaCl (optimum 1% NaCl). Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain ACK0067 belongs to the genus *Alicyclobacillus* with the sequence similarity of 92.3, 92.4, 92.5, and 92.8% to *Alicyclobacillus cycloheptanicus* SCH1, *Alicyclobacillus ferrooxydans* TC-34T, *Alicyclobacillus contaminans* 3-A191T and *Alicyclobacillus disulphidoxidans* SD-11T, respectively. Similarity to other species of the genus *Alicyclobacillus* was 90.3-92.8% and similarity to species of the genus *Tumebacillus* was 85.9-87.8%. The genomic DNA G+C content was 53.7 mol%. The predominant menaquinone was MK-7. Major fatty acids were u-cycloheptane C17:0, iso-C17:0 and anteiso-C17:0. The cell-wall peptidoglycan was the A1γ type; containing meso-diaminopimelic acid as the diagnostic diamino acid. On the basis of polyphasic analysis from this study, strain ACK0067 represents a novel species of the genus *Alicyclobacillus* for which the name *Alicyclobacillus tengchongensis* sp. nov. is proposed. The type strain is ACK0067 (=KCTC 33022 =DSM 25924).

**Keywords**: Acidophilic bacterium; *Alicyclobacillus tengchongensis*; Novel species; Taxonomy

**PMID**: 25037879

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**A tree of cellular life inferred from a genomic census of molecular functions**


Kim KM*, Nasir A, Hwang K, Caetano-Anollés G.

*Co-first: Kyung Mo Kim(kmkim@kribb.re.kr)*

Phylogenomics aims to describe evolutionary relatedness between organisms by analyzing genomic data. The common practice is to produce phylogenomic trees from molecular information in the sequence, order, and content of genes in genomes. These phylogenies describe the evolution of life and become valuable tools for taxonomy. The recent availability of structural and functional data for hundreds of genomes now offers the opportunity to study evolution using more deep, conserved, and reliable sets of molecular features. Here, we reconstruct trees of life from the functions of proteins. We start by inferring rooted phylogenomic trees and networks of organisms directly from Gene Ontology annotations. Phylogenies and networks yield novel insights into the emergence and evolution of cellular life. The ancestor of Archaea originated earlier than the ancestors of Bacteria and Eukarya and was thermophile. In contrast, basal bacterial lineages were non-thermophilic. A close relationship between Plants and Metazoa was also identified that disagrees with the traditional Fungi-Metazoa grouping. While measures of evolutionary reticulation were minimum in Eukarya and maximum in Bacteria, the massive role of horizontal gene transfer in microbes did not materialize in phylogenomic networks. Phylogenies and networks also showed that the best reconstructions were recovered when problematic taxa (i.e., parasitic/symbiotic organisms) and horizontally transferred characters were excluded from analysis. Our results indicate that functionomic data represent a useful addition to the set of molecular characters used for tree reconstruction and that trees of cellular life carry in deep branches considerable predictive power to explain the evolution of living organisms.

**Keywords**: Evolution; Functionomic data; Gene ontology; Phylogenomics; Tree of life

**PMID**: 25128982
Fourier transform infrared (FT-IR) spectroscopy of genomic DNA to discriminate F1 progenies from their paternal lineage of Chinese cabbage (Brassica rapa subsp. pekinensis)


Song SY, Jie EY, Ahn MS, Lee IH, Nou IS, Min BW, Kim SW*.
*Co-corresponding: Suk Weon Kim(kimsw@kribb.re.kr)

Fourier transform infrared spectroscopy (FT-IR) spectroscopy in combination with multivariate analysis was used to discriminate two different F1 hybrid lines from their parental inbred lines. Genomic DNA was isolated from leaves of three parental lines and two F1 hybrid lines of Brassica campestris subsp. pekinensis. Purified genomic DNA was analyzed by FT-IR spectroscopy in the spectral region from 4,000 to 400 cm⁻¹. FT-IR spectra confirmed typical spectral differences between the frequency regions of N-H stretching (amide I) and C=O stretching vibrations (amide II) as well as PO₂⁻ ionized asymmetric and symmetric stretching. Principal component analysis was able to discriminate between F1 hybrid progenies depending on their parental lineages, even though they share the same maternal background. Partial least squares discriminant analysis gave a more clear discrimination between the two parental lines and their hybrid progenies. These FT-IR spectral differences might be directly related to subtle changes in the base functional group and backbone structures of genomic DNA. Considering these results, this technique could provide a solid research foundation for FT-IR spectral-based rapid diagnosis, selection, and discrimination of parental lines from their progenies. Furthermore, this technique could be applied to test purity in the hybrid seed industry.

**Keywords**: Chinese cabbage; Discrimination; FT-IR; Genomic DNA; Multivariate analysis; Progeny

Melatonin prevents gentamicin-induced testicular toxicity and oxidative stress in rats

*Andrologia. 46(9):1032-40.

Kim SH, Lee IC, Back HS, Shin IS, Moon C, Kim SH, Yun WK, Nam KH, Kim HC*, Kim JC.
*Co-corresponding: Hyoung-Chin Kim(hckim@kribb.re.kr)

This study investigated the protective effects of melatonin (MT) against gentamicin (GM)-induced testicular toxicity and oxidative damage in rats. GM (100 mg kg⁻¹) was injected intraperitoneally (i.p.) to rats for 6 days. MT (15 mg kg⁻¹) was administered i.p. to rats for 6 days at 1 hr after the GM treatment. GM caused a decrease in prostate and seminal vesicle weights, sperm count and sperm motility. Histopathological examination showed various morphological alterations in the testis, characterised by degeneration of spermatagonia/spermatocytes, decrease in the number of early spermatogenic cells and vacuolisation. In addition, an increased malondialdehyde concentration and decreased glutathione content and glutathione reductase, catalase and glutathione-S-transferase activities were found in the testis. In contrast, MT treatment significantly attenuated the testicular toxicity of GM, including decreased reproductive organ weights, sperm count, and sperm motility and increased histopathological alterations. MT also had an antioxidant benefit by decreasing the lipid peroxidative product malondialdehyde and increasing the level of the antioxidant glutathione and the activities of antioxidant enzymes in the testis. These results indicate that MT prevents testicular toxicity induced by GM in rats, presumably due to its potent antioxidant activity, and its ability to inhibit lipid peroxidation, and restore antioxidant enzyme activity.

**Keywords**: Gentamicin; Melatonin; Oxidative stress; Protection; Testicular toxicity

PMID: 24188423
Role of NADH: quinone oxidoreductase-1 in the tight junctions of colonic epithelial cells


*Co-corresponding: Chul-Ho Lee(chullee@kribb.re.kr)

NADH:quinone oxidoreductase 1 (NQO1) is known to be involved in the regulation of energy synthesis and metabolism, and the functional studies of NQO1 have largely focused on metabolic disorders. Here, we show for the first time that compared to NQO1-WT mice, NQO1-KO mice exhibited a marked increase of permeability and spontaneous inflammation in the gut. In the DSS-induced colitis model, NQO1-KO mice showed more severe inflammatory responses than NQO1-WT mice. Interestingly, the transcript levels of claudin and occludin, the major tight junction molecules of gut epithelial cells, were significantly decreased in NQO1-KO mice. The colons of NQO1-KO mice also showed high levels of reactive oxygen species (ROS) and histone deacetylase (HDAC) activity, which are known to affect transcriptional regulation. Taken together, these novel findings indicate that NQO1 contributes to the barrier function of gut epithelial cells by regulating the transcription of tight junction molecules.

**Keywords**: Epithelial cell; Gut inflammation; NADH:quinone oxidoreductase 1 (NQO1); NQO1 knockout mouse; Tight junction; Transcription

PMID: 24393524

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Pitx3 deficient mice as a genetic animal model of co-morbid depressive disorder and parkinsonism

Brain Res. 1552:72-81.


*Co-corresponding: Kyoung-Shim Kim(kskim@kribb.re.kr)

Approximately 40-50% of all patients with Parkinson’s disease (PD) show symptoms and signs of depressive disorders, for which neither pathogenic understanding nor rational treatment are available. Using Pitx3-deficient mice, a model for selective nigrostriatal dopaminergic neurodegeneration, we tested depression-related behaviors and acute stress responses to better understand how a nigrostriatal dopaminergic deficit increases the prevalence of depressive disorders in PD patients. Pitx3-deficient mice showed decreased sucrose consumption and preference in the two-bottle free-choice test of anhedonia. Acute restraint stress increased c-Fos (known as a neuronal activity marker) expression levels in various brain regions, including the prefrontal cortex, striatum, nucleus accumbens, and paraventricular nucleus of the hypothalamus (PVN), in both Pitx3+/+ and +/- mice. However, the stress-induced increases in c-Fos levels in the cortex, dorsal striatum, and PVN were significantly greater in Pitx3-/- than +/- mice, suggesting that signs of depressive disorders in parkinsonism are related to altered stress vulnerability. Based on these results, we propose that Pitx3-/- mice may serve as a useful genetic animal model for co-morbid depressive disorder and parkinsonism.

**Keywords**: Depression; Parkinson’s disease (PD); Pitx3 mice; Stress vulnerability; c-Fos

PMID: 24480473
Mechanism for the protective effect of diallyl disulfide against cyclophosphamide acute urotoxicity in rats

Kim SH, Lee IC, Baek HS, Shin IS, Moon C, Bae CS, Kim SH, Kim JC, Kim HC.

*Corresponding: Hyoung-Chin Kim(hckim@kribb.re.kr)

This study investigated the protective effects of diallyl disulfide (DADS) against cyclophosphamide (CP)-induced acute urotoxicity in rats. CP caused severe hemorrhagic cystitis as shown by significant increases in bladder weight, edema, and hemorrhage as well as increased urinary bladder epithelial cell apoptosis, protein expression of nuclear factor erythroid 2-related factor-2 (Nrf-2) and phase II enzymes (i.e., NAD(P)H: quinone oxidoreductase-1 (NQO-1) and heme oxygenase-1 (HO-1)), immunostaining intensity of acrolein-protein adducts, and histopathological changes. The significant decreases in glutathione content and catalase, glutathione-S-transferase, and glutathione reductase activities and a significant increase in malondialdehyde content indicated that CP-induced bladder injury was mediated through oxidative stress. In contrast, pretreatment with DADS significantly attenuated the CP-induced urotoxic effects, including oxidative damage, histopathological lesions, apoptotic changes, and accumulation of acrolein-protein adducts in the bladder. DADS also significantly increased expression of CYP2B1/2, CYP3A1, Nrf-2, NQO-1, and HO-1 and significantly decreased expression of CYP2C11. These results indicate that DADS prevented CP-induced bladder toxicity, in part, by detoxifying acrolein. The protective effects of DADS may be due to its ability to decrease metabolic activation of CP by inhibiting CYP2C11 and inducing CYP3A1, and its potent antioxidant activity and antiapoptotic effects occurred via the Nrf-2-antioxidant response element pathway.

**Keywords**: Cyclophosphamide; Cytochrome P450; Diallyl disulfide; Nrf-2; Oxidative stress; Urotoxicity

PMID: 24291451

Protection of NAD(P)H: Quinone oxidoreductase 1 against renal ischemia/reperfusion injury in mice


*Corresponding: Chul-Ho Lee(chullee@kribb.re.kr)

Ischemia/reperfusion (I/R) is the most common cause of acute renal injury. I/R-induced reactive oxygen species (ROS) are thought to be a major factor in the development of acute renal injury by promoting the initial tubular damage. NAD(P)H: quinone oxidoreductase 1 (NQO1) is a well-known antioxidant protein that regulates ROS generation. The purpose of this study was to investigate whether NQO1 modulates the renal I/R injury (IRI) associated with NADPH oxidase (NOX)-derived ROS production in an animal model. We analyzed renal function, oxidative stress, and tubular apoptosis after IRI. NQO1-/- mice showed increased blood urea nitrogen and creatinine levels, tubular damage, oxidative stress, and apoptosis. In the kidneys of NQO1-/- mice, the cellular NADPH/NADP+ ratio was significantly higher and NOX activity was markedly higher than in those of NQO1+/+ mice. The activation of NQO1 by β-lapachone (βL) significantly improved renal dysfunction and reduced tubular cell damage, oxidative stress, and apoptosis by renal I/R. Moreover, the βL treatment significantly lowered the cellular NADPH/NADP+ ratio and dramatically reduced NOX activity in the kidneys after IRI. From these results, it was concluded that NQO1 has a protective role against renal injury induced by I/R and that this effect appears to be mediated by decreased NOX activity via cellular NADPH/NADP+ modulation. These results provide convincing evidence that NQO1 activation might be beneficial for ameliorating renal injury induced by I/R.

**Keywords**: Ameliorating renal injury; β-L-Lapachone; Free radical; Ischemia/reperfusion injury; NADPH oxidase; NQO1

PMID: 24189322
Opposing effects of prednisolone treatment on T/NKT cell- and hepatotoxin-mediated hepatitis in mice

Hepatology. 59(3):1094-106.

Kwon HJ, Won YS*, Park O, Feng D, Gao B. *Co-first: Young-Suk Won(yswon@kribb.re.kr)

Prednisolone is a corticosteroid that has been used to treat inflammatory liver diseases such as autoimmune hepatitis and alcoholic hepatitis. However, the results have been controversial, and how prednisolone affects liver disease progression remains unknown. In the current study we examined the effect of prednisolone treatment on several models of liver injury, including T/NKT cell hepatitis induced by concanavalin A (ConA) and α-galactosylceramide (α-GalCer), and hepatotoxin-mediated hepatitis induced by carbon tetrachloride (CCL4) and/or ethanol. Prednisolone administration attenuated ConA- and α-GalCer-induced hepatitis and systemic inflammatory responses. Treating mice with prednisolone also suppressed inflammatory responses in a model of hepatotoxic (CCL4)-induced hepatitis, but surprisingly exacerbated liver injury and delayed liver repair. In addition, administration of prednisolone also enhanced acetaminophen-, ethanol-, or ethanol plus CCL4-induced liver injury. Immunohistochemical and flow cytometric analyses demonstrated that prednisolone treatment inhibited hepatic macrophage and neutrophil infiltration in CCL4-induced hepatitis and suppressed their phagocytic activities in vivo and in vitro. Macrophage and/or neutrophil depletion aggravated CCL4-induced liver injury and impeded liver regeneration. Finally, conditional disruption of glucocorticoid receptor in macrophages and neutrophils abolished prednisolone-mediated exacerbation of hepatotoxin-induced liver injury.

CONCLUSION: Prednisolone treatment prevents T/NKT cell hepatitis but exacerbates hepatotoxin-induced liver injury by inhibiting macrophage- and neutrophil-mediated phagocytic and hepatic regenerative functions. These findings may not only increase our understanding of the steroid treatment mechanism but also help us to better manage steroid therapy in liver diseases.

**Keywords**: Cell hepatitis; Inflammatory liver disease; Prednisolone; Steroid therapy

PMID: 24115096

Aldehyde dehydrogenase 2 deficiency ameliorates alcoholic fatty liver but worsens liver inflammation and fibrosis in mice

Hepatology. 60(1):146-57.


Aldehyde dehydrogenase 2 (ALDH2) is the major enzyme that metabolizes acetaldehyde produced from alcohol metabolism. Approximately 40-50% of East Asians carry an inactive ALDH2 gene and exhibit acetaldehyde accumulation after alcohol consumption. However, the role of ALDH2 deficiency in the pathogenesis of alcoholic liver injury remains obscure. In the present study, wild-type and ALDH2−/− mice were subjected to ethanol feeding and/or carbon tetrachloride (CCL4) treatment, and liver injury was assessed. Compared with wild-type mice, ethanol-fed ALDH2−/− mice had higher levels of malondialdehyde-acetaldehyde (MAA) adduct and greater hepatic inflammation, with higher hepatic interleukin (IL)-6 expression but surprisingly lower levels of steatosis and serum alanine aminotransferase (ALT). Higher IL-6 levels were also detected in ethanol-treated precision-cut liver slices from ALDH2−/− mice and in Kupffer cells isolated from ethanol-fed ALDH2−/− mice than those levels in wild-type mice. In vitro incubation with MAA enhanced the lipopolysaccharide (LPS)-mediated stimulation of IL-6 production in Kupffer cells. In agreement with these findings, hepatic activation of the major IL-6 downstream signaling molecule signal transducer and activator of transcription 3 (STAT3) was higher in ethanol-fed ALDH2−/− mice than in wild-type mice. An additional deletion of hepatic STAT3 increased steatosis and hepatocellular damage in ALDH2−/− mice. Finally, ethanol-fed ALDH2−/− mice were more prone to CCL4-induced liver inflammation and fibrosis than ethanol-fed wild-type mice.

CONCLUSION: ALDH2−/− mice are resistant to ethanol-induced steatosis but prone to inflammation and fibrosis by way of MAA-mediated paracrine activation of IL-6 in Kupffer cells. These findings suggest that alcohol, by way of acetaldehyde and its associated adducts, stimulates hepatic inflammation and fibrosis independent from causing hepatocyte death, and that ALDH2-deficient individuals may be resistant to steatosis and blood ALT elevation, but are prone to liver inflammation and fibrosis following alcohol consumption.

**Keywords**: Alcoholic liver; Aldehyde dehydrogenase 2 (ALDH2); ALDH2 deficiency; Hepatic inflammation

PMID: 24492981
Enhanced activation of NAD(P)H: quinone oxidoreductase 1 attenuates spontaneous hypertension by improvement of endothelial nitric oxide synthase coupling via tumor suppressor kinase liver kinase B1/adenosine 5’-monophosphate-activated protein kinase-mediated guanosine 5’-triphosphate cyclohydrolase 1 preservation


Kim YH, Hwang JH, Kim KS, Noh JR, Gang GT, Oh WK, Jeong KH, Kwak TH, Choi HS, Lee IK, Lee CH. *Corresponding: Chul-Ho Leetchullee@kribb.re.kr

AIMS: Guanosine 5’-triphosphate cyclohydrolase-1 (GTPCH-1) is a rate-limiting enzyme in de-novo synthesis of tetrahydrobiopterin (BH4), an essential cofactor for endothelial nitric oxide synthase (eNOS) coupling. Adenosine 5’-monophosphate-activated protein kinase (AMPK) is crucial for GTPCH-1 preservation, and tumor suppressor kinase liver kinase B1 (LKB1), an upstream kinase of AMPK, is activated by NAD+ dependent class III histone deacetylase sirtuin 1 (SIRT1)-mediated deacetylation. β-Lapachone has been shown to increase cellular NAD+/NADH ratio via NAD(P)H: quinone oxidoreductase 1 (NQO1) activation. In this study, we have evaluated whether β-lapachone-induced NQO1 activation modulates blood pressure (BP) through preservation of GTPCH-1 in a hypertensive animal model.

METHODS AND RESULTS: Spontaneously hypertensive rats (SHRs), primary aortic endothelial cells, and endothelial cell line were used to investigate the hypotensive effect of β-lapachone and its action mechanism. β-Lapachone treatment dramatically lowered BP and vascular tension in SHRs and induced eNOS activation in endothelial cells. Consistent with these effects, β-lapachone treatment also elevated levels of both aortic cGMP and plasma nitric oxide in SHRs. Meanwhile, β-lapachone-treated SHRs showed significantly increased levels of aortic NAD+, LKB1 deacetylation, and AMPK Thr172 phosphorylation followed by increased GTPCH-1 and tetrahydrobiopterin/dihydrobiopterin ratio. In vitro study revealed that AMPK inhibition by overexpression of dominant-negative AMPK nearly abolished GTPCH-1 protein conservation. Enhanced LKB1 deacetylation and AMPK activation were also elicited by β-lapachone in endothelial cells. However, inhibition of LKB1 deacetylation by blocking of NQO1 or SIRT1 blunted AMPK activation by β-lapachone.

CONCLUSION: This is the first study demonstrating that eNOS coupling can be regulated by NQO1 activation via LKB1/AMPK/GTPCH-1 modulation, which is possibly correlated with relieving hypertension. These findings provide strong evidence to suggest that NQO1 might be a new therapeutic target for hypertension.

Keywords: AMPK; Endothelial nitric oxide synthase; Hypertension; NAD(P)H: quinone oxidoreductase 1; NQO1 activation
PMID: 24241058

Inhibition of adenylyl cyclase type 5 prevents L-DOPA-induced dyskinesia in an animal model of Parkinson’s disease


Park HY, Kang YM, Kang Y, Park TS, Ryu YK, Hwang JH, Kim YH, Chung BH, Nam KH, Kim MR, Lee CH, Han PL, Kim KS. *Corresponding: Kyoung-Shim Kim(kskim@kribb.re.kr)

The dopamine precursor 3,4-dihydroxyphenylalanine (l-DOPA) is widely used as a therapeutic choice for the treatment of patients with Parkinson’s disease. However, the long-term use of l-DOPA leads to the development of debilitating involuntary movements, called L-DOPA-induced dyskinesia (LID). The cAMP/protein kinase A (PKA) signaling in the striatum is known to play a role in LID. However, from among the nine known adenylyl cyclases (ACs) present in the striatum, the AC that mediates LID remains unknown. To address this issue, we prepared an animal model with unilateral 6-hydroxydopamine lesions in the substantia nigra in wild-type and AC5-knock-out (KO) mice, and examined behavioral responses to short-term or long-term treatment with l-DOPA. Compared with the behavioral responses of wild-type mice, LID was profoundly reduced in AC5-KO mice. The behavioral protection of long-term treatment with l-DOPA in AC5-KO mice was preceded by a decrease in the phosphorylation levels of PKA substrates ERK (extracellular signal-regulated kinase) 1/2, MSK1 (mitogen- and stress-activated protein kinase 1), and histone H3, levels of which were all increased in the lesioned striatum of wild-type mice. Consistently, FosB/DeltaFosB expression, which was induced by long-term l-DOPA treatment in the lesioned striatum, was also decreased in AC5-KO mice. Moreover, suppression of AC5 in the dorsal striatum with lentivirus-shRNA-AC5 was sufficient to attenuate LID, suggesting that the AC5-regulated signaling cascade in the striatum mediates LID. These results identify the AC5/cAMP system in the dorsal striatum as a therapeutic target for the treatment of LID in patients with Parkinson’s disease.

Keywords: Adenylyl cyclase; Dyskinesia; l-DOPA; LID; Parkinson’s disease (PD)
PMID: 25164669
**In vivo** imaging of islet transplantation using PLGA nanoparticles containing iron oxide and indocyanine green


'Corresponding: Chul-Ho Lee(chullee@kribb.re.kr)

PURPOSE: We determined whether poly(lactic-co-glycolic acid) nanoparticles would be a useful reagent for the successful monitoring of isolated islets by magnetic resonance imaging and optical imaging systems, without clinically relevant toxicity in *vitro* or in *vivo*.

METHODS: We used iron oxide for MR imaging and a cyanide dye approved by the Food and Drug Administration (indocyanine green) for optical imaging and estimated the *in vivo* detection of transplanted pancreatic islets.

RESULTS: The poly(lactic-co-glycolic acid) nanoparticles were associated with the islets *in vitro* and were successfully detected by 4.7 T (MR) and optical imaging, without other toxic effects. When labeled islets were transplanted under the mouse kidney capsule, *in vivo* *T*1/*T*2*-weighted scans with 4.7 T MR detected as few as 300 labeled islets by 4 weeks. Optical *in vivo* imaging revealed indocyanine green fluorescence by 2 and 4 days after transplantation of islets containing 250 and 500 µg/mL poly(lactic-co-glycolic acid) nanoparticles, respectively. These results were further supported by the immunohistochemical results for insulin and iron in the recipient mouse kidney and pancreas.

CONCLUSIONS: Taken together, these data indicate that poly(lactic-co-glycolic acid) nanoparticles may be used to label transplanted islets and may be imaged with *in vivo* MR and optical imaging systems.

**Keywords**: Diabetes; Magnetic resonance imaging; Nanoparticle; Transplanted islet

PMID: 23640738

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Ameliorative effects of pine bark extract on spermatotoxicity by α-chlorohydrin in rats


Kim SH, Lee IC, Baek HS, Moon C, Bae CS, Kim SH, Park SC, Kim HC*, Kim JC.

*Corresponding: Hyoung-Chin Kim(hckim@kribb.re.kr)

We investigated the protective effects of pine bark extract (Pycnogenol®, PYC, Horphag Research Ltd., Route de Belis, France) against α-chlorohydrin (ACH)-induced spermatotoxicity in rats. Rats were orally administered ACH (30 mg/kg/day) with or without PYC (20 mg/kg/day) for 7 days. Administration of ACH significantly decreased sperm motility. α-Chlorohydrin also caused histopathological alterations and apoptotic changes in caput epididymides. An increased malondialdehyde concentration and decreased glutathione content, as well as catalase and glutathione peroxidase activities were also found. In contrast, PYC treatment significantly prevented ACH-induced spermatotoxicity, including decreased sperm motility, histopathological lesions, and apoptotic changes in the caput epididymis. Pycnogenol® also had an antioxidant benefit by decreasing malondialdehyde and increasing levels of the antioxidant glutathione and the activities of the antioxidant enzymes catalase and peroxidase in epididymal tissues. These results indicate that PYC treatment attenuated ACH-induced spermatotoxicity through antioxidant and antiapoptotic effects.

**Keywords**: α-chlorohydrin; Oxidative stress; Protective effect; Pycnogenol®; Spermatotoxicity

PMID: 23788506
Two new combinations in the genus Chionanthus L. (Oleaceae)


Quang BH, Choudhary RK, Chinh VT, Bach TT, Anh TTP, Lee J. *Corresponding: Joongku Lee(joongku@kribb.re.kr)

The genus Chionanthus L. is distributed in tropical and subtropical regions of America, Africa, Asia and Australia, and is represented by 80 species (Mabberley, 2008). Indeed, the species number of this genus increased considerably by the merger of the genus Linociera Swartz, based on the survey of morphological and palynological characters by Stearn (1976). Later, Chang et al. (1996) in Flora of China accepted the reduction of Linociera with Chionanthus although the former are deciduous and found in temperate Asia and North America and the later are evergreen and pantropical.

In Vietnam, the genus Chionanthus s.l. is represented by 15 species (Ho, 2000; Ly, 2003). Though most of the species described under the genus Linociera were found to be transferred to Chionanthus, two species Linociera robinsonii Gagnep. and L. subcapitata Merr. described in the year 1933 and 1942 respectively from Indo-China (Vietnam) are yet to be transferred formally, which necessitates the following new combinations.

**Keywords**: Chionanthus; New combination; Oleaceae; Vietnam

Tussilago farfara L. augments TRAIL-induced apoptosis through MKK7/JNK activation by inhibition of MKK7-TIPRL in human hepatocellular carcinoma cells


Lee HJ, Cho HS, Jun SY, Lee JJ, Yoon JY, Lee JH, Song HH, Choi SH, Kim SY, Saloura V, Park CG, Kim NS. *Corresponding: Nam-Soon Kim(nskim37@kribb.re.kr)

Induction of apoptosis through activation of the TRAIL pathway is considered to be a promising anticancer strategy due to its ability to selectively induce apoptosis in cancer cells. However, the ability of cancer cells to acquire TRAIL resistance has limited the clinical translation of this approach. We previously reported that the TOR signaling pathway regulator-like (TIPRL) protein contributes to the resistance to TRAIL-induced apoptosis by inhibiting the MKK7-c-Jun N-terminal kinase (JNK) pathway via MKK7-TIPRL interaction. In the present study, we identified Tussilago farfara L. (TF) as a novel TRAIL sensitizer among 500 natural products using an ELISA system that specifically detects the MKK7-TIPRL interaction, and we validated candidates by GST-pull down assay. Co-treatment of Huh7 cells with TF and TRAIL induced apoptosis via inhibition of the MKK7-TIPRL interaction and an increase in MKK7/JNK phosphorylation. This is the first report to describe TF as a novel TRAIL sensitizer, unveiling a potentially novel therapeutic strategy in cancer therapy.

**Keywords**: Apoptosis; HCC; MKK7; TRAIL resistance; TIPRL; Tussilago farfara

PMID: 24969837
Differential expression pattern of gangliosides during the differentiation of human dental pulp-derived mesenchymal stem cells into dopaminergic neural-like cells


Lee SH, Kwak DH, Ryu JS, Lim MU, Kim JS, Kim SU, Chang KT, Choo YK.

'Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr)

Human dental pulp-derived stem cells (hDPSCs) have been considered as alternative sources of adult stem cells because of their potential to differentiate into multiple cell lineages. Gangliosides, which sialic acid-containing glycosphingolipids are abundantly expressed in the plasma membrane and which play an important role in various processes, such as proliferation and differentiation. This study investigated the possible role of gangliosides in dopaminergic neural differentiation. When hDPSCs were cultured under dopaminergic neural differentiation conditions, the expression of dopaminergic neural cell markers genes such as TH, Pitx-3, Nurr-1, and DAT were detected. Immunofluorescence analysis showed that ganglioside biosynthesis was associated with the dopaminergic neural differentiation of hDPSCs. Specifically, GD3 and GT1b were expressed during dopaminergic neural differentiation. These results suggest that gangliosides may play a role in the dopaminergic neural differentiation process of hDPSCs.

**Keywords**: Adult stem cell; Dopaminergic neural differentiation; Gangliosides; Human dental pulp-derived stem cells (hDPSCs); Markers gene

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Production of cloned mice by aggregation of tetraploid embryo


Sim BW, Min KS.

'First: Bo-Woong Sim(embryont@kribb.re.kr)

Mouse chimeras can also successfully be produced using tetraploid host embryos. This study was conducted to optimize the efficiency of cloning and to produce cloned mice using tetraploid host embryos. Six hours of activation with strontium (SrCl2) was optimal for somatic cell nuclear transfer (SCNT) embryos. Cytochalasin B (CB) concentration (5 µg/ml) during enucleation was evaluated in the efficiency of implantation sites and fetus offspring. Continuous exposure to 5–50 nM trichostatin A (TSA), a histone-deacetylase inhibitor (HDACi), for 10 h is recommended for production of clone mice. Aggregated SCNTs were constructed by aggregation of SCNT embryos with tetraploid embryos to reduce epigenetic errors in the placenta. The pregnancy and implantation rates of aggregated SCNT were significantly higher than those of SCNT alone. The full-term developmental rate of aggregated embryos was also higher than that of SCNT (3.57 vs. 1.16). The placental weight of SCNT clones was significantly higher than that from in vitro fertilization (IVF). However, the placenta weight of aggregated SCNT clones was nearly the same as that of embryos in the IVF group. The placentas of SCNT-only clones appeared to have the hyperplastic histology typical of mouse clones. We produced a total of 36 clone mice, including nine heads derived from aggregated SCNT. One-half of clones derived from aggregated SCNT survived to adulthood, and 14-clones derived from SCNT grew into healthy adults. The aggregated SCNT method was useful for significantly reducing the placental weight of cloned mice and improving the efficiency of SCNT.

**Keywords**: Aggregation; Cloning; Mouse chimera; Placenta; Tetraploid host embryos
Characterization of pig sperm hyaluronidase and improvement of the digestibility of cumulus cell mass by recombinant pSPAM1 hyaluronidase in an in vitro fertilization assay


Although sperm hyaluronidase is thought to play an important role in mammalian fertilization, the molecular function underlying these steps remains largely unknown. In mouse models, sperm-specific SPAM1 and HYAL5 hyaluronidase are believed to function in both sperm penetration of the cumulus matrix and sperm-ZP binding. However, gene-targeting studies for SPAM1 or HYAL5 show that hyaluronidases are not essential for fertilization, despite the fact that exogenous hyaluronidase can disrupt the cumulus matrix. Therefore, to evaluate whether sperm hyaluronidase is essential for mammalian fertilization, it is necessary to generate HYAL5/SPAM1 double-knockout mice. However, generating double-knockout mice is very difficult because these two genes exist on the same chromosome. Recently, investigators have begun to employ the pig model system to study human disease due to its similarities to human anatomy and physiology. In this study, we confirmed that pig SPAM1 exists as a single copy gene on chromosome 18 and is specifically expressed in the testis. In addition, we expressed recombinant pig SPAM1 in human embryonic kidney 293 cells and showed that these enzymes possess hyaluronidase activity. We also demonstrated that a polyclonal antibody against pig sperm hyaluronidase inhibits sperm-egg interactions in an in vitro fertilization (IVF) assay. Our results suggest that pig SPAM1 may play a critical role in pig fertilization and that recombinant SPAM1 can disperse the oocyte-cumulus complex in an IVF assay.

Keywords: Cumulus-oocyte complex; Fertilization; Pig SPAM1; Recombinant protein; Sperm hyaluronidase; Sperm-egg interaction

PMID: 25261076

Developmental competence of bovine early embryos depends on the coupled response between oxidative and endoplasmic reticulum stress


Yoon SB, Choi SA, Sim BW, Kim JS, Mun SE, Jeong PS, Yang HJ, Lee Y, Park YH, Song BS, Kim YH, Jeong KJ, Huh JW, Lee SR, Kim SU, Chang KT. "Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr), Sun-Uk Kim(sunik@kribb.re.kr)

The stress produced by the coupling of reactive oxygen species (ROS) and endoplasmic reticulum (ER) has been explored extensively, but little is known regarding their roles in the early development of mammalian embryos. Here, we demonstrated that the early development of in vitro-produced (IVP) bovine embryos was governed by the cooperative action between ROS and ER stress. Compared with the tension produced by 5% O2 20% O2 significantly decreased the blastocyst formation rate and cell survival, which was accompanied by increases in ROS and in levels of sXBP-1 transcript, which is an ER stress indicator. In addition, treatment with glutathione (GSH), a ROS scavenger, decreased ROS levels, which resulted in increased blastocyst formation and cell survival rates. Importantly, levels of sXBP-1 and ER stress-associated transcripts were reduced by GSH treatment in developing bovine embryos. Consistent with this observation, tauroursodeoxycholate (TUDCA), an ER stress inhibitor, improved blastocyst developmental rate, trophectoderm proportion, and cell survival. Moreover, ROS and sXBP-1 transcript levels were markedly decreased by supplementation with TUDCA, suggesting a possible mechanism governing the mutual regulation between ROS and ER stress. Interestingly, knockdown of XBP-1 transcripts resulted in both elevation of ROS and decrease of antioxidant transcripts, which ultimately reduced in vitro developmental competence of bovine embryos. Based on these results, in vitro developmental competence of IVP bovine embryos was highly dependent on the coupled response between oxidative and ER stresses. These results increase our understanding of the mechanism(s) governing early embryonic development and may improve strategies for the generation of IVP embryos with high developmental competence.

Keywords: Early development; Embryo culture; Endoplasmic reticulum (ER) stress; Reactive oxygen species (ROS)

PMID: 24695629
Inflammation has a pivotal role in the pathogenesis of ischemic stroke, and recent studies posit that inflammation acts as a double-edged sword, not only detrimentally augmenting secondary injury, but also potentially promoting recovery. An initial event of inflammation in ischemic stroke is the activation of microglia, leading to production of both pro- and anti-inflammatory mediators acting through multiple receptor signaling pathways. In this review, we discuss the role of microglial mediators in acute ischemic stroke and elaborate on preclinical and clinical studies focused on microglia in stroke models. Understanding how microglia can lead to both pro- and anti-inflammatory responses may be essential to implement therapeutic strategies using immunomodulatory interventions in ischemic stroke.

Keywords: Acute ischemic stroke; Immunomodulatory intervention; Inflammation; Microglia; Targeting neuroinflammation

PMID: 25089266

Alzheimer's disease and stem cell therapy

Exp Neurol. 23(1):45-52.

Choi SS, Lee SR, Kim SU, Lee HJ.
*Co-first: Sang-Rae Lee(srlee@kribb.re.kr)

The loss of neuronal cells in the central nervous system may occur in many neurodegenerative diseases. Alzheimer's disease is a common senile disease in people over 65 years, and it causes impairment characterized by the decline of mental function, including memory loss and cognitive impairment, and affects the quality of life of patients. However, the current therapeutic strategies against AD are only to relieve symptoms, but not to cure it. Because there are only a few therapeutic strategies against Alzheimer's disease, we need to understand the pathogenesis of this disease. Cell therapy may be a powerful tool for the treatment of Alzheimer's disease. This review will discuss the characteristics of Alzheimer's disease and various available therapeutic strategies.

Keywords: Alzheimer's disease (AD); Cell therapy; Neurodegenerative disease; Stem cell-gene therapy; Transplantation

PMID: 24737939
Beneficial effects of melatonin combined with exercise on endogenous neural stem/progenitor cells proliferation after spinal cord injury


Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr)

Endogenous neural stem/progenitor cells (eNSPCs) proliferate and differentiate into neurons and glial cells after spinal cord injury (SCI). We have previously shown that melatonin (MT) plus exercise (Ex) had a synergistic effect on functional recovery after SCI. Thus, we hypothesized that combined therapy including melatonin and exercise might exert a beneficial effect on eNSPCs after SCI. Melatonin was administered twice a day and exercise was performed on a treadmill for 15 min, six days per week for 3 weeks after SCI. Immunohistochemistry and RT-PCR analysis were used to determine cell population for late response, in conjunction with histological examination and motor function test. There was marked improvement in hindlimb function in SCI+MT+Ex group at day 14 and 21 after injury, as documented by the reduced size of the spinal lesion and a higher density of dendritic spines and axons; such functional improvements were associated with increased numbers of BrdU-positive cells. Furthermore, MAP2 was increased in the injured thoracic segment, while GFAP was increased in the cervical segment, along with elevated numbers of BrdU-positive nestin-expressing eNSPCs in the SCI+MT+Ex group. The dendritic spine density was augmented markedly in SCI+MT and SCI+MT+Ex groups. These results suggest a synergistic effect of SCI+MT+Ex might create a microenvironment to facilitate proliferation of eNSPCs to effectively replace injured cells and to improve regeneration in SCI.

**Keywords**: Endogenous neural stem/progenitor (eNSPCs); Functional recovery; Melatonin; Spinal cord injury; Therapeutic exercise

PMID: 24487506

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Insulin/IGF signaling-related gene expression in the brain of a sporadic Alzheimer's disease monkey model induced by intracerebroventricular injection of streptozotocin


Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr), Sang-Rae Lee(srlee@kribb.re.kr)

We reported previously that the intracerebroventricular streptozotocin (icv-STZ)-treated cynomologus monkey showed regionally specific glucose hypometabolism in FDG-PET imaging, similar to that observed in the early stages of sporadic Alzheimer's disease (sAD). However, further pathological analyses of this model at the molecular level are needed to validate it as a feasible model for sAD. Two cynomologus monkeys were injected with 2 mg/kg STZ into the cerebellomedullary cistern at day 1, 7 and 14. Two control monkeys were given normal saline. At 5 months after injection, the expression levels of genes encoding 9 upstream molecules in insulin/insulin-like growth factor (IGF) signaling and markers for 4 cell-type populations in the frontal cortex, hippocampus, posterior cingulate, precuneus, and occipital cortex of control and icv-STZ treated cynomologus monkeys were examined. Real-time quantitative PCR analyses demonstrated that the overall mRNA expression of insulin/IGF signaling-related genes was mainly impaired in the anterior part of the cerebrum, frontal cortex, and hippocampus, similar to the early stage of sAD. The changes were accompanied by the loss of oligodendrocytes and neurons. The posterior part of the cerebrum did not show degenerative alterations. The present study provides important fundamental information on the icv-STZ monkey model for sAD. These results may help guide future studies using this model for the investigation of pathological mechanisms and the development of drugs for sAD.

**Keywords**: Alzheimer's disease; Brain; Cynomologus monkey; IGF; sAD; Streptozotocin

PMID: 23948941
Melatonin treatment combined with treadmill exercise accelerates muscular adaptation through early inhibition of CHOP-mediated autophagy in the gastrocnemius of rats with intra-articular collagenase-induced knee laxity


*Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr)*

The purpose of this study was to determine the effects of melatonin intervention on gastrocnemius remodeling in rats with collagenase-induced knee instability. Type VII collagenase was injected into the right knee to induce joint laxity with cartilage destruction. Melatonin (MT; 10 mg/kg) injection was performed twice daily subcutaneously, and treadmill exercise (Ex; 11 m/min) was conducted for 1 hr/day at a frequency of 5 days/wk for 4 wks. The gastrocnemius mass, which was reduced with collagenase injection only (Veh), was increased with collagenase injection with melatonin treatment with and without exercise in the early phase, and the mass in both limbs was significantly different in the Veh compared with the MT group. However, there was an increase in the relative muscle weight to body weight ratio in the Veh group at the advanced stage. Insulin-like growth factor receptor (IGF-IR) was downregulated in the Veh group, whereas IGF-IR was upregulated in the MT and MT + Ex groups. Joint laxity induced enhancement of autophagic proteolysis (LC3 II) in the muscle, which was recovered to values similar to those in the normal control group (Con) compared with those in the MT and MT + Ex groups. Although intra-articular collagenase increased the total C/EBP homology protein (CHOP) levels at 1 wk and decreased them at 4 wks in the Veh group, CHOP in the nucleus was upregulated continuously. Prolonged melatonin treatment with and without exercise intervention suppressed nuclear localization of ATF4 and CHOP with less activation of caspase-3, at the advanced phase. Moreover, the interventions promoted the expression of myosin heavy chain (MHC) isoforms under the control of myogenin. Concomitant with a beneficial effect of melatonin with and without exercise, step length of the saline-injected limb and the collagenase-injected supporting side was maintained at values similar to those in control rats. Taken together, the findings demonstrate that melatonin with and without exercise accelerates remodeling of the gastrocnemius through inhibition of nuclear CHOP in rats with collagenase-induced knee instability.

**Keywords**: CHOP; Knee joint instability; Melatonin; Muscle remodeling; Treadmill exercise

PMID: 24313305

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Melatonin treatment induces interplay of apoptosis, autophagy, and senescence in human colorectal cancer cells


Hong Y, Won J, Lee Y, Lee S, Park K, Chang KT*, Hong Y.
*Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr)*

In Asia, the incidence of colorectal cancer has been increasing gradually due to a more Westernized lifestyle. The aim of this study was to determine the interaction between melatonin-induced cell death and cellular senescence. We treated HCT116 human colorectal adenocarcinoma cells with 10 μM melatonin and determined the levels of cell death-related proteins and evaluated cell cycle kinetics. The plasma membrane melatonin receptor, MT1, was significantly decreased by melatonin in a time-dependent manner, whereas the nuclear receptor, RORγ, was increased only after 12 hr treatment. HCT116 cells, which upregulated both pro-apoptotic Bax and anti-apoptotic Bcl-xL in the early response to melatonin treatment, activated autophagic as well as apoptotic machinery within 18 hr. Melatonin decreased the S-phase population of the cells to 57% of the control at 48 hr, which was concomitant with a reduction in BrdU-positive cells in the melatonin-treated cell population. We found not only marked attenuation of E- and A-type cyclins, but also increased expression of p16 and p-p21. Compared to the cardiotoxicity of Trichostatin A in vitro, single or cumulative melatonin treatment induced insignificant detrimental effects on neonatal cardiomyocytes. We found that 10 μM melatonin activated cell death programs early and induced G1-phase arrest at the advanced phase. Therefore, we suggest that melatonin is a potential chemotherapeutic agent for treatment of colon cancer, the effects of which are mediated by regulation of both cell death and senescence in cancerous cells with minimized cardiotoxicity.

**Keywords**: Apoptosis; Autophagy; Cardiotoxicity; Melatonin; Senescence

PMID: 24484372
Salutary effects of melatonin combined with treadmill exercise on cartilage damage


*Co-corresponding: Kyung-Tae Chang(changkt@kribb.re.kr)

Osteoarthritis (OA) is a major cause of disability in the adult population. The purpose of this study was to evaluate the effects of melatonin with graded dosage on extracellular matrix synthesis and cellular death in response to cartilage damage in vitro and in vivo. TNF-α reduced the viability of primary cultured chondrocytes and extracellular matrix protein, but melatonin at concentrations of 1 μm and 1 nm restored them. Rats with knee instability induced by intra-articular collagenase were used for the in vivo study. Joint parameters were significantly augmented in the collagenase injection-only group but not in the melatonin-alone or melatonin-exercise groups, as cartilage degeneration progressed. Serum TNF-α and IL-6 were upregulated by collagenase injection, which was attenuated by melatonin with and without exercise in the early phase. TGF-β1 mRNA was either conserved or enhanced by melatonin with and without exercise at the early phase. In particular, melatonin combined with exercise dramatically decreased the expression of not only catabolic mediators but also cellular death markers with lower mineralization. At the advanced phase, prolonged melatonin treatment promoted mineralization through caspase-3-mediated chondrocyte apoptosis. However, co-intervention induced extracellular matrix remodeling through increases in IL-6, EPAS-1, and MMP-13. Reconstructed micro-CT images showed that collagenase injection induced subchondral bone erosion, formation of paramesenchymal osteophytes, and reduction of trabecular bone thickness regardless of the intervention, which was minimized by combined intervention. In conclusion, we suggest that melatonin with treadmill exercise may have both preventive and synergistic effects on rescue from cartilage degeneration and is more effective in the initial phase.

**Keywords:** Cartilage degeneration; Exercise; Melatonin; MMP-13; Type II collagen

PMID: 24816289

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A cathepsin B inhibitor, E-64, improves the preimplantation development of bovine somatic cell nuclear transfer embryos


Min SH, Song BS*, Yeon JY, Kim JW, Bae JH, Park SY, Lee YH, Chang KT, Koo DB.
*Co-first: Bong-Seok Song(sbs6401@kribb.re.kr)

Bovine somatic cell nuclear transfer (SCNT) is an important and powerful tool for basic research and biomedical and agricultural applications, however, the efficiency of SCNT has remained extremely low. In this study, we investigated the effects of cathepsin B inhibitor (E-64) supplementation of culture medium on in vitro development of bovine SCNT embryos. We initially used three concentrations of E-64 (0.1, 0.5, 1.0 μm), among which 0.5 μm resulted in the highest rate of blastocysts production after in vitro fertilization (IVF), and was therefore used for further experiments. Blastocyst development of SCNT embryos in the E-64 treatment group also increased relative to the control. Moreover, the cryosurvival rates of IVF and SCNT blastocysts were increased in E-64 treatment groups when compared with the control. On the other hand, we found that IVF and SCNT blastocysts derived from E-64-treated groups had increased total cell numbers and decreased apoptotic nuclei. Furthermore, assessment of the expression of apoptosis-related genes (Bax and Bcl-xL) in bovine IVF and SCNT blastocysts treated with E-64 by real-time RT-PCR analysis revealed suppressed expression of the pro-apoptotic gene Bax and stimulated expression of the anti-apoptotic gene Bcl-xL. Taken together, these finding indicate that addition of E-64 to embryo culture medium may have important implications for improving developmental competence and preimplantation quality in bovine IVF and SCNT embryos.

**Keywords:** Apoptosis; Blastocyst; Bovine; Cathepsin B inhibitor (E-64); Somatic cell nuclear transfer (SCNT)

PMID: 24240170
Comparison of surgical methods of transient middle cerebral artery occlusion between rats and mice


Lee S, Hong Y, Park S, Lee SR, Chang KT*, Hong Y. *Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr)

Rodent models of focal cerebral ischemia that do not require craniotomy have been developed by intraluminal suture middle cerebral artery occlusion (MCAo). Mouse MCAo models have been widely used and extended to genetic studies of cell death or recovery mechanisms. Therefore, we compared surgery-related parameters and techniques between such rats and mice. In rodent MCAo models, has to be considered body temperature during the operative period, as well as the need for the use of a standardized tip in terms of the outer diameter of probes. Induction of focal cerebral ischemia was measured by neurological dysfunction parameters. Our methods could induce stable moderate-severity ischemic brain injury models and histological alteration at 24 hr after MCAo surgery. Moreover approximately 80% (rats) and 85% (mice) survival ratios were shown indicating with model engineering success. Finally, we described and compared major parameters between rats and mice, including probe size, thread insert length, operation and occlusion periods, and differences in the procedures.

**Keywords**: Focal cerebral ischemia; Microsurgical procedure; Middle cerebral artery occlusion (MCAo); Rodent model

PMID: 25649935

Progesterone production is affected by unfolded protein response (UPR) signaling during the luteal phase in mice


Park HJ, Park SJ, Koo DB, Lee SR, Kong IK, Ryoo JW, Park YI, Chang KT*, Lee DS. *Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr)

AIMS: We examined whether the three unfolded protein response (UPR) signaling pathways, which are activated in response to endoplasmic reticulum (ER)-stress, are involved in progesterone production in the luteal cells of the corpus luteum (CL) during the mouse estrous cycle.

MAIN METHODS: The luteal phase of C57BL/6 female mice (8 weeks old) was divided into two stages: the functional stage (16, 24, and 48 h) and the regression stage (72 and 96 h). Western blotting and reverse transcription (RT)-PCR were performed to analyze UPR protein/gene expression levels in each stage. We investigated whether ER stress affects the progesterone production by using Tm (0.5 μg/g BW) or TUDCA (0.5 μg/g BW) through intra-peritoneal injection.

KEY FINDINGS: Our results indicate that expressions of Grp78/Bip, p-eIF2α/ATF4, p50ATF6, and p-IRE1/sXBP1 induced by UPR activation were predominantly maintained in functional and early regression stages of the CL. Furthermore, the expression of p-JNK, CHOP, and cleaved caspase3 as ER-stress mediated apoptotic factors increased during the regression stage. Cleaved caspase3 levels increased in the late-regression stage after p-JNK and CHOP expression in the early-regression stage. Additionally, although progesterone secretion and levels of steroidogenic enzymes decreased following intra-peritoneal injection of Tunicamycin, an ER stress inducer, the expression of Grp78/Bip, p50ATF6, and CHOP dramatically increased.

SIGNIFICANCE: These results suggest that the UPR signaling pathways activated in response to ER stress may play important roles in the regulation of the CL function. Furthermore, our findings enhance the understanding of the basic mechanisms affecting the CL life span.

**Keywords**: Corpus luteum; ER stress; Luteal phase; Progesterone production, Unfolded protein response

PMID: 25108065
Protection from hemolytic uremic syndrome by eyedrop vaccination with modified enterohemorrhagic E. coli outer membrane vesicles


Choi KS, Kim SH, Kim ED, Lee SH, Han SJ, Yoon S, Chang KT*, Seo KY.
‘Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr)

We investigated whether eyedrop vaccination using modified outer membrane vesicles (mOMVs) is effective for protecting against hemolytic uremic syndrome (HUS) caused by enterohemorrhagic E. coli (EHEC) O157:H7 infection. Modified OMVs and waaJ-mOMVs were prepared from cultures of MsbB- and Shiga toxin A subunit (STxA)-deficient EHEC O157:H7 bacteria with or without an additional waaJ mutation. BALB/c mice were immunized by eyedrop mOMVs, waaJ-mOMVs, and mOMVs plus polymyxin B (PMB). Mice were boosted at 2 weeks, and challenged peritoneally with wild-type OMVs (wtOMVs) at 4 weeks. As parameters for evaluation of the OMV-mediated immune protection, serum and mucosal immunoglobulins, body weight change and blood urea nitrogen (BUN)/Creatinin (Cr) were tested, as well as histopathology of renal tissue. In order to confirm the safety of mOMVs for eyedrop use, body weight and ocular histopathological changes were monitored in mice. Modified OMVs having penta-acylated lipid A moiety did not contain STxA subunit proteins but retained non-toxic Shiga toxin B (STxB) subunit. Removal of the polymeric O-antigen of O157 LPS was confirmed in waaJ-mOMVs. The mice group vaccinated with mOMVs elicited greater humoral and mucosal immune responses than did the waaJ-mOMVs and PBS-treated groups. Eyedrop vaccination of mOMVs plus PMB reduced the level of humoral and mucosal immune responses, suggesting that intact O157 LPS antigen can be a critical component for enhancing the immunogenicity of the mOMVs. After challenge, mice vaccinated with mOMVs were protected from a lethal dose of wtOMVs administered intraperitoneally, conversely mice in the PBS control group were not. Collectively, for the first time, EHEC O157-derived mOMV eyedrop vaccine was experimentally evaluated as an efficient and safe means of vaccine development against EHEC O157:H7 infection-associated HUS.

Keywords: O157 LPS antigen; Eyedrop vaccination; Hemolytic uremic syndrome (HUS); Modified outer membrane vesicles (mOMVs)

PMID: 25032703

Valproic acid enhances early development of bovine somatic cell nuclear transfer embryos by alleviating endoplasmic reticulum stress


‘Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr), Sun-Uk Kim(sunuk@kribb.re.kr)

Despite the positive roles of histone deacetylase inhibitors in somatic cell nuclear transfer (SCNT), few studies have evaluated valproic acid (VPA) and its associated developmental events. Thus, the present study was conducted to elucidate the effect of VPA on the early development of bovine SCNT embryos and the underlying mechanisms of action. The histone acetylation level of SCNT embryos was successfully restored by VPA, with optimal results obtained by treatment with 3mM VPA for 24h. Importantly, the increases in blastocyst formation rate and inner cell mass and trophectoderm cell numbers were not different between the VPA and trichostatin A treatment groups, whereas cell survival was notably improved by VPA, indicating the improvement of developmental competence of SCNT embryos by VPA. Interestingly, VPA markedly reduced the transcript levels of endoplasmic reticulum (ER) stress markers, including sXBP-1 and CHOP. In contrast, the levels of GRP78/Bip, an ER stress-activating transcript, were significantly increased by VPA. Furthermore, VPA greatly reduced cell apoptosis in SCNT blastocysts, which was further evidenced by the increased levels of the anti-apoptotic transcript Bcl-xL and decreased level of the pro-apoptotic transcript Bax. Collectively, these results suggest that VPA enhances the developmental competence of bovine SCNT embryos by alleviating ER stress and its associated developmental damage.

Keywords: Apoptosis; Blastocyst; Developmental competence; Histone acetylation; Reprogramming

PMID: 23506644
Induction of autophagy during in vitro maturation improves the nuclear and cytoplasmic maturation of porcine oocytes

Reprod Fertil Dev. 26(7):974-81.


*Co-corresponding: Kyu-Tae Chang(changkt@kribb.re.kr), Sun-Uk Kim(sunuk@kribb.re.kr)

While a critical role of autophagy in mammalian early embryogenesis has been demonstrated, few studies have been conducted regarding the role of autophagy in in vitro maturation (IVM) of immature oocytes. In the present study we investigated the effect of rapamycin, a chemical autophagy inducer, on the nuclear and cytoplasmic maturation of porcine oocytes. Rapamycin treatment led to increased expression of LC3-II, an autophagy marker. Compared with the control group, as well as the 5 and 10nM rapamycin treatment groups, the rate of MII oocyte production was higher in the 1nM rapamycin treatment group, indicating improvement in nuclear maturation. In the analyses of cytoplasmic maturation, we found that the level of p34cdc2, a cytoplasmic maturation marker, and the monospermic fertilisation rate were higher in the 1nM rapamycin treatment group than in the other groups. Moreover, the beneficial effect of 1nM rapamycin on cytoplasmic maturation of MII oocytes was further evidenced by increases in blastocyst formation rate, total cell number and cell survival. In the blastocyst embryos, anti-apoptotic Bcl-xL transcript levels were elevated in the 1nM rapamycin-treated group, whereas pro-apoptotic Bax transcript levels were decreased. Collectively, these results suggest that induction of autophagy during IVM contributes to enhancement of the nuclear and cytoplasmic maturation of porcine oocytes.

Keywords: Apoptosis; Autophagy marker; Development; in vitro fertilisation; Porcine oocytes; Rapamycin

PMID: 23902659

Population structure and domestication revealed by high-depth resequencing of Korean cultivated and wild soybean genomes


*Co-corresponding: Soon-Chun Jeong(scjeong@kribb.re.kr), Namshin Kim(deepreds@kribb.re.kr)

Despite the importance of soybean as a major crop, genome-wide variation and evolution of cultivated soybeans are largely unknown. Here, we catalogued genome variation in an annual soybean population by high-depth resequencing of 10 cultivated and 6 wild accessions and obtained 3.87 million high-quality single-nucleotide polymorphisms (SNPs) after excluding the sites with missing data in any accession. Nuclear genome phylogeny supported a single origin for the cultivated soybeans. We identified 10-fold longer linkage disequilibrium (LD) in the wild soybean relative to wild maize and rice. Despite the small population size, the long LD and large SNP data allowed us to identify 206 candidate domestication regions with significantly lower diversity in the cultivated, but not in the wild, soybeans. Some of the genes in these candidate regions were associated with soybean homologues of canonical domestication genes. However, several examples, which are likely specific to soybean or eudicot crop plants, were also observed. Consequently, the variation data identified in this study should be valuable for breeding and for identifying agronomically important genes in soybeans. However, the long LD of wild soybeans may hinder pinpointing causal gene(s) in the candidate regions.

Keywords: Domestication; Linkage disequilibrium (LD); Resequencing; Soybean; Variation

PMID: 24271940
Effects of transgenic poplars expressing increased levels of cellular cytokinin on rhizosphere microbial communities

'Co-corresponding: Chang-Gi Kim(cgkim@kribb.re.kr)

Considerable effort has been made in biotechnology to increase plant biomass. Altering cellular levels of plant hormones, including cytokinin, by genetic modification, has been one way to achieve the goal as it is involved in a variety of processes related to plant growth and development. However, the alteration inevitably may change physiological and biochemical characteristics of plants, and thus could affect the relationship between plants and other organisms interacting with the plants such as microorganisms inhabiting in the rhizosphere. To determine if these indirect effects on rhizosphere microorganisms, mediated by hormonal changes in plants, do occur, we investigated the microbial biomass and community structure associated with transgenic Populus trees with altered cellular cytokinin levels, using phospholipid fatty acid (PLFA) analysis. Three transgenic lines expressing increased levels of cellular cytokinin (T1403, T1410, and T1413) and their non-transgenic isoline (BH) were planted at three locations (Suwon, Cheongwon, and Jinju) in 2011. Soil samples were collected near the base of each tree monthly, from May to September. Indicator PLFAs were utilized to calculate the microbial (bacterial and fungal) biomass, and PLFA profiles were developed to characterize the structure of those communities. Over the growing season, soils from Cheongwon and Jinju had similar microbial biomass (PLFAs indicating functional groups) whereas, at Suwon, the biomass associated with the rhizosphere of Line T1413 was significantly different from that of the other transgenics and the control. At Cheongwon and Jinju, the structure of the rhizosphere microbial communities differed significantly between Lines T1403 or T1410 and BH, but only in May and June. By contrast, those structures were similar in all sampling months for each line at Suwon. Our results indicate that the influence resulting from genetic modification of the poplar trees on the rhizosphere microbial community is only temporary and inconsistent depending upon location and genetic line.

Keywords : Genetically modified tree; Phytohormone; PLFA (phospholipid fatty acid); Rhizosphere; Soil microorganisms

Drought stress-induced compositional changes in tolerant transgenic rice and its wild type

'Corresponding: Chang-Gi Kim(cgkim@kribb.re.kr)

Comparing well-watered versus deficit conditions, we evaluated the chemical composition of grains harvested from wild-type (WT) and drought-tolerant, transgenic rice (Oryza sativa L.). The latter had been developed by inserting AcYp78A7, which encodes a cytochrome P450 protein. Two transgenic Lines, ‘10B-5’ and ‘18A-4’, and the ‘Hwayoung’ WT were grown under a rainout shelter. After the harvested grains were polished, their levels of key components, including proximates, amino acids, fatty acids, minerals and vitamins were analysed to determine the effect of watering system and genotype. Drought treatment significantly influenced the levels of some nutritional components in both transgenic and WT grains. In particular, the amounts of lignoceric acid and copper in the WT decreased by 12.6% and 39.5%, respectively, by drought stress, whereas those of copper and potassium in the transgenics rose by 88.1-113.3% and 10.4-11.9%, respectively, under water-deficit conditions.

Keywords : Chemical composition; Drought tolerance; Food safety assessment; Oryza sativa L.; Rainout shelter; Transgenic crop

PMID: 24491713
Betaine supplementation improves beneficial effects of boxthorn (Lycium chinense Mill.) leaf on body weight/body fat increase and plasma/liver triglycerides accumulation in high-fat diet-fed C57BL/6 mice


Kang MR, Lee CW, Cho IJ, Lee MY, Shin JH, Oh SJ, Yun J, Yoon WK, Han SB, Kim EE, Bok SH, Kang JS. *Corresponding: Jong Soon Kang(kanjon@kribb.re.kr)*

In the present study, we investigated the combinatorial effects of betaine supplementation and boxthorn (Lycium chinense Mill.) leaf on high-fat diet (HFD)-induced body weight/body fat increase, plasma lipid profile and liver damage. Suboptimal dosage of hot water extract of boxthorn leaf (Lycium chinense water extract, LWE) exerted partial inhibitory effect on body weight/body fat increase in HFD-fed mice. Betaine supplementation potentiated the effect of LWE showing a significant inhibition against HFD-induced increase in body weight/body fat. However, both LWE alone and LWE plus betaine had no effect on plasma cholesterol, including total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol in HFD-fed mice. In contrast, HFD-induced increases in plasma and liver triglycerides were partially suppressed by LWE treatment and this was potentiated by betaine supplementation. In addition, both LWE alone and LWE plus betaine significantly suppressed HFD-induced glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase. Collectively, our results suggest that the combination of boxthorn leaf and betaine might be beneficial for the management of obesity and nonalcoholic fatty liver disease.

**Keywords:** Betaine supplementation; Boxthorn leaf; Cholesterol; Liver disease; Lycium chinense; LWE treatment

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Agelaisine D suppresses RANKL-induced osteoclastogenesis via down-regulation of c-Fos, NFATc1 and NF-κB


Kang MR, Jo SA, Yoon YD, Park KH, Oh SJ, Yun J, Lee CW, Nam KH, Kim Y, Han SB, Yu J, Rho J, Kang JS. *Corresponding: Jong Soon Kang(kanjon@kribb.re.kr)*

In the present study, we investigated the effect of agelaisine D (AD) on osteoclastogenesis. Treatment of bone marrow macrophages (BMMs) with receptor activator of nuclear factor κB ligand (RANKL) resulted in a differentiation of BMMs into osteoclasts as evidenced by generation of tartrate-resistant acid phosphatase (TRAP)-positive, multinucleated cells and formation of pits in calcium phosphate-coated plates. However, RANKL-induced osteoclastogenesis was significantly suppressed by AD treatment. We also confirmed the increased mRNA and protein expression of osteoclastic markers, such as TRAP, cathepsin K and matrix metalloproteinase-9, during RANKL-induced osteoclast differentiation and this was down-regulated by AD treatment. Moreover, AD treatment significantly suppressed RANKL-induced mRNA expression of DC-STAMP and OC-STAMP and cell fusion of TRAP-positive mononuclear osteoclast precursors. In addition, AD suppressed RANKL-induced expression of transcription factors, c-Fos and nuclear factor of activated T cells c1 (NFATc1), which are important transcription factors involved in differentiation of BMMs into osteoclasts. Furthermore, RANKL-induced phosphorylation of extracellular signal-related kinase (ERK) and activation of NF-κB were also inhibited by AD treatment. Collectively, these results suggest that AD inhibits RANKL-induced osteoclastogenesis by down-regulation of multiple signaling pathways involving c-Fos, NFATc1, NF-κB and ERK. Our results also suggest that AD might be a potential therapeutic agent for prevention and treatment of osteoporosis.

**Keywords:** Agelaisine D; Osteoclastogenesis; c-Fos; NF-ATc1; NF-κB

PMID: 25421321
Identification of suitable reference genes for the relative quantification of microRNAs in pleural effusion

Oncol Lett. 8(4):1889-95.

Han HS, Jo YN, Lee JY, Choi SY, Jeong Y, Yun J’, Lee OJ.
*Co-corresponding: Jieun Yun(jyun@kribb.re.kr)

Circulating cell-free microRNAs (miRNAs) are potential biomarkers of cancer. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is widely used in miRNA expression studies. The aim of this study was to identify suitable reference genes for RT-qPCR analyses of miRNA expression levels in pleural effusion. The expression levels of candidate reference miRNAs were investigated in 10 benign pleural effusion (BPE) and 10 lung adenocarcinoma-associated malignant pleural effusion (LA-MPE) samples using miRNA microarrays. The expression levels of candidate reference miRNAs, together with those of U6 small nuclear RNA (snRNA), RNU6B, RNU44 and RNU48 small RNAs, in 46 BPE and 45 LA-MPE samples were validated by RT-qPCR, and were analyzed using the NormFinder and BestKeeper algorithms. The impact of different normalization approaches on the detection of differential expression levels of miR-198 in BPE and LA-MPE samples was also assessed. As determined by the miRNA microarray data, five candidate reference miRNAs were identified. Following RT-qPCR validation, U6 snRNA, miR-192, miR-20a, miR-221, miR-222 and miR-16 were evaluated using the NormFinder and BestKeeper software programs. U6 snRNA and miR-192 were identified as single reference genes and the combination of these genes was preferred for the relative quantification of miRNA expression levels in pleural effusion. Normalization of miR-98 expression levels to those of U6 snRNA, miR-192 or a combination of these genes enabled the detection of a significant difference between BPE and LA-MPE samples. Therefore, U6 snRNA and miR-192 are recommended as reference genes for the relative quantification of miRNA expression levels in pleural effusion.

Keywords: microRNAs; miR-192; Pleural effusion; Reference gene; U6 snRNA

PMID: 25202432

Article 184

Chemical aspects of host-acceptance behaviour in the bird cherry-oat aphid Rhopalosiphum padi: Host-acceptance signals used by different morphs with the same genotype


Nam KJ’, Hardie J.
*Corresponding: Ki Jung Nam

Host acceptance by gynoparae and winged virginoparae of the bird cherry-oat aphid Rhopalosiphum padi (L.) is investigated utilizing leaves and aqueous extracts of the primary and secondary hosts, as well as nonhost plants. Gynoparae are specialized to reproduce on bird cherry Prunus padus L., whereas virginoparae reproduce and feed on various grasses. Host acceptance is assessed using levels of reproduction and survival for adults, as well as survival for nymphs. Little is known of host acceptance by nymphs. The data show that gynoparae and winged virginoparae produce nymphs almost exclusively on their host plants, bird cherry and barley leaves, respectively, over 72h. When tested with aqueous plant extracts, however, gynoparae produce nymphs almost exclusively on bird cherry extract and progeny numbers are found to be similar to those on plant leaves. Few nymphs are produced on artificial diet. By contrast, winged virginoparae produce nymphs on aqueous extracts of barley, bird cherry and bean, as well as on artificial diet. The numbers of nymphs deposited by gynoparae are similar on aqueous extracts of bird cherry leaves collected at different times during the growing season. When extracts from leaves of various Prunus species are tested, only leaves of P. padus and Prunus virginiana stimulate parturition. Oviparae, the sexual female nymphs of gynoparae, survive well for 96h on both bird cherry and barley leaves but not on bean seedlings, whereas nymphs of winged virginoparae survive well only on barley leaves. They do not survive for 96h on any plant-leaf extracts, although they do survive on artificial diet.

Keywords: Chemical cue; Host acceptance; Gynoparae; Rhopalosiphum padi; Seasonal morphs; Virginoparae
Pharmacokinetics and metabolism of 4-O-methylhonokiol in rats

Phytother Res. 28(4):568-78.

‘Co-first: Soo Jin Oh(di@kribb.re.kr)

The purpose of this study was to characterize the pharmacokinetics and metabolism of 4-O-methylhonokiol in rats. The absorption and disposition of 4-O-methylhonokiol were investigated in male Sprague-Dawley rats following a single intravenous (2 mg/kg) or oral (10 mg/kg) dose. Its metabolism was studied in vitro using rat liver microsomes and cytosol. 4-O-methylhonokiol exhibited a high systemic plasma clearance and a large volume of distribution. The oral dose gave a peak plasma concentration of 24.1±3.3 ng/mL at 2.9±1.9 h and a low estimated bioavailability. 4-O-methylhonokiol was rapidly metabolized and converted at least in part to honokiol in a concentration-dependent manner by cytochrome P450 in rat liver microsomes, predicting a high systemic clearance consistent with the pharmacokinetic results. It was also shown to be metabolized by glucuronidation and sulfation in rat liver microsomes and cytosol, respectively. 4-O-methylhonokiol showed a moderate permeability with no apparent vectorial transport across Caco-2 cells, suggesting that intestinal permeation process is not likely to limit its oral absorption. Taken together, these results suggest that the rapid hepatic metabolism of 4-O-methylhonokiol could be the major reason for its high systemic clearance and low oral bioavailability.

Keywords: 4-O-methylhonokiol; Honokiol; Metabolism; Neolignan; Pharmacokinetics

PMID: 23824979

Multiple genes confer resistance to soybean mosaic virus in the soybean cultivar Hwangkeum


Jeong N, Jeong SC’.
‘Corresponding: Soon-Chun Jeong(scjeong@kribb.re.kr)

Recombinant inbred lines (RILs) generated from a cross between a cultivated species and its wild progenitor species serve as important germplasm for introgressing valuable genes from a wild species to a cultivated species. During this breeding process, it is equally important to prevent the loss of agronomically important genes in the cultivated species. In an effort to establish an efficient selection system for the single Rsv1 gene conferring durable resistance to soybean mosaic virus (SMV) in the soybean cultivar Hwangkeum (also known as Suweon 97), which is a parent of a RIL population from Hwangkeum (cultivated soybean) × IT182932 (wild soybean), in the present study, we unexpectedly identified an additional necrosis-conditioning gene unmasked by a recombination in the middle of the Rsv1-containing nucleotide-binding leucine-rich repeat gene cluster region and the Rsv3 gene in Hwangkeum. Thus, Hwangkeum contains at least three SMV resistance genes consisting of two tightly linked genes at the Rsv1 locus and the Rsv3 locus. The results of this study provide additional important information for use of the Hwangkeum genome in soybean breeding programmes.

Keywords: Germplasm; Glycine max; Hwangkeum; Resistance gene; Soybean mosaic virus (SMV)
Hepatoprotective effect of aged black garlic extract in rodents

Toxicol Res. 30(1):49-54.

'Corresponding: Jong Soon Kang(ikanjon@kribb.re.kr)

In this study, we investigated the hepatoprotective effects of aged black garlic (ABG) in rodent models of liver injury. ABG inhibited carbon tetrachloride-induced elevation of aspartate transaminase (AST) and alanine transaminase (ALT), which are markers of hepatocellular damage, in SD rats. D-galactosamineinduced hepatocellular damage was also suppressed by ABG treatment. However, ABG does not affect the elevation of alkaline phosphatase (ALP), a marker of hepatobiliary damage, in rats treated with carbon tetrachloride or D-galactosamine. We also examined the effect of ABG on high-fat diet (HFD)-induced fatty liver and subsequent liver damage. ABG had no significant effect on body weight increase and plasma lipid profile in HFD-fed mice. However, HFD-induced increase in AST and ALT, but not ALP, was significantly suppressed by ABG treatment. These results demonstrate that ABG has hepatoprotective effects and suggest that ABG supplementation might be a good adjuvant therapy for the management of liver injury.

**Keywords** : Aged black garlic; Carbon tetrachloride; D-galactosamine; Liver injury; Non-alcoholic fatty liver disease

PMID: 24795800

Inhibition of cytochrome P450 by ethambutol in human liver microsomes


Lee SY, Jang H, Lee JY, Kwon KI, Oh SJ^, Kim SK. 'Co-corresponding: Soo Jin Oh(diatree@kribb.re.kr)

Although cytochrome P450 inhibition is the major drug-drug interaction (DDI) mechanism in clinical pharmacotherapy, DDI of a number of well-established drugs have not been investigated. Rifampicin, isoniazid, pyrazinamide and ethambutol combination therapy inhibits clearance of theophylline in patients with tuberculosis. We determined the inhibitory effects of ethambutol on the activities of nine CYP isoforms including CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 in pooled human liver microsomes (HLM). As measured by liquid chromatography-electrospray ionization tandem mass spectrometry, ethambutol exhibited strong inhibitory potential against CYP1A2 and CYP2E1, moderate against CYP2C19 and CYP2D6 and weak against CYP2A6, CYP2C9 and CYP3A4, based on the IC_{50} values. The K_{i} value of ethambutol for CYP1A2 was 1.4 μM and for CYP2E1 was 2.9 μM. Inhibition of CYP1A2 and CYP2E1 was not increased by preincubation with ethambutol and β-nicotinamideadenine dinucleotide phosphate (NADPH), suggesting that the ethambutol-induced CYP inhibition may not be metabolism-dependent. Kinetic analysis showed that the inhibition of CYP1A2 and CYP2E1 by ethambutol was best fit to a competitive inhibition model. Formation of 1-methylxanthene and 1,3-dimethylxanthene acid from theophylline in HLM was decreased to 47% and 36%, respectively, by 3.0 μM ethambutol, which is comparable to its IC_{50} value against CYP1A2. Considering its maximal plasma concentrations of ~10 μM and long half-life of ~22 h, our findings raise the possibility that ethambutol causes significant DDIs in clinical situations with drugs with narrow therapeutic index, such as theophylline, in clinical situations.

**Keywords** : CYP1A2; CYP2E1; Drug-drug interaction (DDI); Ethambutol; Theophylline

PMID: 24910189
Peptoniphilus rhinitidis sp. nov., isolated from specimens of chronic rhinosinusitis

Anaerobe. 30:30-4.


Chronic rhinosinusitis (CRS) is an inflammatory disorder of the nasal cavity and paranasal sinuses related to bacterial infection. A previous study suggested that a specific bacterial group may have an important role in the course of CRS. In this study, bacteria isolated from CRS patients were characterized. A total of 15 strains were identified as Gram-positive anaerobic cocci (GPAC), which were able to utilize peptone as a sole carbon source. Sequencing of the 16S ribosomal RNA gene revealed that the isolates were closely related to members of the genus Peptoniphilus (>97% similarity) within the Clostridiales Family XI. Incertae Sedis. Genotypic and phenotypic characterization suggests that these isolates represent a novel species of the genus Peptoniphilus associated with CRS. The type strain of Peptoniphilus rhinitidis is 1-13T (=KCTC 59855=JCM 17448).

Keywords: Anaerobic cocci; Chronic rhinosinusitis; Gram-positive; Peptoniphilus

PMID: 25094054

Lysinibacillus composti sp. nov., isolated from compost

Ann Microbiol. 64(3):1081-8.


A Gram-negative, motile, rod-shaped, endospore-forming bacterial strain, designated as NCCP-36*, was isolated from the compost of fruit and vegetable wastes. The strain NCCP-36* grew within a temperature range of 10-45 °C (optimum 28 °C) and a pH range of 6.5-8.5 (optimum 7.0), and its cells tolerated <50 mM boron (optimum growth without boron) and 0-5 % NaCl (w/v) in tryptic soya broth medium. Based on comparative analysis of 16S rRNA gene sequence, strain NCCP-36* showed the highest similarity to Lysinibacillus sinduariensis BLB-1T (97.52 %) and L. xylanilyticus XDB9* (96.96 %), and <97 % similarity with other closely related taxa. However, DNA-DNA relatedness between strain NCCP-36* and the closely related type strains of genus Lysinibacillus was ≤37 %. Phylogenetic and chemotaxonomic analyses [major polar lipids: diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and phospholipids; predominant menaquinone: MK-7; major cellular fatty acids: iso-C15:0, anteiso-C15:0 and iso-C15:0; DNA G+C contents: 37 mol %; Lys-Asp (type A4a) in cell-wall peptidoglycans as diagnostic amino acids] also support the affiliation of strain NCCP-36* to genus Lysinibacillus. Based upon DNA-DNA relatedness as well as distinctive chemotaxonomic, phylogenetic, and genotypic data, we conclude that strain NCCP-36* belongs to a novel species of genus Lysinibacillus, for which the name Lysinibacillus composti sp. nov. is proposed. The type strain is NCCP-36* (JCM 18777T=KCTC 13796=DSMZ 24785).

Keywords: Cell-wall peptidoglycans; Compost; Lysinibacillus composti; Novel species
Sphingobacterium pakistanensis sp. nov., a novel plant growth promoting rhizobacteria isolated from rhizosphere of Vigna mungo


Co-corresponding: Young-Hyo Chang(yhchang@kribb.re.kr)

The taxonomic status of a bacterium, strain NCCP-246\(^7\), isolated from rhizosphere of Vigna mungo, was determined using a polyphasic taxonomic approach. The strain NCCP-246\(^7\) can grow at 16-37 °C (optimum 32 °C), at pH ranges of 6-8 (optimum growth occurs at pH 7) and in 0-4 % (w/v) NaCl. Phylogenetic analysis based upon on 16S rDNA gene sequence revealed that strain NCCP-246\(^7\) belonged to genus Sphingobacterium. Strain NCCP-246\(^7\) showed highest similarity to the type strain of Sphingobacterium canadense CR11\(^7\) (97.67 %) and less than 97 % with other species of the genus. The DNA-DNA relatedness value of strain NCCP-246\(^7\) with S. canadense CR11\(^7\) and Sphingobacterium thalpophilum JCM 21153\(^3\) was 55 and 44.4 %, respectively. The chemotaxonomic data revealed the major menaquinone as MK-7 and dominant cellular fatty acids were summed feature 3 [C\(_{16:1}\) a7c,C\(_{16:1}\) a6c] (37.07 %), iso-C\(_{15:0}\) (28.03 %), C\(_{16:0}\) (11.85 %), C\(_{17:0}\) cyclo (8.84 %) and C\(_{14:0}\) (2.42 %). The G+C content of the strain was 39.2 mol%. On the basis of DNA-DNA hybridization, phylogenetic analyses, physiological and, biochemical data, strain NCCP-246\(^7\) can be differentiated from the validly named members of genus Sphingobacterium and thus represents as a new species, for which the name, Sphingobacterium pakistanensis sp. nov. is proposed, with the type strain NCCP-246\(^7\) (= JCM 18974\(^4\) = KCTC 23914\(^3\)).

Keywords: Antibiotic resistance; nif gene; Novel species; P-solubilization; Sphingobacterium; Vigna mungo

PMID: 24281734

Bacillus pakistanensis sp. nov., a halotolerant bacterium isolated from salt mines of the Karak Area in Pakistan


Co-corresponding: Young-Hyo Chang(yhchang@kribb.re.kr)

A rod shaped, non-motile, endospore forming, Gram-stain positive and moderately halotolerant strain, designated as NCCP-168\(^7\), was isolated from salt mines sampled in the Karak district of Khyber Pakhtunkhwa Province in Pakistan. To delineate its taxonomic position, the strain was subjected to polyphasic characterization. Cells of strain NCCP-168\(^7\) can grow at 10-40 °C (optimum at 30-35 °C), in a pH range of 5.0-9.0 (optimum at pH 8.0) and in 0-17 % (w/v) NaCl on agar medium. The phylogenetic analysis based on the 16S rDNA gene sequence showed that strain NCCP-168\(^7\) belongs to the genus Bacillus with the highest similarity to Bacillus seohaeanensis BH72\(^4\) (97.1 %), and less than 97 % similarity with other closely related taxa (95.6 % with B. subtilis subsp. subtilis NCIB3610\(^9\)). DNA-DNA relatedness between strain NCCP-168\(^7\) and the type strains of closely related species was lower than 30 %. Chemotaxonomic data (major menaquinone, MK-7; cell wall peptidoglycan type, A1L [meso-diaminopimelic acid]; major fatty acids, iso-C\(_{15:0}\) 29.9 %, anteiso-C\(_{15:0}\) 29.3 %, iso-C\(_{16:0}\) 11.4 %, iso-C\(_{14:0}\) 8.9 % and anteiso-C\(_{17:0}\) 7.0 %; major polar lipids, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine) support the affiliation of strain NCCP-168\(^7\) with genus Bacillus. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain NCCP-168\(^7\) can be distinguished from the closely related taxa and thus represents a novel species in the genus Bacillus, for which the name Bacillus pakistanensis sp. nov. is proposed, with the type strain NCCP-168\(^7\) (= KCTC 13786\(^5\) = DSM 24834\(^4\) = JCM 18975\(^3\)).

Keywords: Bacillus pakistanensis; Halotolerant bacterium; Novel species; Salt mines

PMID: 24777297
**Clostridium vulturis** sp. nov., isolated from the intestine of the cinereous vulture (*Aegypius monachus*)

*Antonie Van Leeuwenhoek.* 106(3):577-83.


'Corresponding: Young-Hyo Chang(yhchang@kribb.re.kr)

A Gram-stain positive, strict anaerobe, spore-forming, motile rod-shaped bacterial strain with peritrichous flagella, designated YMB-57, was isolated from the intestine of a cinereous vulture (*Aegypius monachus*) in Korea. Strain YMB-57 was found to show optimal growth at 37 °C, pH 7.5 and 1.0 % (w/v) NaCl. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain YMB-57 belongs to the genus *Clostridium* and is most closely related to the type strains of *Clostridium subterminale* (96.9 % sequence similarity), *Clostridium thiosulfatireducens* (96.7 %) and *Clostridium sulfidigenes* (96.6 %). The main fermentation end-products identified following growth in PYG medium were acetate, butyrate, ethanol, propanol, carbon dioxide and hydrogen. Peptone was converted to ethanol, and butanol, whereas glucose was fermented to ethanol. The major cellular fatty acids were identified as C18:1ω9c, and C16:0c DMA and the DNA G+C content was determined to be 34.0 mol%. Phenotypic and phylogenetic differences indicate that strain YMB-57 is distinct from other *Clostridium* species. It is proposed that strain YMB-57 be classified as the type strain of a novel species of the genus *Clostridium*, with the name *Clostridium vulturis* sp. nov. The type strain is YMB-57 (=KCTC 15114T = JCM 17998T).

**Keywords:** *Clostridium vulturis; Novel species; Polyphasic taxonomy; Vulture intestine*

PMID: 25063360

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**Description of Lysinibacillus pakistanensis**


'Corresponding: Young-Hyo Chang(yhchang@kribb.re.kr)

The purpose of this addendum is to provide the additional information for validation of *Lysinibacillus pakistanensis* sp. nov. as a new name under the procedure described in the *Bacteriological Code* (1990 Revision). The strain NCCP-54 recently published, however it does not meet the basic requirement as it lacks species description according to the rules of International Code of Nomenclature of Prokaryotes. Additionally, the data on DNA-DNA hybridization was required with all the validly recognized species having more than 97% similarity of 16S rRNA gene sequence. The results of this study showed that DNA-DNA relatedness of strain NCCP-54 is below 70% with all the validly recognized species to date. The diagnostic amino acids in cell wall peptidoglycans were re-analyzed and contained Lys-Asp (type A4c). This addendum also provides the formal description of *Lysinibacillus pakistanensis* sp. nov.

**Keywords:** Cell wall peptidoglycans type A4c; DNA-DNA hybridization; *Lysinibacillus pakistanensis*; Species description

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**Diagram:**

- [Diagram of phylogenetic relationships and taxonomic classification](image-url)
Induction of clusterin expression by neuronal cell death in zebrafish


*Co-first: Tae-Eun Jin(tejin@kribb.re.kr)

Clusterin, a protein associated with multiple functions, is expressed in a wide variety of mammalian tissues. Although clusterin is known to be involved in neurodegenerative diseases, ageing, and tumorigenesis, a detailed analysis of the consequences of gain- or loss-of-function approaches has yet to be performed to understand the underlying mechanisms of clusterin functions. Since clusterin levels change in neurological diseases, it is likely that clusterin contributes to cell death and degeneration in general. Zebrafish was investigated as a model system to study human diseases. During development, zebrafish clusterin was expressed in the notochord and nervous system. Embryonic overexpression of clusterin by mRNA microinjection did not affect axis formation, whereas its knock-down by anti-sense morpholino treatment resulted in neuronal cell death. To analyze the function of clusterin in neurodegeneration, a transgenic zebrafish was investigated, in which nitroreductase expression is regulated under the control of a neuron-specific huC promoter which is active between the stages of early neuronal precursors and mature neurons. Nitroreductase turns metronidazole into a cytotoxic agent that induces cell death within 12 h. After metronidazole treatment, transgenic zebrafish showed neuron-specific cell death. Interestingly, we also observed a dramatic induction of clusterin expression in the brain and spinal cord in these fish, suggesting a direct or indirect role of clusterin in neuronal cell death and thus, more generally, in neurodegeneration.

Keywords: Clusterin; Neurodegeneration; Neuronal cell death; Zebrafish

PMID: 25434681
Division of Research & Business Development

Biotechnology Process Engineering Center
Engineering *Escherichia coli* for selective geraniol production with minimized endogenous dehydrogenation


Zhou J, Wang C, Yoon SH, Jang HJ, Choi ES*, Kim SW. *Co-corresponding: Eui-Sung Choi(choi4162@kribb.re.kr)*

Geraniol, a monoterpenic alcohol, has versatile applications in the fragrance industry, pharmacy and agrochemistry. Moreover, geraniol could be an ideal gasoline alternative. In this study, recombinant overexpression of geranyl diprophosphate synthase and the bottom portion of a foreign mevalonate pathway in *Escherichia coli* MG1655 produced 13.3mg/L of geraniol. Introduction of *Ocimum basilicum* geraniol synthase increased geraniol production to 105.2mg/L. However, geraniol production encountered a loss from its endogenous dehydrogenization and isomerization into other geranoids (nerol, neral and geraniol). Three *E. coli* enzymes (YigB, YahK and YddN) were identified with high sequence identity to plant geraniol dehydrogenases. YigB was demonstrated to be the major one responsible for geraniol dehydrogenization. Deletion of yigB increased geraniol production to 129.7mg/L. Introduction of the whole mevalonate pathway for enhanced building block synthesis from endogenously synthesized mevalonate improved geraniol production up to 182.5mg/L in the yigB mutant after 48h of culture, which was a double of that obtained in the wild type control (96.5mg/L). Our strategy for improving geraniol production in engineered *E. coli* should be generalizable for addressing similar problems during metabolic engineering.

**Keywords**: Geraniol dehydrogenation; Geranyl diposphosphate synthase; Mevalonate pathway; Monoterpenic

PMID: 24269531

Digital mRNA profiling of *N*-glycosylation gene expression in recombinant Chinese hamster ovary cells treated with sodium butyrate


Lee SM, Kim YG, Lee EG*, Lee GM. *Co-corresponding: Eun Gyo Lee(eglee@kribb.re.kr)*

To understand the effects of sodium butyrate (NaBu) on protein glycosylation, recombinant Chinese hamster ovary (rCHO) cells producing Fc-fusion glycoprotein were subjected to 3mM NaBu. The addition of NaBu to the cultures reduced the relative proportion of acidic isoforms and sialic acid content of the glycoprotein. Fifty-two *N*-glycosylation-related gene expressions were also assessed by the NanoString nCounter system, which can provide a direct digital readout using custom-designed color-coded probes. Among them, ten genes (ugp, slc35a2, ganc, man1a, man1c, mgat5a, st3gal5, gbl1, neu1, and neu3) were up-regulated and three genes (b4gal1, st3gal3, and neu2) were down-regulated significantly. Altered expression patterns in *st3gal3, neu1*, and *neu3*, which have roles in the sialic acid biosynthesis pathway, correlated with reduced sialic acid content of the glycoprotein by NaBu. Taken together, the results obtained in this study provide a better understanding of the detrimental effect of NaBu on *N*-glycosylation in rCHO cells.

**Keywords**: CHO cell; Gene expression; *N*-glycosylation; NanoString nCounter system; Sodium butyrate

PMID: 24333461
Characterisation of *Pseudomonas aeruginosa* related to bovine mastitis


Park HR, Hong MK*, Hwang SY, Park YK, Kwon KH, Yoon JW, Shin S, Kim JH, Park YH.

*Cor-first: Min Ki Hong*

*Pseudomonas aeruginosa* is one of the causative pathogens of bovine mastitis. Most *P. aeruginosa* strains possess the type III secretion system (TTSS), which may increase somatic cell counts (SCCs) in milk from mastitis-affected cows. Moreover, most of *P. aeruginosa* cells can form biofilms, thereby reducing antibiotic efficacy. In this study, the presence and effect of TTSS-related genotypes on increase of SCCs among 122 *P. aeruginosa* isolates obtained from raw milk samples from mastitis-affected cows and their antibiotic susceptibility at planktonic and biofilm status were investigated. Based on the presence of TTSS-related genes a total of 82.7% of the isolates were found to harbour *exoU* and/or *exoS* genes, including the invasive (*exoU*/*exoS*+, 69.4%), cytotoxic (*exoU*/+/*exoS*−, 8.3%) and cytotoxic/invasive strains (*exoU*/+/−*exoS*+, 5.0%). Milk containing *exoS*-positive isolates had higher SCCs than those containing *exoS*-negative isolates. The majority of isolates showed gentamicin, amikacin, meropenem and ciprofloxacin susceptibility at planktonic status. However, the susceptibility was decreased at the biofilm status. Based on minimum biofilm eradication concentration (MBEC)/minimum inhibitory concentration (MIC) ratios, the range of change in antibiotic susceptibility varied widely depending on the antibiotics (from ≥ 3.1-fold to ≥ 475.0-fold). In conclusion, most *P. aeruginosa* isolates studied here had a genotype related to increase in SCCs. The efficiency of antibiotic therapy against *P. aeruginosa*-related bovine mastitis could be improved by analysing both the MBEC and the MIC of isolates.

**Keywords**: Biofilm; Bovine mastitis; Minimum biofilm eradication concentration (MBEC); *Pseudomonas aeruginosa*; Type three secretion system (TTSS)

**PMID**: 24334080

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**Article 199**

Assessment of mOMV adjuvant efficacy in the pathogenic H1N1 influenza virus vaccine


Lee BJ, Kwon HI, Kim EH, Park SJ, Lee SH, Choi YK, Kim SH*.

*Corresponding: Sang-Hyun Kim*

**PURPOSE**: Since the pandemic (H1N1) 2009 virus has been a seasonal flu which still poses great human health concerns worldwide, vaccination would be considered as the most effective strategy to control the influenza virus spreading. Here, we assessed adjuvant efficacy of modified outer membrane vesicle (mOMV) towards the pandemic H1N1 split antigens.

**MATERIALS AND METHODS**: For this study, mice were vaccinated twice with various amount of antigen (0.05, 0.1, and 0.5 µg/dose hemagglutinin [HA]) that were mixed with mOMV, aluminum hydroxide (alum), and MF59, as well as the combined adjuvant comprising the mOMV plus alum.

**RESULTS**: We found that all the adjuvanted vaccines of A/California/04/09 (CA04, H1N1) containing HA antigen more than 0.1 µg/dose protected effectively from lethal challenge (mA/CA04, H1N1) virus, compared to the antigen only group. Furthermore, vaccinated mice received as low as 0.05 µg/dose of the split vaccine containing the combined adjuvant (10 µg of mOMV plus alum) showed a full protection against lethal challenge with H1N1 virus. Taken together, these results suggest that mOMV can exert not only the self-adjuvancytivity but also a synergy effect for the vaccine efficacy when combined with alum.

**CONCLUSION**: Our results indicate that mOMV could be a promising vaccine adjuvant by itself and it could be used as a vaccine platform for development of various vaccine formulations to prepare future influenza pandemic.

**Keywords**: A/California/04/09 (CA04, H1N1); Adjuvant; Influenza A virus; mOMV; Vaccine platform

**PMID**: 25003093
Evolution and ecology of influenza A viruses

Yoon SW*, Webby RJ, Webster RG.

*First: Sun Woo Yoon(syoon@kribb.re.kr)

Wild aquatic bird populations have long been considered the natural reservoir for influenza A viruses with virus transmission from these birds seeding other avian and mammalian hosts. While most evidence still supports this dogma, recent studies in bats have suggested other reservoir species may also exist. Extensive surveillance studies coupled with an enhanced awareness in response to H5N1 and pandemic 2009 H1N1 outbreaks is also revealing a growing list of animals susceptible to infection with influenza A viruses. Although in a relatively stable host-pathogen interaction in aquatic birds, antigenic, and genetic evolution of influenza A viruses often accompanies interspecies transmission as the virus adapts to a new host. The evolutionary changes in the new hosts result from a number of processes including mutation, reassortment, and recombination. Depending on host and virus these changes can be accompanied by disease outbreaks impacting wildlife, veterinary, and public health.

Keywords: Influenza A virus; Interspecies transmission; Pandemic 2009 H1N1; Reservoir species; Wild aquatic bird

Novel porcine epidemic diarrhea virus variant with large genomic deletion, South Korea

Park S, Kim S, Song D*, Park B.

*Co-corresponding: Daesub Song

Since 1992, porcine epidemic diarrhea virus (PEDV) has been one of the most common porcine diarrhea-associated viruses in South Korea. We conducted a large-scale investigation of the incidence of PEDV in pigs with diarrhea in South Korea and consequently identified and characterized a novel PEDV variant with a large genomic deletion.

Keywords: Genomic deletion; Pig; PEDV variant; Porcine epidemic diarrhea virus (PEDV); South Korea

PMID: 25424875
**Isolation and genetic characterization of naturally NS-truncated H3N8 equine influenza virus in South Korea**


'Co-corresponding: Daesub Song

Equine influenza virus (EIV) causes a highly contagious respiratory disease in equids, with confirmed outbreaks in Europe, America, North Africa, and Asia. Although China, Mongolia, and Japan have reported equine influenza outbreaks, Korea has not. Since 2011, we have conducted a routine surveillance programme to detect EIV at domestic stud farms, and isolated H3N8 EIV from horses showing respiratory disease symptoms. Here, we characterized the genetic and biological properties of this novel Korean H3N8 EIV isolate. This H3N8 EIV isolate belongs to the Florida sublineage clade I of the American H3N8 EIV lineage, and surprisingly, possessed a non-structural protein (NS) gene segment, where 23 bases of the NS1-encoding region were naturally truncated. Our preliminary biological data indicated that this truncation did not affect virus replication; its effect on biological and immunological properties of the virus will require further study.

**Keywords**: Equine influenza virus; H3N8 EIV; Non-structural protein (NS) gene; NS1 truncation; South Korea

PMID: 23800580

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**Preliminary study about sublingual administration of bacteria-expressed pandemic H1N1 influenza vaccine in miniature pigs**

*J Microbiol.* 52(9):794-800.


'Co-corresponding: Daesub Song

Sublingual (SL) administration of influenza vaccine would be non-invasive and effective way to give human populations protective immunity against the virus, especially when pandemic influenza outbreaks. In this study, the efficacy of pandemic influenza virus-based subunit vaccines was tested after sublingual (SL) adjuvant administration in pigs. Eight specific pathogen-free Yucatan pigs were divided into 4 groups: nonvaccinated but challenged (A) and vaccinated and challenged (B, C, and D). The vaccinated groups were subdivided by vaccine type and inoculation route: SL subunit vaccine (hemagglutinin antigen 1 [HA1] + wild-type cholera toxin [wtCT]), B; IM subunit vaccine (HA1 + aluminum hydroxide, C); and IM inactivated vaccine (+ aluminum hydroxide, D). The vaccines were administered twice at a 2-week interval. All pigs were challenged with pandemic influenza virus (A/swine/GCVP-KS01/2009 [H1N1]) and monitored for clinical signs, serology, viral shedding, and histopathology. After vaccination, hemagglutination inhibition titre was higher in group D (320) than in the other vaccinated groups (40-80) at the time of challenge. The mobility and feed intake were reduced in group C. Both viral shedding and histopathological lesions were reduced in groups B and D. Although this study has limitation due to the limited number of pigs (2 pigs per a group), the preliminary data in this study provided the protective potential of SL administration of bacteria-expressed pandemic H1N1 influenza vaccine in pigs. There should be additional animal studies about effective adjuvant system and vaccine types for the use of SL influenza vaccination.

**Keywords**: Influenza; HA1; Pandemic; Pig; Sublingual vaccine

PMID: 25079956
Comparative pathology of pigs infected with Korean H1N1, H1N2, or H3N2 swine influenza A viruses

Lyoo KS, Kim JK, Jung K, Kang BK, Song D*. Corresponding: Daesub Song

BACKGROUND: The predominant subtypes of swine influenza A virus (SIV) in Korea swine population are H1N1, H1N2, and H3N2. The viruses are genetically close to the classical U.S. H1N1 and triple-reassortant H1N2 and H3N2 viruses, respectively. Comparative pathogenesis caused by Korean H1N1, H1N2, and H3N2 SIV was evaluated in this study.

FINDINGS: The H3N2 infected pigs had severe scores of gross and histopathological lesions at post-inoculation days (PID) 2, and this then progressively decreased. Both the H1N1 and H1N2 infected pigs lacked gross lesions at PID 2, but they showed moderate to severe pneumonia on PID 4, 7 and 14. The pigs infected with H1N1 had significant scores of gross and histopathological lesions when compared with the other pigs infected with H1N2, H3N2, and mock at PID 14. Mean SIV antigen-positive scores were rarely detected for pigs infected with H1N2 and H3N2 from PID 7, whereas a significantly increased amount of viral antigens were found in the bronchioles and alveolar epithelium of the H1N1 infected pigs at PID 14.

CONCLUSIONS: We demonstrated that Korean SIV subtypes had different pulmonary pathologic patterns. The Korean H3N2 rapidly induced acute lung lesions such as broncho-interstitial pneumonia, while the Korean H1N1 showed longer course of infection as compared to other strains.

Keywords: Comparative pathogenesis; Korean; Pathology; Pneumonia; Subtypes; Swine influenza A virus (SIV)

PMID: 25253051
Small molecules enable OCT4-mediated direct reprogramming into expandable human neural stem cells

*Co-corresponding: Janghwan Kim(janghwan.kim@kribb.re.kr)*

We developed a novel chemical cocktail that enables the generation of expandable hiNSCs from human fibroblasts transduced with OCT4 alone. We found that SOX2 overexpression combined with the chemical cocktail treatment was not sufficient to reprogram adult fibroblasts, suggesting SOX2-mediated hiNSC reprogramming may follow a different reprogramming trajectory from our OCT4-mediated hiNSC reprogramming. These results further highlight the unique ability of the OCT4/CASD strategy and chemical cocktail in hiNSC reprogramming. In the OCT4/CASD reprogramming paradigm, environmental cues were found to be critical for committing cell fates. Thus, the novel chemical cocktail can facilitate future investigations into the mechanistic basis of CASD reprogramming. Discovery of more small molecules and fine-tuning their combinations following the logic and strategy described here may increase the efficiency of hiNSC reprogramming and kinetics of this transition, and ultimately enable hiNSC reprogramming with only small molecules.

**Keywords**: Adult fibroblast; Chemical cocktail treatment; hiNSC reprogramming; Neural stem cell; OCT4-mediated direct reprogramming  
**PMID:** 24296783

Comparative receptor tyrosine kinase profiling identifies a novel role for AXL in human stem cell pluripotency

*Son MY, Seol B, Han YM, Cho YS*.  
*Corresponding: Yee Sook Cho(june@kribb.re.kr)*

The extensive molecular characterization of human pluripotent stem cells (hPSCs), human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs) is required before they can be applied in the future for personalized medicine and drug discovery. Despite the efforts that have been made with kinase analyses, we still lack in-depth insights into the molecular signatures of receptor tyrosine kinases (RTKs) that are related to pluripotency. Here, we present the first detailed and distinct repertoire of RTK characteristic for hPSC pluripotency by determining both the expression and phosphorylation profiles of RTKs in hESCs and hiPSCs using reverse transcriptase-polymerase chain reaction with degenerate primers that target conserved tyrosine kinase domains and phospho-RTK array, respectively. Among the RTKs tested, the up-regulation of EPHA1, ERBB2, FGFR4 and VEGFR2 and the down-regulation of AXL, EPHA4, PDGFRB and TYRO3 in terms of both their expression and phosphorylation levels were predominantly related to the maintenance of hPSC pluripotency. Notably, the specific inhibition of AXL was significantly advantageous in maintaining undifferentiated hESCs and hiPSCs and for the overall efficiency and kinetics of hiPSC generation. Additionally, a global phosphoproteomic analysis showed that ∼30% of the proteins (293 of 970 phosphoproteins) showed differential phosphorylation upon AXL inhibition in undifferentiated hPSCs, revealing the potential contribution of AXL-mediated phosphorylation dynamics to pluripotency-related signaling networks. Our findings provide a novel molecular signature of AXL in pluripotency control that will complement existing pluripotency-kinome networks.  
**Keywords**: AXL inhibition; hPSC pluripotency; Human pluripotent stem cells (hPSCs); Receptor tyrosine kinases (RTKs)  
**PMID:** 24218367
The direct lineage reprogramming of somatic cells to other lineages by defined factors has led to innovative cell-fate-change approaches for providing patient-specific cells. Recent reports have demonstrated that four pluripotency factors (Oct4, Sox2, Klf4, and c-Myc) are sufficient to directly reprogram fibroblasts to other specific cells, including induced neural stem cells (iNSCs). Here, we show that mouse fibroblasts can be directly reprogrammed into midbrain dopaminergic neuronal progenitors (iDPS) by temporal expression of the pluripotency factors and environment containing sonic hedgehog and fibroblast growth factor (FGF) 8. Within thirteen days, self-renewing and functional induced DPs (iDPS) were generated. Interestingly, the inhibition of both Jak and Gsk3β notably enhanced the iDP reprogramming efficiency. We confirmed the functionality of the iDPS by showing that the dopaminergic neurons generated from iDPS express midbrain markers, release dopamine, and show typical electrophysiological profiles. Our results demonstrate that the pluripotency factors-mediated direct reprogramming is an invaluable strategy for supplying functional and proliferating iDPS and may be useful for other neural progenitors required for disease modeling and cell therapies for neurodegenerative disorders.

Keywords: Direct lineage reprogramming; Dopaminergic neuronal progenitor; Functional induced DPs (iDPS); Mouse fibroblast; Pluripotency factor

PMID: 24145188
Expression signature defined by FOXM1-CCNB1 activation predicts disease recurrence in non-muscle-invasive bladder cancer

Kim SK, Roh YG, Park K, Kang TH, Kim WJ, Lee JS, Leem SH, Chu IS.*
*Co-corresponding: In-Sun Chu(chu@kribb.re.kr)

PURPOSE: Although standard treatment with transurethral resection and intravesical therapy (IVT) is known to be effective, to address the clinical behavior of non-muscle-invasive bladder cancer (NMIBC), many patients fail to respond to the treatment and frequently experience disease recurrence. Here, we aim to identify a prognostic molecular signature that predicts the NMIBC heterogeneity and response to IVT.

EXPERIMENTAL DESIGN: We analyzed the genomic profiles of 102 patients with NMIBC to identify a signature associated with disease recurrence. The validity of the signature was verified in three independent patient cohorts (n = 658). Various statistical methods, including a leave-one-out cross-validation and multivariate Cox regression analyses, were applied to identify a signature. We confirmed an association between the signature and tumor aggressiveness with experimental assays using bladder cancer cell lines.

RESULTS: Gene expression profiling in 102 patients with NMIBC identified a CCNB1 signature associated with disease recurrence, which was validated in another three independent cohorts of 658 patients. The CCNB1 signature was shown to be an independent risk factor by a multivariate analysis and subset stratification according to stage and grade [HR, 2.93; 95% confidence intervals (CI), 1.302-6.594; P = 0.009]. The subset analysis also revealed that the signature could identify patients who would benefit from IVT. Finally, gene network analyses and experimental assays indicated that NMIBC recurrence could be mediated by FOXM1-CCNB1-Fanconi anemia pathways.

CONCLUSIONS: The CCNB1 signature represents a promising diagnostic tool to identify patients with NMIBC who have a high risk of recurrence and to predict response to IVT.

Keywords: Disease recurrence; FOXM1-CCNB1 activation; Intravesical therapy (IVT); Non-muscle-invasive bladder cancer (NMIBC)

Identification of ethnically specific genetic variations in pan-asian ethnos

*Co-corresponding: Byungwook Lee(bulee@kribb.re.kr), Jin Ok Yang(joy@kribb.re.kr)

Asian populations contain a variety of ethnic groups that have ethnically specific genetic differences. Ethnic variants may be highly relevant in disease and human differentiation studies. Here, we identified ethnically specific variants and then investigated their distribution across Asian ethnic groups. We obtained 58,960 Pan-Asian single nucleotide polymorphisms of 1,953 individuals from 72 ethnic groups of 11 Asian countries. We selected 9,306 ethnic variant single nucleotide polymorphisms (ESNPs) and 5,167 ethnic variant copy number polymorphisms (ECNPs) using the nearest shrunk centroid method. We analyzed ESNPs and ECNPs in 3 hierarchical levels: superpopulation, subpopulation, and ethnic population. We also identified ESNP- and ECNP-related genes and their features. This study represents the first attempt to identify Asian ESNP and ECNP markers, which can be used to identify genetic differences and predict disease susceptibility and drug effectiveness in Asian ethnic populations.

Keywords: DNA copy number variation; Classification; Ethnic group; Genotype; Single nucleotide polymorphism

PMID: 24748860
2-cys peroxiredoxins: emerging hubs determining redox dependency of Mammalian signaling networks

*First: Jinah Park

Mammalian cells have a well-defined set of antioxidant enzymes, which includes superoxide dismutases, catalase, glutathione peroxidases, and peroxiredoxins. Peroxiredoxins are the most recently identified family of antioxidant enzymes that catalyze the reduction reaction of peroxides, such as H$_2$O$_2$. In particular, typical 2-Cys peroxiredoxins are the featured peroxidase enzymes that receive the electrons from NADPH by coupling with thioredoxin and thioredoxin reductase. These enzymes distribute throughout the cellular compartments and, therefore, are thought to be broad-range antioxidant defenders. However, recent evidence demonstrates that typical 2-Cys peroxiredoxins play key signal regulatory roles in the various signaling networks by interacting with or residing near a specific redox-sensitive molecule. These discoveries help reveal the redox signaling landscape in mammalian cells and may further provide a new paradigm of therapeutic approaches based on redox signaling.

**Keywords**: 2-Cys peroxiredoxins; Antioxidant enzyme; Cellular compartment; Redox signaling; Signaling network

PMID: 24672551
Protein NMR structures refined without NOE data


*Corresponding: Jinhyuk Lee@kribb.re.kr

The refinement of low-quality structures is an important challenge in protein structure prediction. Many studies have been conducted on protein structure refinement; the refinement of structures derived from NMR spectroscopy has been especially intensively studied. In this study, we generated flat-bottom distance potential instead of NOE data because NOE data have ambiguity and uncertainty. The potential was derived from distance information from given structures and prevented structural dislocation during the refinement process. A simulated annealing protocol was used to minimize the potential energy of the structure. The protocol was tested on 134 NMR structures in the Protein Data Bank (PDB) that also have X-ray structures. Among them, 50 structures were used as a training set to find the optimal "width" parameter in the flat-bottom distance potential functions. In the validation set (the other 84 structures), most of the 12 quality assessment scores of the refined structures were significantly improved (total score increased from 1.215 to 2.044). Moreover, the secondary structure similarity of the refined structure was improved over that of the original structure. Finally, we demonstrate that the combination of two energy potentials, statistical torsion angle potential (STAP) and the flat-bottom distance potential, can drive the refinement of NMR structures.

**Keywords**: Flat-bottom distance potential; NMR structure; NOE data; Statistical torsion angle potential (STAP)

PMID: 25279564

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**Article 215**

Mg²⁺ effect on argonaute and RNA duplex by molecular dynamics and bioinformatics implications


*Corresponding: Jinhyuk Lee@kribb.re.kr

RNA interference (RNAi), mediated by small non-coding RNAs (e.g., miRNAs, siRNAs), influences diverse cellular functions. Highly complementary miRNA-target RNA (or siRNA-target RNA) duplexes are recognized by an Argonaute family protein (Ago2), and recent observations indicate that the concentration of Mg²⁺ ions influences miRNA targeting of specific miRNAs, thereby modulating miRNA-mRNA networks. In the present report, we studied the thermodynamic effects of differential [Mg²⁺] on slicing (RNA silencing cycle) through molecular dynamics simulation analysis, and its subsequent statistical analysis. Those analyses revealed different structural conformations of the RNA duplex in Ago2, depending on Mg²⁺ concentration. We also demonstrate that cation effects on Ago2 structural flexibility are critical to its catalytic/functional activity, with low [Mg²⁺] favoring greater Ago2 flexibility (e.g., greater entropy) and less miRNA/mRNA duplex stability, thus favoring slicing. The latter finding was supported by a negative correlation between expression of an Mg²⁺ influx channel, TRPM7, and one miRNA's (miR-378) ability to downregulate its mRNA target, TMEM245. These results imply that thermodynamics could be applied to siRNA-based therapeutic strategies, using highly complementary binding targets, because Ago2 is also involved in RNAi slicing by exogenous siRNAs. However, the efficacy of a siRNA-based approach will differ, to some extent, based on the Mg²⁺ concentration even within the same disease type; therefore, different siRNA-based approaches might be considered for patient-to-patient needs.

**Keywords**: Argonaute family protein (Ago2); miRNA targeting; RNA duplex; RNA interference (RNAi)

PMID: 25330448
Positive feedback loop between Sox2 and Sox6 inhibits neuronal differentiation in the developing central nervous system

*Co-first: Jihae Seo

How a pool of undifferentiated neural progenitor cells is maintained in the developing nervous system is an issue that remains unresolved. One of the key transcription factors for self-renewal of these cells is Sox2, the forced expression of which has been shown to inhibit neuronal differentiation in vivo. To dissect the molecular mechanisms of Sox2 activity, a ChIP-on-chip assay has been carried out for Sox2, and multiple candidate direct target genes have been isolated. In this report, we provide evidence indicating that Sox6, which like Sox2 belongs to the SRY-related HMG box transcription factor family, is a bona-fide direct regulatory target of Sox2. In vivo, Sox6 expression is seen with a temporal lag in Sox2-positive neural precursor cells in the ventricular zone, and Sox2 promotes expression of Sox6 as a transcriptional activator. Interestingly, gain- and loss-of-function assays indicate that Sox6 in turn is required for the maintenance of Sox2 expression, suggesting that a positive feedback loop, which functions to inhibit premature neuronal differentiation, exists between the two transcription factors.

**Keywords**: CNS; SoxB1; SoxD; Neural development; Neural stem cell

PMID: 24501124

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Effects of osmolytes on arginine kinase from Euphausia superba: A study on thermal denaturation and aggregation

Fang NY, Lee J*, Yin SJ, Wang W, Wang ZJ, Yang JM, Qian GY, Si YX, Park YD.
*Co-first: Jinhyuk Lee(jinhyuk@kribb.re.kr)

Investigations of energy-related enzymatic properties may provide valuable information about the mechanisms that are involved in the adaptation to extreme climatic environments. The protective effects of osmolytes on the thermal denaturation and aggregation of arginine kinase from E. superba (ESAK) was investigated. When the concentration of glycine, proline and glycerol increased, the relative activation was significantly enhanced, while the aggregation of ESAK during thermal denaturation was decreased. Spectrofluorometry results showed that the presence of these three osmolytes significantly decreased the tertiary structural changes of ESAK and that thermal denaturation directly induced ESAK aggregation. The results demonstrated that glycine, proline and glycerol not only prevented ESAK from inactivation and unfolding but also inhibited aggregation by stabilizing the ESAK conformation. We measured the ORF gene sequence of ESAK by RACE, and built the 3D structure of ESAK and osmolytes by homology models. The results showed that the docking energy was relatively low and that the clustering groups were spread to the surface of ESAK, indicating that osmolytes directly protect the surface of the protein. Our study provides important insight into the protective effects of osmolytes on ESAK folding.

**Keywords**: Arginine kinase; Docking simulation; Euphausia superba; Osmolytes; RACE; Thermal denaturation
Effects of hydroxysafflor yellow A on ALDH1: Inhibition kinetics and molecular dynamics simulation


Zhang X, Shen D, Lu ZR, Zhan Y, Si N, Li MM, Yang JM, Zhou HM, Park YD, Zhang Q, Lee J.* Co-corresponding: Jinhyuk Lee(jinhyuk@kribb.re.kr)

Hydroxysafflor yellow A (HSYA) is a potent natural antioxidant that displays important neuroprotective activity. Inhibition of aldehyde dehydrogenase 1 (ALDH1) has attracted the attention of researchers due to its overexpression in several types of cancers. We studied the effects of HSYA on ALDH1 by evaluating the inhibitory kinetics based on its antioxidant properties and performing computational simulation integrating methods. HSYA reversibly inhibited human recombinant ALDH1 via non-competitive inhibition ($K_i = 0.267 \pm 0.024$ mM). We also investigated the tertiary structural changes via measuring intrinsic and ANS-binding fluorescence. The results indicated that the inactivation induced by HSYA was associated with structural changes. To obtain further information, we simulated the 3D structure of ALDH1 and conducted computational docking simulations as well as molecular dynamics simulations. The results indicated that 4 rings of HSYA interact with several residues near the ALDH1 active site. Our study provides insight into the inhibition of ALDH1 accompanied by structural changes. Based on its ALDH1-inhibiting effect and its potential as a natural antioxidant, HSYA is a potential agent for treating ALDH1-associated cancers.

**Keywords:** ALDH1; Docking simulation; Hydroxysafflor yellow A; Inhibition kinetics; Molecular dynamics; Non-competitive inhibitor

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The inhibitory role of Co$^{2+}$ on α-glucosidase: Inhibition kinetics and molecular dynamics simulation integration study


Li X, Lu ZR, Shen D, Zhan Y, Yang JM, Park YD, Zhou HM, Sheng Q, Lee J.* Co-corresponding: Jinhyuk Lee(jinhyuk@kribb.re.kr)

It is important to study enzyme inhibition of α-glucosidase (EC 3.2.1.20) due to its clinical relevance as a target enzyme for the treatment of type 2 diabetes mellitus. In this study, we investigated Co$^{2+}$-induced inhibition as well as structural changes of α-glucosidase integrated with computational simulations. α-Glucosidase activity was inhibited by Co$^{2+}$ in a dose-dependent manner. Co$^{2+}$ inhibited α-glucosidase in a parabolic non-competitive inhibition reaction ($K_i = 0.78 \pm 0.08$ mM) and directly induced regional unfolding of the enzyme resulting in a slight decrease in hydrophobic surface. The computational simulations using molecular dynamics showed that simulation with Co$^{2+}$ resulted in a loss of secondary structure by positioning Co$^{2+}$ near the active site for glucose production, implying that the Co$^{2+}$ stimulate enzyme unfolding. Our study revealed the mechanism of Co$^{2+}$ ligand binding mediated structural changes as well as inhibition of α-glucosidase activity, and suggested that Co$^{2+}$ could act as a potent inhibitor of α-glucosidase for the treatment of type 2 diabetes mellitus.

**Keywords:** Co$^{2+}$; Inhibition kinetics; Molecular dynamics; Unfolding; α-Glucosidase
Bio-Therapeutics Research Institute

- Natural Medicine Research Center
- Chemical Biology Research Center
- Incurable Diseases Therapeutics Research Center
Homoeogonol attenuates the asthmatic responses induced by ovalbumin challenge

Arch Pharm Res. 37(9):1201-10.

Shin IS, Ahn KS, Shin NR, Jeon CM, Kwon OK, Chin YW, Lee K, Oh SR*. Corresponding: Sei-Ryang Oh(seiryang@kribb.re.kr)

Homoeogonol is a lignan derived from styrrax lignolide A, which was isolated from Styrax japonica, a medicinal plant widely used for treatment of inflammatory diseases in Korea. We investigated the efficacy of homoeogonol for the treatment of allergic asthma using an ovalbumin (OVA)-induced murine asthma model. The mice were sensitized through intraperitoneal injections of OVA on days 0 and 14. On days 21, 22 and 23 after the initial OVA sensitization, the mice were received OVA airway challenge. Homoeogonol was administered by oral gavage at a dose of 30 mg/kg 1 h prior to the OVA challenge. The homoeogonol-treated mice exhibited reduced inflammatory cell counts and Th2 cytokines in BALF, AHR, and IgE in the serum compared with the OVA-sensitized/challenged mice. The histological analysis of the lung tissue revealed that the administration of homoeogonol attenuated the airway inflammation and the mucus overproduction in airway epithelial lesions induced by OVA through a reduction in expression of inducible nitric oxide synthase and matrix metalloproteinase-9. These findings indicate that homoeogonol effectively suppresses the asthmatic responses induced by OVA challenge and suggests that homoeogonol exhibits potential as therapeutic drug for allergic asthma.

Keywords: Asthma; Cytokine; Homoeogonol; Inducible nitric oxide synthase; Matrix metalloproteinase-9

PMID: 24424605

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Anti-inflammatory effect of mangostenone F in lipopolysaccharide-stimulated RAW264.7 macrophages by suppressing NF-κB and MAPK activation

Biomol Ther. 22(4):288-94.

Cho BO, Ryu HW*, So Y, Lee CW, Jin CH, Yook HS, Jeong YW, Park JC, Jeong IY. Co-first: Hyung Won Ryu(ryuhw@kribb.re.kr)

Mangostenone F (MF) is a natural xanthone isolated from Garcinia mangostana. However, little is known about the biological activities of MF. This study was designed to investigate the anti-inflammatory effect and underlying molecular mechanisms of MF in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. MF dose-dependently inhibited the production of NO, iNOS, and pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) in LPS-stimulated RAW264.7 macrophages. Moreover, MF decreased the NF-κB luciferase activity and NF-κB DNA binding capacity in LPS-stimulated RAW264.7 macrophages. Furthermore, MF suppressed the NF-κB activation by inhibiting the degradation of IκBα and nuclear translocation of p65 subunit of NF-κB. In addition, MF attenuated the AP-1 luciferase activity and phosphorylation of ERK, JNK, and p38 MAP kinases. Taken together, these results suggest that the anti-inflammatory effect of MF is associated with the suppression of NO production and iNOS expression through the down-regulation of NF-κB activation and MAPK signaling pathway in LPS-stimulated RAW264.7 macrophages.

Keywords: Anti-inflammatory effect; iNOS; MAPK; Mangostenone F; NF-κB; NO

PMID: 25143806
The involvement of Nrf2 in the protective effects of diallyl disulfide on carbon tetrachloride-induced hepatic oxidative damage and inflammatory response in rats


*Co-corresponding: In-Sik Shin

This study investigated the potential effect of diallyl disulfide (DADS) against carbon tetrachloride (CCL4)-induced oxidative hepatic damage and inflammatory response in rat liver. DADS at doses of 50 and 100 mg/kg/day was administered orally once daily for 5 days, prior to CCL4 administration. Pretreatment with DADS attenuated CCL4-induced elevated serum transaminase activities and histopathological alterations in liver. It prevented the hepatocellular apoptotic changes with induction of Bcl-2-associated X (Bax), cytochrome c, and caspase-3 caused by CCL4. An increase in the nuclear translocation of nuclear factor-kappaB (NF-κB) and phosphorylation of I kappaB alpha (IκBα) was observed in the livers of CCL4-treated rats that coincided with induction of inflammatory mediators or cytokines. In contrast, DADS inhibited NF-κB translocation and IκBα phosphorylation, and that subsequently decreased inflammatory mediators. Furthermore, DADS prevented CCL4-induced depletion of cytosolic nuclear factor E2-related factor 2 (Nrf2) and suppression of nuclear translocation of Nrf2, which, in turn, up-regulated phase II/antioxidant enzyme activities. Taken together, these results demonstrate that DADS increases the expression of phase II/antioxidant enzymes and simultaneously decreases the expression of inflammatory mediators in CCL4-induced liver injury. These findings indicate that DADS induces antioxidant defense mechanism by activating Nrf2 pathway and reduces inflammatory response by inhibiting NF-κB activation.

Keywords: Carbon tetrachloride; Diallyl disulfide; Hepatotoxicity; Nuclear factor E2-related factor 2; Nuclear factor kappaB

Induction of cytochrome P450 3A1 expression by diallyl disulfide: protective effects against cyclophosphamide-induced embryo-fetal developmental toxicity


Kim SH, Lee IC, Back HS, Moon C, Kim SH, Yoo JC, Shin IS', Kim JC.

*Co-corresponding: In-Sik Shin

The protective effects of diallyl disulfide (DADS) on cyclophosphamide (CP)-induced developmental toxicity and the possible mechanisms involved in this protection were investigated in rats. In order to study the mechanisms involved in the protection, we examined the effects of DADS on the expression of cytochrome P450 (CYP) 3A1 in the maternal liver and placenta and oxidative stress in the maternal hepatic tissues caused by CP. CP caused severe embryo-fetal developmental toxicity and hepatic oxidative stress. In contrast, DADS treatment significantly attenuated CP-induced developmental toxicity and oxidative damage in the maternal liver. DADS also significantly increased expression of CYP3A1 in the maternal liver and placenta. These results indicate that the protective effects of DADS against CP-induced developmental toxicity may be due to its ability to promote detoxification of CP, primarily by inducing CYP3A1 expression in the maternal liver and placenta, and its potent antioxidant effects.

Keywords: Cyclophosphamide; Cytochrome P450 3A1; Developmental toxicity; Diallyl disulfide; Oxidative stress

PMID: 24769015
Melatonin reduces airway inflammation in ovalbumin-induced asthma


Corresponding: Kyung-Seop Ahn(ksahn@kribb.re.kr)

Asthma is a common chronic inflammatory airway disease that is recognized as a major public health problem. In this study, we evaluated the effects of melatonin on allergic asthma using a murine model of ovalbumin (OVA)-induced allergic asthma and BEAS-2B cells. To induce allergic asthma, the mice were sensitized and airway-challenged with OVA. Melatonin was administered by intraperitoneal injection once per day at doses of 10 and 15 mg/kg from days 21 to 23 after the initial OVA sensitization. We investigated the effects of melatonin on proinflammatory cytokines and matrix metalloproteinase-9 (MMP-9) activity and expression in tumor necrosis factor (TNF)-α-stimulated BEAS-2B cells. The administration of melatonin significantly decreased the number of inflammatory cells, airway hyperresponsiveness, and immunoglobulin (Ig) E with reductions in interleukin (IL)-4, IL-5, and IL-13. Melatonin attenuated the airway inflammation and the mucus production in lung tissue and significantly suppressed elevated MMP-9 expression and activity induced by an OVA challenge. In TNF-α-stimulated BEAS-2B cells, treatment with melatonin significantly reduced the levels of proinflammatory cytokines and lowered the expression and activity of MMP-9. These results indicate that melatonin effectively suppressed allergic asthma induced by an OVA challenge. The results suggest a potential role for melatonin in treating asthma.

**Keywords**: Airway inflammation; Allergic asthma; Melatonin; Metalloproteinase-9 (MMP-9) activity; Ovalbumin (OVA)

PMID: 25161126

EC-18, a synthetic monoacetyldiglyceride (1-palmitoyl-2-linoleoyl-3- acetylglycerol), attenuates the asthmatic response in an aluminum hydroxide/ovalbumin-induced model of asthma


Shin IS, Shin NR, Jeon CM, Kwon OK, Sohn KY, Lee TS, Kim JW, Ahn KS, Oh SR'.
Corresponding: Sei-Ryang Oh(seiryang@kribb.re.kr)

EC-18 is a synthetic monoacetyldiglyceride that is a major constituent in antlers of Sika deer (*Cervus nippon* Temminck). In this study, we evaluated the protective effects of EC-18 on Th2-type cytokines, eosinophil infiltration, and other factors in an aluminum hydroxide/ovalbumin (OVA)-induced murine asthma model. Mice were sensitized on days 0 and 14 by intraperitoneal injection of OVA with aluminum hydroxide. On days 21, 22 and 23 after the initial sensitization, the mice received an airway challenge with OVA for 1h using an ultrasonic nebulizer. EC-18 was administered to mice by oral gavage at doses of 30mg/kg and 60mg/kg once daily from day 18 to 23. Methacholine responsiveness was measured 24h after the final OVA challenge, and the bronchoalveolar lavage fluid (BALF) was collected 48h after the final OVA challenge. EC-18 significantly reduced methacholine responsiveness, T helper type 2 (Th2) cytokines, eotaxin-1, immunoglobulin (Ig) E, IgG, and the number of inflammatory cells. In addition, EC-18-treated mice exhibited the reduction in the expression of inducible nitric oxide synthase (iNOS) in lung tissue. In the histological analysis using hematoxylin-eosin stain and periodic acid-Schiff stain, EC-18 attenuated the infiltration of inflammatory cells into the airway and reduced the level of mucus production. Our results showed that EC-18 effectively suppressed the asthmatic response induced by OVA challenge. These effects were considered to be associated with iNOS suppression. In conclusion, this study suggests that EC-18 may be a therapeutic agent for allergic asthma.

**Keywords**: Asthma; Cytokine; EC-18; Inducible nitric oxide synthase; Monoacetyldiacylglyceride

PMID: 24269625
**Siegesbeckia glabrescens** attenuates allergic airway inflammation in LPS-stimulated RAW 264.7 cells and OVA induced asthma murine model


Jeon CM, Shin IS, Shin NR, Hong JM, Kwon OK, Kim HS, Oh SR, Myung PK, Ahn KS.  
*C-responding: Kyung-Seop Ahn(ksahn@kribb.re.kr)

**Siegesbeckia glabrescens** (SG) is a plant growing in Korea that is used as a traditional medicine for various inflammatory diseases. In this study, we investigated the protective effects of SG extract on allergic asthma in an ovalbumin (OVA)-induced asthma murine model and lipopolysaccharide (LPS)-stimulated RAW264.7 cells. Female BALB/c mice were sensitized by intraperitoneal injection of OVA on days 0 and 14 and then challenged with OVA from days 21 to 23. SG (30mg/kg) was administered by oral gavage 1h before the OVA challenge. LPS-stimulated RAW264.7 cells were evaluated to determine their levels of nitric oxide (NO). The SG significantly reduced the number of inflammatory cells in bronchoalveolar lavage (BAL) fluid and also reduced IL-4, IL-5, IL-13, eotaxin and immunoglobulin E in OVA-sensitized/challenged mice. SG also effectively reduced airway inflammation and mucus overproduction in lung tissue in addition to decreasing the expression of iNOS and COX-2. In LPS-stimulated RAW264.7 cells, SG treatment significantly reduced the levels of NO. These findings indicate that SG effectively suppressed inflammatory responses, and its effects appear to be related to reduction in iNOS and COX-2 expression. Therefore, we suggest that SG may have potential use as a therapeutic agent for inflammatory diseases such as allergic asthma.

**Keywords**: Asthma; Cytokine; Inducible nitric oxide synthase; Inflammation; *Siegesbeckia glabrescens

PMID: 25066761

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**Rapid identification of cholinesterase inhibitors from the seedcases of mangosteen using an enzyme affinity assay**


*Co-first: Hyung Won Ryu(ryuhw@kribb.re.kr), Sei-Ryang Oh(seiryang@kribb.re.kr)

Enzyme binding affinity has been recently introduced as a selective screening method to identify bioactive substances within complex mixtures. We used an assay which identified small molecule binders of acetylcholinesterase (AChE) using the following series of steps: incubation of enzyme with extract; centrifugation and filtration; identification of small molecule content in the flow through. The crude extract contained 10 peaks in the UPLC chromatogram. However, after incubation the enzyme, six peaks were reduced, indicating these compounds bound AChE. All these isolated compounds (2, 3, and 5-8) significantly inhibited human AChE with IC₅₀ = 5.4-15.0 μM and butrylcholinesterase (IC₅₀ = 0.7-11.0 μM). All compounds exhibited reversible mixed kinetics. Consistent with the binding screen and fluorescence quenching, γ-mangostin 6 had a much higher affinity for AChE than 9-hydroxyxalabaxanthone 9. This validates this screening protocol as a rapid method to identify inhibitors of AChE.

**Keywords**: Cholinesterase; Enzyme binding affinity; Fluorescence quenching; *Garcinia mangostana*; UPLC-PDA-QTOF-MS

PMID: 24446804
Discrimination of white ginseng origins using multivariate statistical analysis of data sets


Song HH, Moon JY, Ryu HW, Noh BS, Kim JH, Lee HK, Oh SR.
’Corresponding: Sei-Ryang Oh(sciryang@kribb.re.kr)

BACKGROUND: White ginseng (Panax ginseng Meyer) is commonly distributed as a health food in food markets. However, there is no practical method for distinguishing Korean white ginseng (KWG) from Chinese white ginseng (CWG), except for relying on the traceability system in the market.

METHODS: Ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry combined with orthogonal partial least squares discrimination analysis (OPLS-DA) was employed to discriminate between KWG and CWG.

RESULTS: The origins of white ginsengs in two test sets (1.0 μL and 0.2 μL injections) could be successfully discriminated by the OPLS-DA analysis. From OPLS-DA S-plots, KWG exhibited tentative markers derived from ginsenoside Rf and notoginsenoside R3 isomer, whereas CWG exhibited tentative markers derived from ginsenoside Ro and chikusetsusaponin Iva.

CONCLUSION: Results suggest that ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry coupled with OPLS-DA is an efficient tool for identifying the difference between the geographical origins of white ginsengs.

Keywords: Panax ginseng Meyer; Ginsenoside; Metabolomics; OPLS-DA analysis; White ginseng

PMID: 25378993

Melatonin inhibits MUC5AC production via suppression of MAPK signaling in human airway epithelial cells


Shin IS, Park JW, Shin NR, Jeon CM, Kwon OK, Lee MY, Kim HS, Kim JC, Oh SR, Ahn KS.
’Corresponding: Kyung-Seop Ahn(ksahn@kribb.re.kr)

Mucus acts as a primary defense system in the airway against various stimuli. However, excess mucus production causes a reduction in lung function via limitation of the airflow in the airway of patients suffering from asthma or chronic obstructive pulmonary disease (COPD). In this study, we evaluated the effects of melatonin on the production of MUC5AC, a major constituent of the mucin that is secreted from the airway, using epidermal growth factor (EGF)-stimulated NCI-H292 cells, a human mucoepidermoid carcinoma cell line, and an ovalbumin (OVA)-induced asthma murine model. Melatonin treatment significantly reduced the mRNA and protein levels of MUC5AC and reduced interleukin (IL)-6 production in EGF-stimulated H292 cells. Melatonin markedly decreased the phosphorylation of MAPKs, including ERK1/2, JNK, and p-38, induced by EGF stimulation. These findings were consistent with the results using MAPK inhibitors. Particularly, co-treatment with melatonin and a MAPK inhibitor more effectively suppressed MAPK phosphorylation than treatment with a MAPK inhibitor alone, which resulted in a reduction in MUC5AC expression. In the asthma murine model, melatonin-treated mice exhibited a marked reduction in MUC5AC expression in the airway compared with the OVA-induced mice. These reductions were accompanied by reductions in proinflammatory cytokine production and inflammatory cell infiltration. Collectively, these findings indicate that melatonin effectively inhibits MUC5AC expression. These effects may be closely associated with the inhibition of MAPK phosphorylation. Furthermore, our study suggests that melatonin could represent a potential therapeutic for chronic airway diseases, such as asthma and COPD.

Keywords: Asthma; Chronic obstructive pulmonary disease (COPD); MAPK; Melatonin; MUC5AC; NCI-H292

PMID: 24720799
**Metabolomics investigation of flavonoid synthesis in soybean leaves depending on the growth stage**

**Metabolomics.** 10:833-41.

Song HH, Ryu HW, Lee KJ, Jeong IY, Kim DS, Oh SR.

Soybean (*Glycine max* L.) leaves have unique nutraceutical and pharmacological benefits, and have been widely used as a source of healthy and functional food stuffs in Korea. In this study, we investigated the phytochemical metabolomic changes of soybean leaves depending on growth stages (maturation period) assessed based on UPLC-QTOF-MS analysis. Principal component analysis was carried out to trace the metabolite profiles of the phytochemicals from the vegetable stage (1D) through the seven reproductive stages (R1-R7). On the loading plot, significant changes in the contents of metabolites were found during the growth, and eight flavonoid kaempferol glycosides (2, 3, 6, 8, and 10), daidzein (14), genistein (17), and coumestrol (19) were evaluated as growth markers among the 19 isolated metabolites. The kaempferol glycosides were increasingly synthesized from the 1D to the R6 stage but decreased rapidly at stages R7-R8. The extensively synthesized daidzein and genistein were shown during seed growth in the pod (R5-R6), while coumestrol was increased significantly at stages R7-R8 (maturity period). The synthetic pathway of the flavonoids could be elucidated based on the concentration of the individual metabolites. These results demonstrate that the metabolite production changed depending on the growth stage; a possible pathway could be deduced using metabolomic analysis to provide information regarding physiological characterization and optimal harvesting time for crops.

**Keywords:** Growth stage; Isoflavone; Kaempferol glycone; Pterocarpans; Soybean leaf; UPLC-QTOF-MS

**Anti-inflammatory activity of a methanol extract from *Ardisia tinctoria* on mouse macrophages and paw edema**


Kim HS, Park JW, Kwon OK, Kim JH, Oh SR, Lee HK, Bach TT, Quang BH, Ahn KS.

*Ardisia tinctoria* (AT) is a plant of the Myrsinaceae family. No studies on its anti-inflammatory effects have yet been reported. This study investigated the anti-inflammatory activity of AT. A non-cytotoxic methanol extract of AT inhibited the expression of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2), leading to significantly reduced levels of nitric oxide (NO) and prostaglandin E2 (PGE2) and of two proteins regulated by these, interleukin-1β (IL-1β) and IL-6, in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. The thickness of paw edema induced *in vivo* in mice by carrageenan administration was effectively reduced by the AT extract. Translocation of the nuclear factor-κB (NF-κB) subunit 65 (p65) into the nucleus and phosphorylation of mitogen-activated protein kinase kinase kinase (MEK) and extracellular signal-related kinase (ERK) were inhibited by the AT extract. Our results indicated that a methanol extract of AT downregulates the inflammatory response by blocking phosphorylation of MEK and ERK and activation of NF-κB. To the best of our knowledge, this is the first study of anti-inflammatory effects of an AT extract, and demonstrates its potential in the treatment of inflammatory diseases.

**Keywords:** *Ardisia tinctoria* (AT); Inflammation; MAPK; NF-κB; Nitric oxide (NO)

PMID: 24534870
**Article 232**

**Inhibitory effects of Picrasma quassioides (D.Don) Benn. on airway inflammation in a murine model of allergic asthma**


Shin NR, Shin IS, Jeon CM, Hong JM, Oh SR, Hahn KW, Ahn KS.*

*Co-corresponding: Kyung-Soop Ahn(ksahn@kribb.re.kr)

Picrasma quassioides (D.Don) Benn. (PQ) is used in traditional medicine for the treatment of inflammatory conditions, including gastritis. This study aimed to evaluate the inhibitory effects of PQ on the inflammatory responses in mice with allergic asthma induced by ovalbumin (OVA) and in lipopolysaccharide (LPS)-stimulated RAW264.7 cells.

To induce allergic asthma, the mice underwent OVA sensitization on days 0 and 14 and then were challenged with OVA from days 21-23. The mice were administered 15 and 30 mg/kg doses of PQ 1 h prior to the OVA challenge. The PQ treatment decreased the inflammatory cell count in the bronchoalveolar lavage fluid of the mice and reduced the levels of interleukin (IL)-4, IL-5, IL-13 and immunoglobulin (Ig)E when compared with those in the OVA group. The PQ treatment also reduced the airway hyperresponsiveness induced by the OVA challenge, attenuated the recruitment of inflammatory cells and the mucus production in the airways of the mice. In the LPS-stimulated RAW264.7 cells, the PQ treatment reduced the overexpression of inducible nitric oxide synthase (iNOS). The results indicated that PQ inhibits inflammatory responses in mice with OVA-sensitized/challenged allergic asthma and in LPS-stimulated RAW264.7 cells. These effects were considered to be associated with the suppression of iNOS expression. Therefore, PQ may have the potential to treat airway inflammatory diseases, including allergic asthma.

**Keywords**: Allergic asthma; Inflammatory response; iNOS expression; Picrasma quassioides (PQ)

PMID: 24927487

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**Article 233**

**Indacaterol inhibits tumor cell invasiveness and MMP-9 expression by suppressing IKK/NF-κB activation**


Lee SU, Ahn KS, Sung MH, Park JW, Ryu HW, Lee HJ, Hong ST, Oh SR.*

*Co-corresponding: Sei-Ryang Oh(seiryang@kribb.re.kr)

The β2 adrenergic receptor (ADRB2) is a G protein-coupled transmembrane receptor expressed in the human respiratory tract and widely recognized as a pharmacological target for treatments of asthma and chronic obstructive pulmonary disorder (COPD). Although a number of ADRB2 agonists have been developed for use in asthma therapy, indacaterol is the only ultra-long-acting inhaled β2-agonist (LABA) approved by the FDA for relieving the symptoms in COPD patients. The precise molecular mechanism underlying the pharmacological effect of indacaterol, however, remains unclear. Here, we show that β-arrestin-2 mediates the internalization of ADRB2 following indacaterol treatment. Moreover, we demonstrate that indacaterol significantly inhibits tumor necrosis factor-α (TNF-α)-induced NF-κB activity by reducing levels of both phosphorylated-IKK and -IκBα, thereby decreasing NF-κB nuclear translocation and the expression of MMP-9, an NF-κB target gene. Subsequently, we show that indacaterol significantly inhibits TNF-α/NF-κB-induced cell invasiveness and migration in a human cancer cell line. In conclusion, we propose that indacaterol may inhibit NF-κB activity in a β-arrestin2-dependent manner, preventing further lung damage and improving lung function in COPD patients.

**Keywords**: ADRB2; Asthma; Chronic obstructive pulmonary disease (COPD); MMP-9; NF-κB; Indacaterol; Invasion

PMID: 25134539
Chemical Biology Research Center

Article 234

Chemical constituents of the Korean endangered species 
*Rhododendron brachycarpum*


Zhou W, Oh J, Li W, Kim DW, Yang MH, Jang JH, Ahn JS*, Lee SH, Na M.

*Co-corresponding: Jong Seog Ahn(jsahn@kribb.re.kr)*

We herein describe the isolation and structural characterization of a new kaurane-type diterpenoid glycoside, β-sophorosyl ent-16-a-hydroxykauran-18-oate, along with 52 known compounds and discuss the chemotaxonomic significance of these identified compounds. This study is the first confirmation of the presence of 21 compounds isolated from the family Ericaceae, two of these from the genus *Rhododendron*. Of the 53 compounds reported in this study, 22 compounds were obtained from *R. brachycarpum* for the first time. Highly oxygenated diterpenoids have been reported from genera belonging to the Ericaceae family such as *Kalmia, Leucothoe, Lyonia, Fieris* and *Rhododendron*, whereas ent-kaurane diterpenoids, believed to be a biosynthetic precursor of the aforementioned diterpenoids, have been rarely isolated from this family. Our study is the first validation of the presence of triterpenoid saponins from the Ericaceae even though similar triterpenoid saponins were identified in *R. luteum* and *R. molle*. The Korean endangered species *R. brachycarpum* is of taxonomic importance given our study identifying a wide range of phytochemical compounds that complement the current chemotaxonomic profile of the family Ericaceae. These results also exhibit that rare and endangered plant species can serve as a new resource for providing chemotaxonomically significant entities, which might warrant future conservation efforts. Our study has shown that the *R. brachycarpum* contains compounds that differ from other species in the genus *Rhododendron*, which requires more research to evaluate which compounds can be serve as chemotaxonomic markers for the identification of *R. brachycarpum*.

**Keywords**: β-sophorosyl ent-16-a-hydroxykauran-18-oate; Chemotaxonomy; Endangered species; Ericaceae; *Rhododendron brachycarpum*

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Article 235

**Title**: Boseongazepines A-C, pyrrolobenzodiazepine derivatives from a *Streptomyces* sp. 11A057


*Co-corresponding: Jong Seog Ahn(jsahn@kribb.re.kr)*, Bo-Yeon Kim(bykim@kribb.re.kr)

Three new pyrrolobenzodiazepine derivatives, boseongazepines A-C (1-3), were isolated from a culture broth of *Streptomyces* sp. 11A057, together with the known compound usabamycin B (4). The structures of 1-4 were determined through the analysis of spectroscopic data including extensive 1D-, 2D-NMR, and MS techniques. Cell growth inhibition effects of these compounds were evaluated against Jurkat, K-562, HL-60, and HepG2 cell lines.

**Keywords**: Boseongazepines; Cytotoxicity; Pyrrolobenzodiazepine; *Streptomyces*

PMID: 24613164

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**Figure 1. Structures of compounds 1-4.**
Biosynthesis of methylated resveratrol analogs through the construction of an artificial biosynthetic pathway in *E. coli*


*Corresponding: Young-Soo Hong(hongsoo@kribb.re.kr)*

**BACKGROUND:** Methylated resveratrol analogs show similar biological activities that are comparable with those of the resveratrol. However, the methylated resveratrol analogs exhibit better bioavailability as they are more easily transported into the cell and more resistant to degradation. Although these compounds are widely used in human health care and in industrial materials, at present they are mainly obtained by extraction from raw plant sources. Accordingly their production can suffer from a variety of economic problems, including low levels of productivity and/or heterogeneous quality. On this backdrop, large-scale production of plant metabolites via microbial approaches is a promising alternative to chemical synthesis and extraction from plant sources.

**RESULTS:** An *Escherichia coli* system containing an artificial biosynthetic pathway that produces methylated resveratrol analogues, such as pinostilbene (3,4′-dihydroxy-5-methoxystilbene), 3,5-dihydroxy-4′-methoxystilbene, 3,4′-dimethoxy-5-hydroxystilbene, and 3,5,4′-trimethoxystilbene, from simple carbon sources is developed. These artificial biosynthetic pathways contain a series of codon-optimized O-methytransferase genes from sorghum in addition to the resveratrol biosynthetic genes. The *E. coli* cells that harbor pET-opTLO1S or pET-opTLO3S produce the one-methyl resveratrol analogues of 3,5-dihydroxy-4′-methoxystilbene and pinostilbene, respectively. Furthermore, the *E. coli* cells that harbor pET-opTLO13S produce 3,5-dihydroxy-4′-methoxystilbene, *bis*-methyl resveratrol (3,4′-dimethoxy-5-hydroxystilbene), and *tri*-methyl resveratrol (3,5,4′-trimethoxystilbene).

**CONCLUSIONS:** Our strategy demonstrates the first harness microorganisms for *de novo* synthesis of methylated resveratrol analogs used as a single vector system joined with resveratrol biosynthetic genes and sorghum two resveratrol O-methytransferase genes. Thus, this is also the first report on the production of the methylated resveratrol compounds *bis*-methyl and *tri*-methyl resveratrol (3,4′-dimethoxy-5-hydroxystilbene and 3,5,4′-trimethoxystilbene) in the *E. coli* culture. Thus, the production of the methylated resveratrol compounds was performed on the simple *E. coli* medium without precursor feeding in the culture.

**Keywords:** Artificial biosynthetic pathway; Harness microorganism; Methylated resveratrol compound; Plant metabolites

PMID: 25033820

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Anti-tumor activity of WK88-1, a novel geldanamycin derivative, in gefitinib-resistant non-small cell lung cancers with Met amplification


Jang WJ, Jung SK, Kang JS, Jeong JW, Bae MK, Joo SH, Park GH, Kundu JK, Hong YS*, Jeong CH.  
*Co-corresponding: Young-Soo Hong(hongsoo@kribb.re.kr)*

Although epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) have been introduced for the treatment of non-small cell lung cancer (NSCLC), the emergence of secondary T790M mutation in EGFR or amplification of the Met proto-oncogene restrain the clinical success of EGFR-TKIs. Since heat shock protein-90 (Hsp90) stabilizes various oncoproteins including EGFR and c-Met, the inhibition of Hsp90 activity appears as a rational strategy to develop anticancer drugs. Despite preclinical efficacy of geldanamycin/masnaycin (GA)-derivatives containing benzoquinone moiety as Hsp90 inhibitors, the hepatotoxicity of these GA-derivatives restricts their therapeutic benefit. We have prepared WK-88 series of GA-derivatives, which lack the benzoquinone moiety. In this study, we have examined the anticancer effects of WK88-1 in Met-amplified- and gefitinib-resistant (HCC827GR) NSCLC cells and its parental HCC827 cells. Treatment with WK88-1 reduced the cell viability in both HCC827 and HCC827GR cells, which was associated with marked decrease in the constitutive expression of Hsp90 client proteins, such as EGFR, ErbB2, ErbB3, Met and Akt. Moreover, WK88-1 attenuated phosphorylation of these Hsp90 client proteins and reduced the anchorage-independent growth of HCC827GR cells. Administration of WK88-1 did not cause hepatotoxicity in animals and significantly reduced the growth of HCC827GR cells xenograft tumors in nude mice. Our study provides evidence that ErbB3 might be a client for Hsp90 in Met-amplified NSCLCs. In conclusion, we demonstrate that inhibition of Hsp90 dampens the activation of EGFR- or c-Met-mediated survival of Met-amplified NSCLCs and that WK88-1 as a Hsp90 inhibitor alleviates gefitinib resistance in HCC827GR cells.

**Keywords:** Gefitinib; Hsp90; Non-benzoquinone GA; Non-small cell lung cancer (NSCLC); WK88-1

PMID: 25117641
Inhibition of indoleamine 2,3-dioxygenase by thielavin derivatives from a soil fungus, Coniochaeta sp. 10F058


*Co-corresponding: Jong Seog Ahn(jsahn@kribb.re.kr), Bo-Yeon Kim(bykim@kribb.re.kr)

Indoleamine 2,3-dioxygenase (IDO) is an intracellular monomeric heme-containing protein that catalyzes the initial step of tryptophan catabolism via the kynurenine pathway (KP). We have commenced a screening program to identify and develop new IDO inhibitors derived from microbial metabolites. New benzoate trimer, named thielavin Q together with two known thielavin F and B, were isolated from the fermentation broth of Coniochaeta sp. 10F058. Thielavin derivatives have been reported as the inhibitors of prostaglandin biosynthesis and phospholipase A2. The thielavins have also shown inhibitory activity against telomerase, phospholipase C, and glucose-6-phosphatase. In addition, recently thielavin B methyl ester exhibited moderate cytotoxicity. Structurally related compounds, such as gyrophoric acid and amidines have been reported as inhibitors against cancer cell growth and diacylglycerol acyltransferase, respectively. Our study is the first one showing that thielavins have IDO inhibitory activity. Further investigation and optimization of thielavins might lead to the finding of new IDO inhibitors potentially useful in the treatment of cancer and neurological disorders.

**Keywords**: 3-dioxygenase; Benzoate trimer; Coniochaeta sp.; IDO; Indoleamine 2; Thielavin

PMID: 24326340

Construction of artificial biosynthetic pathways for resveratrol glucoside derivatives


*Corresponding: Young-Soo Hong(hongsou@kribb.re.kr)

Resveratrol, which is a polyphenolic antioxidant, is dose-dependent when used to provide health benefits, to enhance stress resistance, and to extend lifespans. However, even though resveratrol has therapeutic benefits, its clinical therapeutic effect is limited owing to its low oral bioavailability. An Escherichia coli system was developed that contains an artificial biosynthetic pathway that produces resveratrol glucoside derivatives, such as resveratrol-3-O-glucoside (piceid) and resveratrol-4'-O-glucoside (resveratroloside), from simple carbon sources. This artificial biosynthetic pathway contains a glycosyltransferase addition (YjiC from Bacillus) with resveratrol biosynthetic genes. The produced glucoside compounds were verified through the presence of a product peak(s) and also through LC/MS analyses. The strategy used in this research demonstrates the first harnessing of E. coli for de novo synthesis of resveratrol glucoside derivatives from a simple sugar medium.

**Keywords**: Artificial biosynthesis; Glycosylation; Piceid; Resveratrol

PMID: 24561723
Anticancer effects of the Hsp90 inhibitor 17-demethoxy-reblastatin in human breast cancer MDA-MB-231 cells


Zhao Q, Wu CZ, Lee JK, Zhao SR, Li HM, Huo Q, Ma T, Zhang J, Hong YS*, Liu H.
*Co-corresponding: Young-Soo Hong(hongsoo@kribb.re.kr)

Triple-negative breast cancer (TNBC) possesses a higher rate of distant recurrence and a poorer prognosis than other breast cancer subtypes. Interestingly, most of the heat shock protein 90 (Hsp90) client proteins are oncoproteins, and some are closely related to unfavorable factors of TNBC patients. 17-Demethoxy-reblastatin (17-DR), a novel nonbenzoquinone-type geldanamycin analog, exhibited potent Hsp90 ATPase inhibition activity. In this study, the anticancer effects of 17-DR on TNBC MDA-MB-231 cells were investigated. These results showed that 17-DR inhibited cell proliferation, induced apoptosis, and suppressed cell invasion and migration in the MDA-MB-231 cells. Down-regulation of the key Hsp90-dependent tumor-driving molecules, such as RIP1 and MMP-9, by 17-DR may be related to these effects. Taken together, our results suggest that 17-DR has potential as a therapeutic agent for the treatment of TNBC.

Keywords: 17-demethoxy-reblastatin; Anticancer effect; Hsp90; Triple-negative breast cancer (TNBC)

PMID: 24705874

Illumins C2 and C3 stimulate lipolysis in 3T3-L1 adipocytes and suppress adipogenesis in 3T3-L1 preadipocytes


*Co-corresponding: Jong Seog Ahn(jsahn@kribb.re.kr), Bo-Yeon Kim(bykim@kribb.re.kr), Jae-Hyuk Jang (jangjh@kribb.re.kr)

The secondary metabolites illumins C2 (1) and C3 (2), obtained from the culture broth of Coprinus atramentarius, have been shown to possess antimicrobial activity. In the present study, we discovered novel biological activities of 1 and 2 in lipolysis of differentiated 3T3-L1 adipocytes and adipogenesis of 3T3-L1 preadipocytes. Compounds 1 and 2 exhibit a dose-dependent increase in glycerol release and thereby reduce intracellular lipid accumulation. The stimulatory effects of 1 and 2 on lipolysis are prevented by cAMP-dependent protein kinase (PKA) and extracellular signal-regulated kinase (ERK) inhibitors. Compounds 1 and 2 down-regulated perilipin and also affected the mRNA and protein levels of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). However, 1 and 2 treatment leads to a significant increase in PKA-mediated phosphorylation of HSL at S563 and S660. In addition, 1 and 2 treatment in 3T3-L1 preadipocytes induces down-regulation of the critical transcription factors, CCAAT/enhancer binding protein α and β (C/EBPα and C/EBPβ), and peroxisome proliferator activated receptor γ (PPARγ), which are required for adipogenesis, and accordingly inhibits adipogenesis. These results suggest that 1 and 2 might be useful for treating obesity due to their modulatory effects on fat by affecting adipocyte differentiation and fat mobilization.

Keywords: Adipogenesis; Antimicrobial activity; Coprinus atramentarius; Illumins; Obesity

PMID: 24597820
Transcriptional responses of the Bdtf1-deletion mutant to the phytoalexin brassinin in the necrotrophic fungus *Alternaria brassicicola*

*Brassica* species produce the antifungal indolyl compounds brassinin and its derivatives, during microbial infection. The fungal pathogen *Alternaria brassicicola* detoxifies brassinin and possibly its derivatives. This ability is an important property for the successful infection of brassicaceous plants. Previously, we identified a transcription factor, Bdtf1, essential for the detoxification of brassinin and full virulence. To discover genes that encode putative brassinin-digesting enzymes, we compared gene expression profiles between a mutant strain of the transcription factor and wild-type *A. brassicicola* under two different experimental conditions. A total of 170 and 388 genes were expressed at higher levels in the mutants than the wild type during the infection of host plants and saprophytic growth in the presence of brassinin, respectively. In contrast, 93 and 560 genes were expressed, respectively, at lower levels in the mutant than the wild type under the two conditions. Fifteen of these genes were expressed at lower levels in the mutant than in the wild type under both conditions. These genes were assumed to be important for the detoxification of brassinin and included Bdtf1 and 10 putative enzymes. This list of genes provides a resource for the discovery of enzyme-coding genes important in the chemical modification of brassinin.

**Keywords**: *Alternaria brassicicola*; Antifungal indolyl compounds; Necrotrophic fungus; Phytoalexin
detoxification

PMID: 25061722

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Synthetic ion transporters can induce apoptosis by facilitating chloride anion transport into cells


*Co-first: Sung-Kyun Ko(ksk1230@kribb.re.kr)*

Anion transporters based on small molecules have received attention as therapeutic agents because of their potential to disrupt cellular ion homeostasis. However, a direct correlation between a change in cellular chloride anion concentration and cytotoxicity has not been established for synthetic ion carriers. Here we show that two pyridine diamide-strapped calix[4]pyrroles induce coupled chloride anion and sodium cation transport in both liposomal models and cells, and promote cell death by increasing intracellular chloride and sodium ion concentrations. Removing either ion from the extracellular media or blocking natural sodium channels with amiloride prevents this effect. Cell experiments show that the ion transporters induce the sodium chloride influx, which leads to an increased concentration of reactive oxygen species, release of cytochrome c from the mitochondria and apoptosis via caspase activation. However, they do not activate the caspase-independent apoptotic pathway associated with the apoptosis-inducing factor. Ion transporters, therefore, represent an attractive approach for regulating cellular processes that are normally controlled tightly by homeostasis.

**Keywords**: Anion transporter; Apoptotic pathway; Cellular chloride anion concentration; Cytotoxicity; Ion transporter

PMID: 25242483
Heat shock protein 90 (Hsp90) is a molecular chaperone for numerous client proteins, many of which are crucial for the pathogenesis of non-small cell lung cancers (NSCLCs). To date, therapeutic approaches using epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) such as gefitinib or erlotinib for the treatment of NSCLCs have been limited due to the emergence of acquired drug resistance mainly mediated by a secondary T790M mutation in EGFR. Considering this, Hsp90 inhibition seems promising as it leads to overall degradation of the oncogenic EGFR family proteins. In this regard, the present study provides the preclinical basis for a new Hsp90 inhibitor, WK88-1, for the treatment of NSCLCs harboring the T790M mutation in EGFR. Our data revealed that inhibition of Hsp90 by WK88-1 induced overall degradation of multiple oncogenic signaling molecules including EGFR, ErbB2 and ErbB3, leading to subsequent growth arrest and apoptosis in the gefitinib-resistant H1975 cell line. In addition, treatment with WK88-1 markedly inhibited proliferation, migration and invasion in H1975 cells. Moreover, an in vivo xenograft assay indicated that WK88-1 markedly suppressed tumor growth in the H1975 xenografts, highlighting the potential efficacy of WK88-1 for overcoming gefitinib resistance in NSCLCs harboring the T790M mutation in EGFR.

**Keywords**: EGFR-TKIs; Gefitinib resistance; Heat shock protein 90 (Hsp90); Non-small cell lung cancer (NSCLC); WK88-1

PMID: 24789511

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**Incurable Diseases Therapeutics Research Center**

**Article 245**

The ubiquitin ligase human TRIM71 regulates let-7 microRNA biogenesis via modulation of Lin28B protein


let-7 microRNA (miRNA) is implicated in various biological processes, and its downregulation essentially linked to human malignancy. Regulation of gene expression of the let-7 family is critically linked to RNA-binding proteins. For instance, Lin28B and its paralog, Lin28A, inhibit the pre-let-7 precursor from being processed to mature miRNA by recruiting terminal uridylyltransferase, TUT4, which adds oligomeric U at the 3’ end, suggesting deregulation of Lin28B, together with Lin28A, may alter various biological processes through modulation of let-7 expression. Here, we showed that the Lin28B protein level is regulated via ubiquitin-mediated proteasomal degradation, and identified the ubiquitin ligase as human TRIM-NHL domain-containing TRIM71. In cells, TRIM71 negatively regulates Lin28B protein stability by catalyzing polyubiquitination. Compared with its paralog, Lin28A, a C-terminal unique ~50 amino acid stretch of Lin28B is essential for TRIM71 interactions and subsequent polyubiquitination. Moreover, the N-terminal RING finger motif of TRIM71 is critical for protein-protein interactions and polyubiquitination of Lin28B, and consequent let-7 expression. Consistent with the let-7 stimulatory role of TRIM71 via Lin28B polyubiquitination, specific knockdown of TRIM71 led to downregulation of let-7 expression. Expression of one of the known let-7 targets, HMGA2, was derepressed after knockdown of TRIM71. We additionally showed that enhanced expression of let-7 is part of a feedback loop that targets TRIM71 3’UTR, which contains two conserved let-7 target sites. Our findings collectively reveal critical aspects of regulatory complexity of let-7 biogenesis at the posttranscriptional level.

**Keywords**: let-7 microRNA; Lin28B; miRNA; TRIM71; Ubiquitination

PMID: 24602972
Diacylglycerol acyltransferase 2 (DGAT2), which catalyzes the final step in triacylglycerol (TG) synthesis, is a key enzyme associated with hepatic steatosis and insulin resistance. Here, using an in vitro screen of 20000 molecules, we identified a class of compounds with a substituted 1H-pyrrolo[2,3-b]pyridine core which proved to be potent and selective inhibitors of human DGAT2. Of these compounds, H2-003 and -005 exhibited a considerable reduction in TG biosynthesis in HepG2 hepatic cells and 3T3-L1 preadipose cells. These compounds exert DGAT2-specific-inhibitory activity, which was further confirmed in DGAT2- or DGAT1-overexpressing HEK293 cells. In addition, these compounds almost completely abolished lipid droplet formation in 3T3-L1 cells when co-treated with a DGAT1 inhibitor, which was not attained using either a DGAT2 or DGAT1 inhibitor alone. Collectively, we identified two DGAT2 inhibitors, H2-003 and -005. These compounds will aid in DGAT2-related lipid metabolism research as well as in therapeutic development for the treatment of metabolic diseases associated with excessive TG.

**Keywords**: Diacylglycerol acyltransferase 2 (DGAT2); Metabolic disease; Small molecule inhibitor; Triacylglycerol

**PMID**: 25099343

**Perspectives on the therapeutic potential of short-chain fatty acid receptors**


Kim S’, Kim JH, Park BO, Kwak YS.

‘Corresponding: Sunhong Kim(sunhong@kribb.re.kr)

There is rapidly growing interest in the human microbiome because of its implication in metabolic disorders and inflammatory diseases. Consequently, understanding the biology of short chain fatty acids and their receptors has become very important for identifying novel therapeutic avenues. GPR41 and GPR43 have been recognized as the cognate receptors for SCFAs and their roles in metabolism and inflammation have drawn much attention in recent years. GPR43 is highly expressed on immune cells and has been suggested to play a role in inflammatory diseases such as inflammatory bowel disease. Both GPR41 and GPR43 have been implicated in diabetes and obesity via the regulation of adipose tissue and gastrointestinal hormones. So far, many studies have provided contradictory results, and therefore further research is required to validate these receptors as drug targets. We will also discuss the synthetic modulators of GPR41 and GPR43 that are critical to understanding the functions of these receptors.

**Keywords**: Human microbiome; Inflammatory disease; Metabolic disorder; Short chain fatty acids (SCFAs)

**PMID**: 24499669
Mitogen- and stress-activated kinase 1 (MSK1) is a nuclear serine/threonine protein kinase that acts downstream of both extracellular signal-regulated kinases and p38 mitogen-activated protein kinase in response to stress or mitogenic extracellular stimuli. Increasing evidence has shown that MSK1 is closely associated with malignant transformation and cancer development. MSK1 should be an effective target for cancer chemoprevention and chemotherapy. However, very few MSK1 inhibitors, especially natural compounds, have been reported. We used virtual screening of a natural products database and the active conformation of the C-terminal kinase domain of MSK1 (PDB id 3KN) as the receptor structure to identify chrysin and its derivative, compound 69407, as inhibitors of MSK1. Compared with chrysin, compound 69407 more strongly inhibited proliferation and 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced neoplastic transformation of JB6 P+ cells with lower cytotoxicity. Western blot data demonstrated that compound 69407 suppressed phosphorylation of the MSK1 downstream effector histone H3 in intact cells. Knocking down the expression of MSK1 effectively reduced the sensitivity of JB6 P+ cells to compound 69407. Moreover, topical treatment with compound 69407 before TPA application significantly reduced papilloma development in terms of number and size in a two-stage mouse skin carcinogenesis model. The reduction in papilloma development was accompanied by the inhibition of histone H3 phosphorylation at Ser10 in tumors extracted from mouse skin. The results indicated that compound 69407 exerts inhibitory effects on skin tumorigenesis by directly binding with MSK1 and attenuates the MSK1/histone H3 signaling pathway, which makes it an ideal chemopreventive agent against skin cancer.

**Keywords:** Malignant transformation; Mitogen- and stress-activated kinase 1 (MSK1); MSK1 inhibitor; Skin cancer

**PMID:** 24169959
Antiviral activity of ginsenosides against coxsackievirus B3, enterovirus 71, and human rhinovirus 3


Song JH, Choi HJ, Song HH, Hong EH, Lee BR, Oh SR, Choi K, Yeo SG, Lee YP, Cho S*, Ko HJ.

Co-corresponding: Sungchan Cho(sungchan@kribb.re.kr)

BACKGROUND: Ginsenosides are the major components responsible for the biochemical and pharmacological actions of ginseng, and have been shown to have various biological activities. In this study, we investigated the antiviral activities of seven ginsenosides [protopanaxatriol (PT) type: Re, Rf, and Rg2; protopanaxadiol (PD) type: Rb1, Rb2, Rc, and Rd] against coxsackievirus B3 (CVB3), enterovirus 71 (EV71), and human rhinovirus 3 (HRV3).

METHODS: Assays of antiviral activity and cytotoxicity were evaluated by the sulforhodamine B method using the cytopathic effect (CPE) reduction assay.

RESULTS: The antiviral assays demonstrated that, of the seven ginsenosides, the PT-type ginsenosides (Re, Rf, and Rg2) possess significant antiviral activities against CVB3 and HRV3 at a concentration of 100 μg/mL. Among the PT-type ginsenosides, only ginsenoside Rg2 showed significant anti-EV71 activity with no cytotoxicity to cells at 100 μg/mL. The PD-type ginsenosides (Rb1, Rb2, Re, and Rd), by contrast, did not show any significant antiviral activity against CVB3, EV71, and HRV3, and exhibited cytotoxic effects to virus-infected cells. Notably, the antiviral efficacies of PT-type ginsenosides were comparable to those of ribavirin, a commonly used antiviral drug.

CONCLUSION: Collectively, our findings suggest that the ginsenosides Re, Rf, and Rg2 have the potential to be effective in the treatment of CVB3, EV71, and HRV3 infection.

Keywords: Antiviral activity; CVB3; EV71; Ginsenoside; HRV3

PMID: 25378991

Efficient lytic induction of kaposi's sarcoma-associated herpesvirus (KSHV) by the anthracyclines

Oncotarget. 5(18):8515-27.


Corresponding: Sungchan Cho(sungchan@kribb.re.kr)

Lytic induction of latent Kaposi's sarcoma-associated herpesvirus (KSHV) has been considered as a therapeutic option for efficient treatment of several KSHV-associated malignancies. Here, we developed a robust high-throughput screening system that allows an easy and quantitative measurement of lytic induction of latent KSHV and discovered three anthracyclines as potent inducers from screen of FDA-approved drugs. Lytic induction of latent KSHV by three compounds was verified by the significant induction of lytic genes and subsequent production of infectious KSHV. Importantly, lytic induction by three compounds was much more efficient than that by sodium butyrate, a well-characterized inducer of KSHV lytic cycle. Mechanistically, the anthracyclines caused lytic induction of KSHV through apoptosis induced by their DNA intercalation rather than topoisomerase II inhibition. Consequently, our results clearly demonstrated a role of anthracyclines as effective lytic inducers of KSHV and also provided a molecular basis of their use for efficient treatment of diseases associated with KSHV infection.

Keywords: Anthracyclines; Kaposi's sarcoma-associated herpesvirus (KSHV); KSHV infection; Lytic induction; Malignancy

PMID: 25237786
Identification of a novel function of CX-4945 as a splicing regulator


*Corresponding: Sungchan Cho(sungchan@kribb.re.kr)

Alternative splicing is a nearly ubiquitous versatile process that controls gene expression and creates numerous protein isoforms with different functions from a single gene. The significance of alternative splicing has been confirmed by the increasing number of human diseases that are caused by misregulation of splicing events. Very few compounds, however, have been reported to act as inhibitors of alternative splicing, and their potential clinical use needs to be evaluated. Here, we report that CX-4945, a previously well-characterized inhibitor of casein kinase 2 (CK2) and a molecule currently in clinical trials (Phase II) for cancer treatment, regulates splicing in mammalian cells in a CK2-independent manner. Transcriptome-wide analysis using exon array also showed a widespread alteration in alternative splicing of numerous genes. We found that CX-4945 potently inhibits the Cdc2-like kinases (Clks) \textit{in vitro} and in turn, leads to suppression of the phosphorylation of serine/arginine-rich (SR) proteins in mammalian cells. Surprisingly, the overall efficacy of CX-4945 on Clks ($IC_{50} = 3-90$ nM) was stronger than that of TG-003, the strongest inhibitor reported to date. Of the Clks, Clk2 was most strongly inhibited by CX-4945 in an ATP-competitive manner. Our research revealed an unexpected activity of the drug candidate CX-4945 as a potent splicing modulator and also suggested a potential application for therapy of diseases caused by abnormal splicing.

Keywords: Alternative splicing; Cdc2-like kinases (Clks); CK2-independent manner; CX-4945; Serine/arginine-rich (SR)

PMID: 24743259
Integrated Biorefinery Research Institute

- Sustainable Bioresource Research Center
- Bioenergy and Biochemical Research Center
- Industrial Microbiology and Bioprocess Research Center
- Eco-friendly Biomaterial Research Center
- Other Articles
Overexpression and self-assembly of virus-like particles in *Nicotiana benthamiana* by a single-vector DNA replicon system


*Co-corresponding: Hyun-Soon Kim(hyuns@kribb.re.kr),
Jae-Heung Jeon(jeonjh@kribb.re.kr)*

Based on recent developments, virus-like particles (VLPs) are considered to be perfect candidates as nanoplatforms for applications in materials science and medicine. To succeed, mass production of VLPs and self-assembly into a correct form in plant systems are key factors. Here, we report expression of synthesized coat proteins of the three viruses, Brome mosaic virus, Cucumber mosaic virus, and Maize rayado fino virus, in *Nicotiana benthamiana* and production of self-assembled VLPs by transient expression system using agroinfiltration. Each coat protein was synthesized and cloned into a pBYR2fp single replicon vector. Target protein expression in cells containing p19 was fourfold higher than that of cells lacking p19. After agroinfiltration, protein expression was analyzed by SDS-PAGE and quantitative image analyzer. Quantitative analysis showed that BMVCP, CMVCP, and MRFVCP concentrations were 0.5, 1.0, and 0.8 mg · g⁻¹ leaf fresh weight, respectively. VLPs were purified by sucrose cushion ultracentrifugation and then analyzed by transmission electron microscopy. Our results suggested that BMVCP and CMVCP proteins expressed in *N. benthamiana* leaves were able to correctly self-assemble into particles. Moreover, we evaluated internal cavity accessibility of VLPs to load foreign molecules. Finally, plant growth conditions after agroinfiltration are critical for increasing heterologous protein expression levels in a transient expression system.

**Keywords**: Biomaterial; pBYR2fp viral vector; Plant virus; Self-assembly; Virus-like particle

PMID: 24965559

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**Role of *Rhizobium*, a plant growth promoting bacterium, in enhancing algal biomass through mutualistic interaction**


Kim BH, Ramanan R, Cho DH, Oh HM, Kim HS*.

*Corresponding: Hee-Sik Kim(hkim@kribb.re.kr)*

*Rhizobium* plays the pivotal role in mutualistic interactions with plants and this study extends this mutualism to several species of green algae. Denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene clone library experiments of *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Scenedesmus* sp., and *Botryococcus braunii* revealed that the dominant phycosphere bacteria hosted by these green algae were *Rhizobium*, *Mesorhizobium*, and *Shinella* within Rhizobiales, *Flavobacterium* within Flavobacteriales, and *Pseudomonas* within Pseudomonadales. When *Rhizobium* sp., most prevalent and dominant bacterium isolated from *C. vulgaris*, was co-cultured with green algae, it promoted algal cell count by ~72%. The qPCR analysis of 16S rRNA, *Rhizobium* specific rirA and rhtA genes, performed to understand the effect of green algae on growth of *Rhizobium* sp., showed a significant increase in copy numbers indicating sustained growth of *Rhizobium* upon co-culture with green algae. Likewise, growth rates of algae and *Rhizobium* increased by an average of ~11% and ~110%, respectively, confirming mutualistic interaction. Considering the presence of *Rhizobium* sp. in a variety of green algae, this must be a major mutualistic relationship among green algae and this ubiquitous association might serve as a model for elucidating the mutualistic mechanism between green algae and rhizobacter. This interaction could be also utilized in enhancing microalgal biomass, especially slow growing organisms like *B. braunii*, to augment their bioenergy productivity.

**Keywords**: Green algae; Mutualism; Phycosphere bacteria; Plants; *Rhizobium* sp.
Plastid and mitochondrion genomic sequences from Arctic Chlorella sp. ArM0029B

BMC Genomics. 15:286.

Jeong H, Lim JM, Park J, Sim YM, Choi HG, Lee J1, Jeong WJ.
*Co-corresponding: Won-Joong Jeong(wonjoong@kribb.re.kr)

BACKGROUND: Chlorella is the representative taxon of Chlorellales in Trebouxiiophyceae, and its chloroplast (cp) genomic information has been thought to depend only on studies concerning Chlorella vulgaris and GenBank information of C. variabilis. Mitochondrial (mt) genomic information regarding Chlorella is currently unavailable. To elucidate the evolution of organelle genomes and genetic information of Chlorella, we have sequenced and characterized the cp and mt genomes of Arctic Chlorella sp. ArM0029B.

RESULTS: The 119,989-bp cp genome lacking inverted repeats and 65,049-bp mt genome were sequenced. The ArM0029B cp genome contains 114 conserved genes, including 32 tRNA genes, 3 rRNA genes, and 79 genes encoding proteins. Chlorella cp genomes are highly rearranged except for a Chlorella-specific six-gene cluster, and the ArM0029B plastid resembles that of Chlorella variabilis except for a 15-kb gene cluster inversion. In the mt genome, 62 conserved genes, including 27 tRNA genes, 3 rRNA genes, and 32 genes encoding proteins were determined. The mt genome of ArM0029B is similar to that of the non-photosynthetic species Prototheca and Helicosporidium. The ArM0029B mt genome contains a group I intron, with an ORF containing two LAGLIDADG motifs, in cox1. The intronic ORF is shared by C. vulgaris and Prototheca. The phylogeny of the plastid genome reveals that ArM0029B showed a close relationship of Chlorella to Parachlorella and Oocystis within Chlorellales. The distribution of the cox1 intron at 721 support membership in the order Chlorellales. Mitochondrial phylogenetic analyses, however, indicated that ArM0029B shows a greater affinity to MX-AZII and Coccomyxa than to the Helicosporidium-Prototheca clade, although the detailed phylogenetic relationships among the three taxa remain to be resolved.

CONCLUSIONS: The plastid genome of ArM0029B is similar to that of C. variabilis. The mt sequence of ArM0029B is the first genome to be reported for Chlorella. Chloroplast genome phylogeny supports monophyly of the seven investigated members of Chlorellales. The presence of the cox1 intron at 721 in all four investigated Chlorellales taxa indicates that the cox1 intron had been introduced in early Chlorellales as a cis-splice form and that the cis-splicing intron was inherited to recent Chlorellales and was recently trans-spliced in Helicosporidium.

Keywords: Arctic Chlorella; Chlorellales; cox1 intron; Genomic sequence
PMID: 24735464
Nutrient removal and biofuel production in high rate algal pond using real municipal wastewater


Kim BH, Kang Z, Ramanan R, Choi JE, Cho DH, Oh HM, Kim HS.
Corresponding: Hee-Sik Kim(hkim@kribb.re.kr)

This study evaluated the growth and nutrient removal ability of an indigenous algal consortium on real untreated municipal wastewater in a high rate algal pond (HRAP). The HRAP was operated semicontinuously under different hydraulic retention times (HRT: 2, 4, 6, and 8 days). The average removal efficiencies of chemical oxygen demand, and total nitrogen and phosphate of real municipal wastewater were maintained at 85.44 ± 5.10%, 92.74 ± 5.82%, and 82.85 ± 8.63%, respectively, in 2 day HRT. Algae dominated the consortium and showed high settling efficiency (99%), and biomass and lipid productivity of 0.500 ± 0.03 g/l/day and 0.103 ± 0.0083 g/l/day (2 day HRT), respectively. Fatty acid methyl ester analysis revealed a predominance of palmitate (C16:0), palmitoleate (C16:1), linoleate (C18:2), and linolenate (C18:3). Microalgal diversity analyses determined the presence of Chlorella, Scenedesmus, and Stigeoclonium as the dominant microalgae. The algal consortium provides significant value not only in terms of energy savings and nutrient removal but also because of its bioenergy potential as indicated by the lipid content (20-23%) and FAME profiling.

Keywords: Algal consortium; Chlorella sp.; High rate algal pond; Municipal wastewater; Scenedesmus sp.

PMID: 24759425

Expression of A beta-Fc fusion protein in transgenic potato

Kor J Horticul Sci. 32(3):375-81.

Kim HS*, Youn JW, Lee JH, Jeon JH, Ko K.
Corresponding: Hyun-Soon Kim(hyuns@kribb.re.kr)

Transgenic potato was generated to express recombinant 5 repeated beta-amyloid (Aβ) peptides, potential antigens to be applied as a preventiveaccine for Alzheimer's disease using Agrobacterium mediated transformation. The Aβ peptides were fused to the human IgG Fc fragment enhancing protein and KDEL, which is the endoplasmic reticulum (ER) retention signal (5Aβ-FcK). The 5Aβ-FcK, was expressed under the control of the duplicated 35S promoter. PCR analysis confirmed the presence of the transgene in several transgenic potato lines. Southern blot analysis showed only a single gene copy number in transgenic line 22, whereas multiple gene copy numbers were shown for transgenic lines 31 and 44. Northern blot analysis showed that line 22 had stronger mRNA levels when compared to lines 31 and 44. Immunoblot analysis confirmed that the 5Aβ-FcK protein was expressed in the transgenic potato plant. These results indicate that 5Aβ fused to Fc can be expressed in potato plants.

Keywords: 5Aβ-FcK; Alzheimer's disease; β-amyloid (Aβ); Fusion protein; Transgenic potato

5Aβ42-FcK pBINPLUS

Expected protein structure

Expected glycan structure
AtFKBP16-1, a chloroplast luminal immunophilin, mediates response to photosynthetic stress by regulating PsAL stability


Seok MS, You YN, Park HJ, Lee SS, Aigen F, Luan S, Ahn JC, Cho HS.  
*Co-corresponding: Hye Sun Cho(hscho@kribb.re.kr)

Abidopsis contains 16 putative chloroplast lumen-targeted immunophilins (IMMs). Proteomic analysis has enabled the subcellular localization of IMMs experimentally, but the exact biological and physiological roles of most luminal IMMs remain to be discovered. FK506-binding protein (FKBP) 16-1, one of the luminal IMMs containing poorly conserved amino acid residues for peptidyl-prolyl isomerase (PPIase) activity, was shown to play a possible role in chloroplast biogenesis in Abidopsis, and was also found to interact with PsAL in wheat. In this study, further evidence is provided for the notion that Abidopsis FKBP16-1 (AtFKBP16-1) is transcriptionally and post-transcriptionally regulated by environmental stresses including high light (HL) intensity, and that overexpression of AtFKBP16-1 plants exhibited increased photosynthetic stress tolerance. A blue native-polyacrylamide gel electrophoresis/two-dimensional (BN-PAGE/2-D) analysis revealed that the increase of AtFKBP16-1 affected the levels of photosystem I (PSI)-light harvesting complex I (LHC1) and PSII-LHC1-light harvesting complex II (LHCII) supercomplex, and consequently enhanced tolerance under conditions of HL stress. In addition, plants overexpressing AtFKBP16-1 showed increased accumulation of PsAL protein and enhanced drought tolerance. Using a protease protection assay, AtFKBP16-1 protein was found to have a role in PsAL stability. The AtPsAL levels also responded to abiotic stresses derived from drought, and from methyl viologen stresses in wild-type plants. Taken together, these results suggest that AtFKBP16-1 plays a role in the acclimation of plants under photosynthetic stress conditions, probably by regulating PsAL stability.

Keywords: Chloroplast luminal immunophilin; Luminal IMMs; Photosynthetic stress; PsAL stability; Stress tolerance

PMID: 24124981

Overexpression of stearoyl-ACP desaturase enhances accumulations of oleic acid in the green alga Chlamydomonas reinhardtii


Hwangbo K, Ahn JW, Lim JM, Park YI, Liu JR, Jeong WJ.  
*Corresponding: Won-joong Jeong(wojoong@kribb.re.kr)

FAB2, which encodes stearoyl-acyl carrier protein desaturase, catalyzes the conversion of stearic acid (18:0) to oleic acid (18:1) in fatty acid biosynthesis. In this study, we isolated FAB2 from Chlamydomonas reinhardtii, named CrFAB2, and generated CrFAB2-overexpressing transgenic lines to identify a major role of CrFAB2 in fatty acid biosynthesis of C. reinhardtii. In CrFAB2-overexpressing lines, oleic acid (18:1) content was increased by approximately 2.4-fold compared to the wild-type control plants. Interestingly, CrFAB2 overexpression resulted in the induction of CrFAD2 expression. Consistent with this result, the induction of linoleic acid (18:2) was also detected in CrFAB2-overexpressing lines, and total fatty acid content in these lines was induced by approximately 28% by CrFAB2 overexpression compared to the wild-type control. Our results indicate that CrFAB2 overexpression enhances the synthesis of oleic acid (18:1) and that CrFAB2 may also play a key role in regulating total fatty acid content in the green alga C. reinhardtii.

Keywords: Chlamydomonas reinhardtii; Desaturase; Fatty acid; Oleic acid
A simple and non-invasive method for nuclear transformation of intact-walled Chlamydomonas reinhardtii


Kim S, Lee YC, Cho DH, Lee HU, Huh YS, Kim GI, Kim HS.

*Co-corresponding: Hee-Sik Kim(hkim@kribb.re.kr)

Genetic engineering in microalgae is gaining attraction but nuclear transformation methods available so far are either inefficient or require special equipment. In this study, we employ positively charged nanoparticles, 3-aminopropyl-functionalized magnesium phyllosilicate (aminoclay, approximate unit cell composition of [H2Ni(Ni(CH3)]3Si2MgO2(OH)]2, for nuclear transformation into eukaryotic microalgae. TEM and EDX analysis of the process of transformation reveals that aminoclay coats negatively-charged DNA biomolecules and forms a self-assembled hybrid nanostructure. Subsequently, when this nanostructure is mixed with microalgal cells and plated onto selective agar plates with high friction force, cell wall is disrupted facilitating delivery of plasmid DNA into the cell and ultimately to the nucleus. This method is not only simple, inexpensive, and non-toxic to cells but also provides efficient transformation (5.03×1024 transforming m2;µg DNA), second only to electroporation which needs advanced instrumentation. We present optimized parameters for efficient transformation including pre-treatment, friction force, concentration of foreign DNA/aminomoly, and plasticity of agar plates. It is also confirmed the successful integration and stable expression of foreign gene in Chlamydomonas reinhardtii through molecular methods.

**Keywords**: Agar plates; Chlamydomonas reinhardtii; Nanoparticle; Nuclear transformation

PMID: 24988123

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RNA-seq analysis and de novo transcriptome assembly of Jerusalem artichoke (Helianthus tuberosus var. lineae)


Jung WY, Lee SS, Kim CW, Kim HS, Min SR, Moon JS, Kwon SY, Jeon JH*, Cho HS*.

*Co-corresponding: Hye Sun Cho(hscho@kribb.re.kr), Jae-Heung Jeon(jeonjh@kribb.re.kr)

Jerusalem artichoke (Helianthus tuberosus L.) has long been cultivated as a vegetable and as a source of fructans (inulin) for pharmaceutical applications in diabetes and obesity prevention. However, transcriptomic and genomic data for Jerusalem artichoke remain scarce. In this study, Illumina RNA sequencing (RNA-Seq) was performed on samples from Jerusalem artichoke leaves, roots, stems and two different tuber tissues (early and late tuber development). Data were used for de novo assembly and characterization of the transcriptome. In total 206,215,632 paired-end reads were generated. These were assembled into 66,322 loci with 272,548 transcripts. Loci were annotated by querying against the NCBI non-redundant, Phytozome and UniProt databases, and 40,215 loci were homologous to existing database sequences. Gene Ontology terms were assigned to 19,848 loci, 15,434 loci were matched to 25 Clusters of Eukaryotic Orthologous Groups classifications, and 11,844 loci were classified into 142 Kyoto Encyclopedia of Genes and Genomes pathways. The assembled loci also contained 10,778 potential simple sequence repeats. The newly assembled transcriptome was used to identify loci with tissue-specific differential expression patterns. In total, 670 loci exhibited tissue-specific expression, and a subset of these were confirmed using RT-PCR and qRT-PCR. Gene expression related to inulin biosynthesis in tuber tissue was also investigated. Existing genetic and genomic data for H. tuberosus are scarce. The sequence resources developed in this study will enable the analysis of thousands of transcripts and will thus accelerate marker-assisted breeding studies and studies of inulin biosynthesis in Jerusalem artichoke.

**Keywords**: Assembled transcriptome; Gene ontology; Inulin biosynthesis; Jerusalem artichoke

PMID: 25375764
**Bioenergy and Biochemical Research Center**

**Article 264**

*Ferruginibacter profundus* sp. nov., a novel member of the family *Chitinophagaceae*, isolated from freshwater sediment of a reservoir


Jin L, Lee HG, La HJ, Ko SR, Ahn CY³, Oh HM³.
³Co-corresponding: Hee-Mock Oh(heemock@kribb.re.kr), Chi-Yong Ahn(cyahn@kribb.re.kr)

A Gram-negative, aerobic, non-motile, and rod-shaped bacterium, designated strain DS48-5-3, was isolated from a 48 m sediment sample taken from Daehung Reservoir, Republic of Korea. Comparative 16S rRNA gene sequence studies showed a clear affiliation of this strain to the *Bacteroidetes*, notably most closely related to *Ferruginibacter alkalitolerans* HU1-GD23³, *Ferruginibacter lapisanis* HU1-HG42³ and *Ferruginibacter yonginensis* HME8442³, showing 16S rRNA gene sequence similarities to the type strains of these species of 95.2-96.4 % similarity. The predominant ubiquinone was identified as MK-7. The major fatty acids were identified as iso-C₁₅:₀, iso-C₁₇:₀ 3-OH, and iso-C₁₆:₁ G. The G+C content of the genomic DNA of strain DS48-5-3 was determined to be 37.2 %. On the basis of polyphasic evidence, it is proposed that strain DS48-5-3 should belong to a novel species, for which the name *Ferruginibacter profundus* sp. nov. (type strain DS48-5-3 = KCTC 32478T = JCM 19431¹), is proposed.

**Keywords**: 16S rRNA gene sequence; *Bacteroidetes; Ferruginibacter profundus; Freshwater sediment; Novel species; Polyphasic taxonomy

PMID: 24917388

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**Article 264**

Status, alert system and prediction of cyanobacterial bloom in South Korea


³Corresponding: Hee-Mock Oh(heemock@kribb.re.kr)

Bloom-forming freshwater cyanobacteria generat pose a major ecological problem due to their ability to produce toxins and other bioactive compounds, which can have important implications in illnesses of humans and livestock. Cyanobacteria such as *Microcystis, Anabaena, Oscillatoria, Phormidium*, and *Aphanizomenon* species producing microcysts and anatoxin-a have been predominantly documented from most South Korean lakes and reservoirs. With the increase in frequency of such blooms, various monitoring approaches, treatment processes, and prediction models have been developed in due course. In this paper we review the field studies and current knowledge on toxin producing cyanobacterial species and ecological variables that regulate toxin production and bloom formation in major rivers (Han, Geum, Nakdong, and Yeongsan) and reservoirs in South Korea. In addition, development of new, fast, and high-throughput techniques for effective monitoring is also discussed with cyanobacterial bloom advisory practices, current management strategies, and their implications in South Korea freshwater bodies.

**Keywords**: Anatoxin-a; Bloom formation; Cyanobacteria; Microcysts; Toxin production

PMID: 25705675
Functional innovations of three chronological mesohexaploid Brassica rapa genomes

BMC Genomics. 15:606.

*Corresponding: HyeRan Kim (kimhr@kribb.re.kr)

BACKGROUND: The Brassicaceae family is an exemplary model for studying plant polyploidy. The Brassicaceae knowledge-base includes the well-annotated Arabidopsis thaliana reference sequence; well-established evidence for three rounds of whole genome duplication (WGD); and the conservation of genomic structure, with 24 conserved genomic blocks (GBs). The recently released Brassica rapa draft genome provides an ideal opportunity to update our knowledge of the conserved genomic structures in Brassica, and to study evolutionary innovations of the mesohexaploid plant, B. rapa.

RESULTS: Three chronological B. rapa genomes (recent, young, and old) were reconstructed with sequence divergences, revealing a trace of recursive WGD events. A total of 636 fast evolving genes were unevenly distributed throughout the recent and young genomes. The representative Gene Ontology (GO) terms for these genes were 'stress response' and 'development' both through a change in protein modification or signaling, rather than by enhancing signal recognition. In retention patterns analysis, 98% of B. rapa genes were retained as collinear gene pairs; 77% of those were singly-retained in recent or young genomes resulting from death of the ancestral copies, while others were multi-retained as long retention genes. GO enrichments indicated that single retention genes mainly function in the interpretation of genetic information, whereas, multi-retention genes were biased toward signal response, especially regarding development and defense. In the recent genome, 13,302, 5,790, and 20 gene pairs were multi-retained following Brassica whole genome triplication (WGT) events with 2, 3, and 4 homoeologous copies, respectively. Enriched GO-slim terms from B. rapa homoeologues imply that a major effect of the B. rapa WGT may have been to acquire environmental adaptability or to change the course of development. These homoeologues seem to more frequently undergo subfunctionalization with spatial expression patterns compared with other possible events including nonfunctionalization and neofunctionalization.

CONCLUSION: We refined Brassicaceae GB information using the latest genomic resources, and distinguished three chronologically ordered B. rapa genomes. B. rapa genes were categorized into fast evolving, single- and multi-retention genes, and long retention genes by their substitution rates and retention patterns. Representative functions of the categorized genes were elucidated, providing better understanding of B. rapa evolution and the Brassica genus.

Keywords: Brassica rapa; Chronological genome; Fast evolving; Long retention; Multi retention

PMID: 25033750

Rpi-blb2-mediated late blight resistance in Nicotiana benthamiana requires SGT1 and salicylic acid-mediated signaling but not RAR1 or HSP90


Oh SK, Kim H*, Choi D.
*Co-corresponding: HyeRan Kim (kimhr@kribb.re.kr)

The Rpi-blb2 recognizes the presence of the Phytophthora infestans AVRblb2 and initiates effector-triggered immunity (ETI). We performed gain-of-function and loss-of-function studies in Nicotiana benthamiana to elucidate Rpi-blb2-mediated resistance to P. infestans. Rpi-blb2 triggered a hypersensitive response through SGT1-mediated, but not RAR-mediated or HSP90-mediated, pathways. NbSGT1 was also required for basal and ETI-mediated by Rpi-blb2 in N. benthamiana. Moreover, salicylic acid (SA) affected basal defense and Rpi-blb2-mediated resistance against P. infestans. The increased susceptibility of Rpi-blb2-transgenic plants in the NahG-background correlated with reduced levels of SA. These findings provide evidence for the roles of SGT1- and SA-signaling in Rpi-blb2-mediated resistance against P. infestans.

Keywords: AVRblb2; Nicotiana benthamiana; Phytophthora infestans; Resistance; Rpi-blb2; Salicylic acid; SGT1

PMID: 24582656
Caulobacter profunda sp. nov., isolated from deep freshwater sediment


Jin L, La HJ, Lee HG, Lee JJ, Lee S, Ahn CY, Oh HM.
'Co-corresponding: Hee-Mock Oh(heemock@kribb.re.kr),
Chi-Yong Ahn(cyahn@kribb.re.kr)

The Gram-stain-negative, aerobic, non-spore-forming, motile, with a single polar flagellum, or non-motile (stalked) and rod-shaped bacteria, DS48-5-2 and DS48-6-3, were isolated from a sediment sample collected from a depth of 48 m taken from Daechung Reservoir, Republic of Korea. Comparative 16S rRNA gene sequence studies showed that the two isolates had clear affiliation with Alphaproteobacteria and the closest relatedness to Caulobacter mirabilis FWC 38, Caulobacter fusciformis ATCC 15257 and Caulobacter daechungensis H-E3-2 showing 98.5%, 97.3% and 97.3% 16S rRNA gene sequence similarity, respectively, and 96.1-96.7% similarity to all other species of the genus Caulobacter. The two isolates shared 100 % 16S rRNA gene sequence similarity. The predominant ubiquinone was Q-10. The major fatty acids were summed feature 8 (C16:0 6f0c and/or C18:0 7c), C16:0, C18:0 7c 11-methyl and summed feature 3 (C16:0 6f0c and/or C16:0 7c). The G+C contents of the genomic DNA of strains DS48-5-2 and DS48-6-3 were 66.7 mol% and 66.2 mol%, respectively. DNA-DNA hybridization values of strains DS48-5-2 and DS48-6-3 with C. mirabilis FWC 38, C. fusciformis ATCC 15257 and C. daechungensis H-E3-2 were 19.3%-24.4%. Thus, based on the evidence from polyphasic studies, it is proposed that strains DS48-5-2 and DS48-6-3 are representatives of a novel species in the genus Caulobacter, for which the name Caulobacter profunda sp. nov. is proposed. The type strain is DS48-5-2T (= KCTC 32480T = JCM 19440T).

Keywords: 16S rRNA gene sequence; Caulobacter profunda; Freshwater sediment; Novel species

PMID: 24198059

Hymenobacter ruber sp. nov., isolated from grass soil


Jin L, Lee HG, Kim SG, Lee KC, Ahn CY, Oh HM.
'Co-corresponding: Hee-Mock Oh(heemock@kribb.re.kr),
Chi-Yong Ahn(cyahn@kribb.re.kr)

A taxonomic study using a polyphasic approach was performed on a Gram-stain-negative, aerobic, non-motive, non-spore-forming, rod-shaped bacterium, designated strain PB156T, isolated from grass soil. Comparative 16S rRNA gene sequence studies showed that the isolate was clearly affiliated with the phylum Bacteroidetes, and most closely related to Hymenobacter soli PB17T, Hymenobacter antarcticus VUG-A42aaT and Hymenobacter glaciei VUG-A130T, showing 96.4, 96.2 and 95.9 % 16S rRNA gene sequence similarity, respectively, while all other species of the genus shared only 89.3-95.2 % similarity. The main polyamine present was sym-homospermidine. The predominant menaquinone was MK-7. The major fatty acids were C15:0 iso, summed feature 3 (C16:0 6f0c and/or C16:0 7c), C16:0 5c and C15:0 7c. The G+C content of the genomic DNA of strain PB156T was 61.7 mol%. The combined genotypic and phenotypic data supported the conclusion that strain PB156T represents a novel species of the genus Hymenobacter, for which the name Hymenobacter ruber sp. nov. is proposed. The type strain is PB156T (= KCTC 32477T = JCM 19433T).

Keywords: 16S rRNA gene sequence; Grass soil; Hymenobacter ruber; Novel species

PMID: 24425816
**Sphingomonas daechungensis** sp. nov., isolated from sediment of a eutrophic reservoir


*Co-corresponding: Hee-Mock Oh(heemock@kribb.re.kr), Chi-Yong Ahn(cychn@kribb.re.kr)

Strain CH15-11ᵀ, isolated from a sediment sample taken from Daechung Reservoir, South Korea, during the late-blooming period of cyanobacteria, was found to be a Gram-stain-negative, non-motile, non-spore-forming, rod-shaped and aerobic bacterium. Strain CH15-11ᵀ grew optimally at pH 7 and 28-30 °C. According to a phylogenetic tree based on 16S rRNA gene sequences, strain CH15-11ᵀ belonged to the genus *Sphingomonas* and clustered with *Sphingomonas sediminicola* Dae 20¹, with which it shared the highest 16S rRNA gene sequence similarity (97.6 %). Chemotaxonomic analysis showed that strain CH15-11ᵀ had characteristics typical of members of the genus *Sphingomonas*, such as the presence of sphingoglycolipid, ubiquinone Q-10 and sym-homospermidine. Plus, strain CH15-11ᵀ included summed feature 8 (C₁₀-10c7 and/or C₁₈:1ω6c) and C₁₆:0 as the major fatty acids. The genomic DNA G+C content was 65.6 mol%. Sequence data showed that strain CH15-11ᵀ was most closely related to *Sphingomonas sediminicola* Dae 20¹ (97.6 %), *Sphingomonas ginsengisoli* Gsoil 634² (97.2 %) and *Sphingomonas jaspi* TDMA-16-¹ (97.0 %). However, the DNA-DNA relatedness values between strain CH15-11ᵀ and the most closely related type strains were within a range of 35-59 %. Thus, based on the phylogenetic, phenotypic and genetic data, strain CH15-11ᵀ was classified as a member of the genus *Sphingomonas* as a representative of a novel species, for which the name *Sphingomonas daechungensis* sp. nov. is proposed. The type strain is CH15-11ᵀ ( = KCTC 23718ᵀ = JCM 17887ᵀ).

**Keywords**: 16S rRNA gene sequence; Cyanobacteria; Novel species; Sediment; *Sphingomonas daechungensis*; PMID: 24449789

**Flaviflexus salsibiostraticola** sp. nov., an actinobacterium isolated from a biofilm reactor


Jin L, Ko SR, Lee HG, Kim BH, Kim HS, Ahn CY, Oh HM*.

*Corresponding: Hee-Mock Oh(heemock@kribb.re.kr)

A Gram-stain-positive, aerobic, non-motile, non-spore-forming, coccobacillus-shaped actinobacterium, designated strain EBR4-1-², was isolated from a biofilm reactor in Korea. Comparative 16S rRNA gene sequence studies showed the isolate was clearly affiliated with the class *Actinobacteria*, and was related most closely to *Flaviflexus hungaensis* H5, showing 98.9 % similarity. Cells of strain EBR4-1-² formed yellow colonies on R2A agar, contained MK-9(H₄) as the predominant menaquinone, and included C₁₈:1ω9c, C₁₆:0, C₁₆:1ω9c and C₁₄:0 as the major fatty acids. The cell-wall peptidoglycan type was A5α (L-Lys-L-Ala-L-Lys-D-Glu). The G+C content of the genomic DNA of strain EBR4-1-² was 65.6 mol%. Thus, the combined genotypic and phenotypic data supported the conclusion that strain EBR4-1-² represents a novel species of the genus *Flaviflexus*, for which the name *Flaviflexus salsibiostraticola* sp. nov. is proposed. The type strain is EBR4-1-² ( = KCTC 33148ᵀ = JCM 19016ᵀ).

**Keywords**: 16S rRNA gene sequence; Actinobacterium; Biofilm reactor; *Flaviflexus salsibiostraticola*; Novel species

PMID: 24994776
Enhanced production of n-alkanes in *Escherichia coli* by spatial organization of biosynthetic pathway enzymes


Rahmana Z, Sung BH*, Yi JY, Bui le M, Lee JH, Kim SC. *Co-first: Bong Hyun Sung(bhsung@kribb.re.kr)*

Alkanes chemically mimic hydrocarbons found in petroleum, and their demand as biofuels is steadily increasing. Biologically, n-alkanes are produced from fatty acyl-ACP s by acyl-ACP reductases (AARs) and aldehyde deformylating oxygenases (ADOs). One of the major impediments in n-alkane biosynthesis is the low catalytic turnover rates of ADOs. Here, we studied n-alkane biosynthesis in *Escherichia coli* using a chimeric ADO-AAR fusion protein or zinc finger protein-guided ADO/AAR assembly on DNA scaffolds to control their stoichiometric ratios and spatial arrangements. Bacterial production of n-alkanes with the ADO-AAR fusion protein was increased 4.8-fold (24 mg/L) over a control strain expressing ADO and AAR separately. Optimal n-alkane biosynthesis was achieved when the ADO:AAR binding site ratio on a DNA scaffold was 3:1, yielding an 8.8-fold increase (44 mg/L) over the control strain. Our findings indicate that the spatial organization of alkane-producing enzymes is critical for efficient n-alkane biosynthesis in *E. coli*.

**Keywords**: Alkanes; Biofuel; Chimeric expression; DNA scaffold; Synthetic biology

PMID: 25456061

Aeration effects on metabolic events during sporulation of *Bacillus thuringiensis*


Sarrafzadeh MH, Schorr-Galindo S, La HJ, Oh HM*. *Corresponding: Hee-Mock Oh(heemock@kribb.re.kr)*

The metabolism of *Bacillus thuringiensis* during its sporulation process was investigated under different concentrations of oxygen. At the beginning of sporulation, the aeration conditions were regulated to obtain different oxygen transfer rates (OTR) in four separate fermentations, representing interrupted, limited, non-limited, and saturated oxygenation, respectively. A higher OTR resulted in a higher pH, up to about 9 in the case of saturated oxygenation, while the interrupted oxygenation resulted in a significantly acidic culture. In contrast, the absence of oxygen resulted in rapid sporangia lysis and caused acidification of the medium, indicating a distinctly different sporangia composition and different metabolism. The bacterium also showed different CO₂ production rates during sporulation, although a maximum point was observed in every case. With a higher OTR, the maximal value was observed after a longer time and at a lower value (40, 26, and 13 mmol/L/h for limited, non-limited, and saturated cases, respectively). Despite the exhaustion of glucose prior to the sporulation phase, the interrupted oxygenation resulted in acetate, lactate, and citrate in the medium with a maximum concentration of 4.8, 1.3, and 5.0 g/L, respectively. Notwithstanding, while the metabolic events differed visibly in the absence of oxygen, once sporulation was triggered, it was completed, even in the case of an interrupted oxygen supply.

**Keywords**: Aeration condition; *Bacillus thuringiensis*; Bioinsecticide; Metabolism; Sporulation

PMID: 24972809
Development of novel microsatellite markers for strain-specific identification of *Chlorella vulgaris*


Jo BH, Lee CS, Song HR, Lee HG, Oh HM*.  
*Corresponding: Hee-Mock Oh(heemock@kribb.re.kr)

A strain-specific identification method is required to secure *Chlorella* strains with useful genetic traits, such as a fast growth rate or high lipid productivity, for application in biofuels, functional foods, and pharmaceuticals. Microsatellite markers based on simple sequence repeats can be a useful tool for this purpose. Therefore, this study developed five novel microsatellite markers (mChl-001, mChl-002, mChl-005, mChl-011, and mChl-012) using specific loci along the chloroplast genome of *Chlorella vulgaris*. The microsatellite markers were characterized based on their allelic diversities among nine strains of *C. vulgaris* with the same 18S rRNA sequence similarity. Each microsatellite marker exhibited 2~5 polymorphic allele types, and their combinations allowed discrimination between seven of the *C. vulgaris* strains. The two remaining strains were distinguished using one specific interspace region between the mChl-001 and mChl-005 loci, which was composed of about 27 single nucleotide polymorphisms, 13~15 specific sequence sites, and (T)n repeat sites. Thus, the polymorphic combination of the five microsatellite markers and one specific locus facilitated a clear distinction of *C. vulgaris* at the strain level, suggesting that the proposed microsatellite marker system can be useful for the accurate identification and classification of *C. vulgaris*.

**Keywords**: Chloroplast; *Chlorella vulgaris*; Marker; Microsatellite; Polymorphism; Sequence repeat

PMID: 24931503

Higher biomass productivity of microalgae in an attached growth system, using wastewater


Lee SH, Oh HM, Jo BH, Lee SA, Shin SY, Kim HS, Lee SH, Ahn CY*.  
*Corresponding: Chi-Yong Ahn(cyahn@kribb.re.kr)

Although most algae cultivation systems are operated in suspended culture, an attached growth system can offer several advantages over suspended systems. Algal cultivation becomes light-limited as the microalgal concentration increases in the suspended system; on the other hand, sunlight penetrates deeper and stronger in attached systems owing to the more transparent water. Such higher availability of sunlight makes it possible to operate a raceway pond deeper than usual, resulting in a higher areal productivity. The attached system achieved 2.8-times higher biomass productivity and total lipid productivity of 9.1 g m⁻² day⁻¹ and 1.9 g m⁻² day⁻¹, respectively, than the suspended system. Biomass productivity can be further increased by optimization of the culture conditions. Moreover, algal biomass harvesting and dewatering were made simpler and cheaper in attached systems, because mesh-type substrates with attached microalgae were easily removed from the culture and the remaining treated wastewater could be discharged directly. When the algal biomass was dewatered using natural sunlight, the palmitic acid (C₁₆:0) content increased by 16% compared with the freeze-drying method. There was no great difference in other fatty acid composition. Therefore, the attached system for algal cultivation is a promising cultivation system for mass biodiesel production.

**Keywords**: Attached growth; Biodiesel; Cultivation; Microalgae; Wastewater

PMID: 25112320
**Article 275**

**Phenotypic profiling and gene expression analyses for aromatic and volatile compounds in Chamoeas (Cucumis melo)**


*Co-corresponding: HyeRan Kim(kimhr@kribb.re.kr)*

Gotgam chamoe (GgC), a native oriental melon in Korea, is known to possess the aroma of a dried persimmon, an agronomic relevance for melon breeding program. The volatile compounds and the transcript levels of aromatic compound genes in cultivar (Ohbokgulg chamoe [OC]) and GgC were profiled. A total of 62 volatile compounds were identified and quantified. Twenty-eight volatile compounds were specific to either the OC or the GgC. The amounts of volatile alcohol, saturated hydrocarbon, and unsaturated hydrocarbon compounds were 2.2, 2.7, and 1.1 times higher in OC, respectively. The amounts of ketone volatiles were 1.2 times higher in GgC, whereas the total amounts of esters were similar. In the shikimate pathway, transcriptional patterns with the fruit parts were different between the two chamoeas for CmDAHPS, CmDHDSDH, and CmEPSPS. The expression levels of all six genes investigated, especially CmCS, were highest in the peel of both chamoeas compared to the other parts. The transcript levels of the aromatic amino acid biosynthesis genes demonstrate that phenylalanine and tyrosine are present more in edible parts of the chamoe, while tryptophan may be accumulated low in the chamoe. In addition, phenylalanine and tryptophan are synthesized more in GgC than the OC.

**Keywords**: Aromatic amino acids; Chamoe; Cucumis melo; Shikimate pathway; Volatile aromatic compound

PMID: 24515385

**Article 276**

**A MORN-domain protein regulates growth and seed production and enhances freezing tolerance in Arabidopsis**


Lee J', Han CT, Kim H, Hur Y.

*First: Jeongyeo Lee(leejy@kribb.re.kr)*

AtRGP (AT4G17080, Arabidopsis thaliana reduction in growth and productivity) contains two N-terminal transmembrane helices and seven membrane occupation and recognition nexus motifs at its C-terminus, and associates with phosphatidylinositol phosphate kinase. To elucidate the function of AtRGP, we employed mutant plants to analyze gene expression, plant phenotypes, protein localization, structure and function of the chloroplast, and freezing tolerance. Overexpression of AtRGP increased growth rate, hypocotyl elongation, leaf size, seed production, photosynthetic rate, and freezing tolerance, and promoted chloroplast organization and stacking of grana. By contrast, Atrpg null mutants exhibited a smaller plant size, reduced seed production, photosynthetic rate, and freezing tolerance, and displayed abnormal chloroplast organization with insufficient stacking of grana. Considering these data, we postulate that AtRGP may bind transiently to the chloroplast envelope and interact with other proteins under certain conditions, thereby regulating cellular processes involved in growth and abiotic stress responses.

**Keywords**: Chloroplast targeting; Freezing tolerance; Grana stacking; MORN domain; RGP
Accumulation of anthocyanin and related genes expression during the development of cabbage seedlings


Li X, Uddin MR, Park WT, Kim YB, Seo JM, Kim SJ, Nou IS, Lee J, Kim H*, Park SU.
*Co-corresponding: HyeRan Kim(kimhr@kriib.re.kr)

In this study, we investigated anthocyanin accumulation and gene expression in response to light and dark conditions during the development of white and red cabbage seedlings. Two-day-old white cabbage seedlings expressed the highest transcript level for most of the genes under light conditions. Red cabbage also showed higher expression under light than under dark conditions, although gene expression (evaluated based on transcript levels normalized to that of a housekeeping gene) in 2-day-old red cabbage sprouts was much lower than that in the corresponding white cabbage sprouts. Trends in anthocyanin accumulation were similar for red and white cabbage but much greater accumulation was observed in red cabbage. Anthocyanin levels were higher in seedlings grown under light conditions compared to those grown under dark conditions for both cabbage cultivars. Especially, red cabbage accumulated 1.94-4.05 times greater total anthocyanin in 4-, 6-, 8-, and 10-day-old seedlings, when compared to white cabbage cultivar under light/dark and dark conditions. Our findings can improve understanding of the effects of light on accumulation of secondary metabolites in the seedling stages of various crops.

**Keywords**: Anthocyanin; Cabbage seedling; Cultivar; Gene expression; Light treatment

Production of 2-butanol from crude glycerol by a genetically-engineered *Klebsiella pneumoniae* strain


Oh BR, Heo SY, Lee SM, Hong WK, Park JM, Jung YR, Kim DH, Sohn JH, Seo JW, Kim CH*
*Corresponding: Chul Ho Kim(kim3641@kriib.re.kr)

*Klebsiella pneumoniae* was engineered to produce 2-butanol from crude glycerol as a sole carbon source by expressing acetolactate synthase (*ilvH*), keto-acid reducto-isomerase (*ilvC*) and dihydroxy-acid dehydratase (*ilvD*) from *K. pneumoniae*, and α-ketoisovalerate decarboxylase (*kivd*) and alcohol dehydrogenase (*adhA*) from *Lactococcus lactis*. Engineered *K. pneumoniae, ΔilvHΔpBR::iBO* (*ilvH-ilvC-ilvD-kivd-adhA*), produced 2-butanol (160 mg l⁻¹) from crude glycerol. To increase the yield of 2-butanol, we eliminated the 2,3-butanediol pathway from the recombinant strain by inactivating α-acetolactate decarboxylase (*adc*). This further engineering step improved the yield of 2-butanol from 160 to 320 mg l⁻¹. This represents the first successful attempt to produce 2-butanol from crude glycerol.

**Keywords**: 2-Butanol/isobutanol; Crude glycerol; Genetic engineering; *Klebsiella pneumoniae*;

PMID: 24078128
Enhancement of 1,3-propanediol production by expression of pyruvate decarboxylase and aldehyde dehydrogenase from Zymomonas mobilis in the acetalactate-synthase-deficient mutant of Klebsiella pneumoniae


Lee SM, Hong WK, Heo SY, Park JM, Jung YR, Oh BR, Joe MH, Seo JW, Kim CH.

*Co-corresponding: Chul Ho Kim(kim3641@kribb.re.kr), Jeong-Woo Seo(jwseo@kribb.re.kr)

The acetalactate synthase (als)-deficient mutant of Klebsiella pneumoniae fails to produce 1,3-propanediol (1,3-PD) or 2,3-butanediol (2,3-BD), and is defective in glycerol metabolism. In an effort to recover production of the industrially valuable 1,3-PD, we introduced the Zymomonas mobilis pyruvate decarboxylase (pdc) and aldehyde dehydrogenase (aldB) genes into the als-deficient mutant to activate the conversion of pyruvate to ethanol. Heterologous expression of pdc and aldB efficiently recovered glycerol metabolism in the 2,3-BD synthesis-defective mutant, enhancing the production of 1,3-PD by preventing the accumulation of pyruvate. Production of 1,3-PD in the pdc- and aldB-expressing als-deficient mutant was further enhanced by increasing the aeration rate. This system uses metabolic engineering to produce 1,3-PD while minimizing the generation of 2,3-BD, offering a breakthrough for the industrial production of 1,3-PD from crude glycerol.

**Keywords**: 1,3-propanediol; Acetalactate synthase; Glycerol; Klebsiella pneumoniae; Metabolic engineering

PMID: 24841211

Identification and characterization of a short-chain acyl dehydrogenase from Klebsiella pneumoniae and its application for high-level production of L-2,3-butandiol


Park JM, Hong WK, Lee SM, Heo SY, Jung YR, Kang IY, Oh BR, Seo JW, Kim CH.

*Co-corresponding: Chul Ho Kim(kim3641@kribb.re.kr), Jeong-Woo Seo(jwseo@kribb.re.kr)

Klebsiella pneumoniae synthesize large amounts of L-2,3-butandiol (L-2,3-BD), but the underlying mechanism has been unknown. In this study, we provide the first identification and characterization of an L-2,3-BD dehydrogenase from K. pneumoniae, demonstrating its reductive activities toward diacetyl and acetoin, and oxidative activity toward L-2,3-BD. Optimum pH, temperature, and kinetics determined for reductive and oxidative reactions support the preferential production of 2,3-BD during cell growth. Synthesis of L-2,3-BD was remarkably enhanced by increasing gene dosage, reaching levels that, to the best of our knowledge, are the highest achieved to date.

**Keywords**: Gene dosage; Klebsiella pneumoniae; L-2,3-BD dehydrogenase; L-2,3-butandiol; Reductive activity; Stereospecificity

PMID: 25037723
Production of mixed acids from non-pretreated red algae Gelidium amansii


Lee SM, Choi MH, Hong WK, Park JM, Yu A, Lee JS, Seo JW, Kim CH. *Corresponding: Chul Ho Kim(kim3641@kribb.re.kr)

The present study investigates the production of mixed acids by anaerobic fermentation using non-pretreated red algae Gelidium amansii as a substrate. The production levels of mixed acids were increased 1.6-fold in enrichment cultures of anaerobic digester sludge and tidal field soil compared with fresh sludge. The enrichment culture was composed of four major groups of microbial strains belonging to Clostridium sp. and Enterococcus sp. A defined mixed culture established based on the identified isolates increased mixed acids production level by up to 50.9% (g/g). Semi-continuous fermentation with a biomass-loading rate of 2.77g/L/d and hydraulic retention time of 30 days yielded a productivity of 1.41g/L/d. This is the first report of the production of mixed acids from the red alga G. amansii.

Keywords: Anaerobic fermentation; Enrichment culture; Gelidium amansii; Mixed acids; Red algae

Evaluation of whole Jerusalem artichoke (Helianthus tuberosus L.) for consolidated bioprocessing ethanol production

Renew Energ. 65:83-91.

Kim S*, Kim CH. *Corresponding: Seonghun Kim(seonghun@kribb.re.kr)

For consolidated bioprocessing (CBP), components of Jerusalem artichoke (Helianthus tuberosus L.) tubers and stalks as a potential bioenergy crop were analyzed as carbon and nutrient sources, respectively. The effectiveness of chemical pretreatment with dilute acid or alkali was evaluated to develop a CBP method. Cellulose content, delignification, and enzymatic hydrolysis efficiency of the pretreated stalks were increased more effectively by NaOH treatment than dilute H2SO4 treatment. However, weight loss was greater during alkali pretreatment. Additionally, large volumes of water were required to wash the alkali-treated biomass. Therefore, CBP using the dilute acid-pre-treated stalk and the ground tuber of Kluyveromyces marxianus were investigated. Fermentation of both pretreated stalks and tubers by K. marxianus with no nutrient supplementation proceeded acceptably. At 10% (w/v) stalk and 8% (w/v) tuber loading, K. marxianus produced 45.3g/L ethanol after 30h. The ethanol yield was 0.252g ethanol per g dry biomass, or 0.32g ethanol per g fermentable sugars, with a fermentable sugar conversion rate of 60%. These results suggest a cost-effective CBP strategy for bioethanol production from the whole Jerusalem artichoke plant.

Keywords: Alkali pretreatment; Consolidated bioprocessing; Diluted acid pretreatment; Jerusalem artichoke; Kluyveromyces marxianus; Whole plant utilization
**Salvia plebeia** suppresses atopic dermatitis-like skin lesions


*Co-corresponding: Mun-Chual Rho(rho-m@kribb.re.kr)*

Salvia plebeia R. Br. (Lamiaceae) has been used for folk medicines in Asian countries, including Korea and China, to treat skin inflammatory diseases and asthma. In this study, we investigated the effects of *S. plebeia* extract (SPE) on atopic dermatitis (AD)-like skin lesions and defined underlying mechanisms of action. We established an AD model in BALB/c mice by repeated local exposure of house dust mite extract (*Dermatophagoides farinae*, extract, DFE) and 2,4-dinitrochlorobenzene (DNCB) to the ears. Repeated alternative treatment of DFE/DNCB caused AD-like skin lesions. The oral administration of SPE decreased AD symptoms based on ear thickness and histopathological analysis, in addition to serum IgE and IgG2a levels. SPE suppressed mast cell infiltration into the ear and serum histamine level. SPE inhibited Th1/Th2/Th17 phenotype CD4+ T lymphocytes expansion in the lymph node and the expression of Th1/Th2/Th17 cytokines in the ear tissue. To define the underlying mechanisms of action, the tumor necrosis factor (TNF)-α and interferon (IFN)-γ activated human keratinocytes (HaCaT) model was used. SPE significantly suppressed the expression of cytokines and chemokines through the down-regulation of mitogen-activated protein kinases, nuclear factor-κB, and STAT1 in HaCaT cells. Taken together, our results suggest that SPE might be a candidate for the treatment of AD.

**Keywords**: Asthma; Atopic dermatitis (AD); Keratinocytes; *Salvia plebeia*; Skin inflammatory; *S. plebeia* extract (SPE)

PMID: 25004886
Neuraminidase inhibitory activities of quaternary isoquinoline alkaloids from Corydalis turtschaninovii rhizome

Bioorg Med Chem. 22(21):6047-52.

Kim JH, Ryu YB, Lee WS*, Kim YH.
*Corresponding: Woo Song Lee(wslee@kribb.re.kr)

Clostridium perfringens is a Gram-positive spore-forming bacterium that causes food poisoning. The neuraminidase (NA) protein of C. perfringens plays a pivotal role in bacterial proliferation and is considered a novel antibacterial drug target. Based on screens for novel NA inhibitors, a 95% EtOH extract of Corydalis turtschaninovii rhizome showed NA inhibitory activity (68% at 30 μg/ml), which resulted in the isolation of 10 isoquinoline alkaloids; namely, palmatine (1), berberine (2), coptisine (3), pseudodehydrocorydaline (4), jatrorrhizine (5), dehydrocorybulbine (6), pseudocoptisine (7), glaucine (8), corydaline (9) and tetrahydrocorypin (10). Interestingly, seven quaternary isoquinoline alkaloids 1-7 (IC₅₀ = 12.8 ± 1.5 to 65.2 ± 4.5 μM) showed stronger NA inhibitory activity than the tertiary alkaloids 8-10. In addition, highly active compounds 1 and 2 showed reversible non-competitive behavior based on a kinetic study. Molecular docking simulations using the Autodock 4.2 software increased our understanding of receptor-ligand binding of these compounds. In addition, we demonstrated that compounds 1 and 2 suppressed bacterial growth.

Keywords: Bacterial neuraminidase; Corydalis turtschaninovii; Fumariaceae; Isoquinoline alkaloids; Non-competitive inhibition

PMID: 25277281

A new stereoisomeric acetogenic glycoside from the flower buds of Buddleja officinalis

Bull Kor Chem Soc. 35(7):2159-61.

Lee C’, Hwang KW, Park SY.
*First: Chul Lee(leechul@kribb.re.kr)

Buddleja officinalis Maximowicz, which belongs to the Buddlejaceae, is a shrub tree and widely distributed in Asia, Africa, and America. It has been regarded as a traditional herbal medicine for the treatment of inflammation, conjunctivitis, headache, and clustered nebulae. Previous phytochemical studies have led to the discovery of terpenoids, phenethyl glycosides, flavonoids and saponins. In our continuing search for new constituents of B. officinalis, a new stereoisomeric acetogenic glycoside along with four phenethyl glycosides. Eight known isolates were identified by the extensive analysis of spectroscopic data and comparison with the literature values. Compound 1 was isolated as a white powder, and the molecular formula, C18H32O11, was demonstrated by HRESIMS data coupled with 1D NMR spectra. Also, compounds 3, 4, and 6 were isolated from the genus of Buddlejaceae for the first time. Among isolated compounds, some biological investigations on syringin have been reported that it has an ability to raise the release of ACh from nerve terminals, and inhibit the production of TNF-α and cytotoxic T cell proliferation.

Keywords: Acetogenic glycoside; Buddleja officinalis; Buddlejaceae; Hydrolysis; NMR; Syringin
Production of pinostilbene compounds by the expression of resveratrol O-methyltransferase genes in Escherichia coli

Enzyme Microb Technol. 54:8-14.

Jeong YJ, An CH, Woo SG, Jeong HJ, Kim YM, Park SJ, Yoon BD, Kim CY*. Corresponding: Cha Young Kim(kimcy@kribb.re.kr)

Resveratrol (3',4',5'-trans-trihydroxystilbene) is a polyphenolic phytoalexin that belongs to a family of naturally occurring stilbenes. It has been reported that the health-promoting activities of certain methylated resveratrol derivatives are more effective than those of unmodified resveratrol. In this study, we isolated two candidate genes with resveratrol O-methyltransferase (ROMT) activity from grape (Vitis riparia) and sorghum (Sorghum bicolor). To assess their ROMT activities in vivo, we synthesized VrROMT and ShROMT3 following codon-optimization and expressed the VrROMTsyn and ShROMT3syn genes using a dual expression vector system. Furthermore, we attempted to produce pterostilbene from resveratrol as a substrate by the expression of two putative ROMT proteins in Escherichia coli. Unexpectedly, expression of the ShROMT3syn gene in E. coli led to the production of mono-methylated stilbene (3,4''-dihydroxy-5-methoxy-trans-stilbene, pinostilbene) from resveratrol compounds. However, a very small amount of di-methylated stilbene (3,5-dimethoxy-4'-hydroxy-trans-stilbene, pterostilbene) was also detected. Consistently, we found that in vitro methylation assays of resveratrol by recombinant ShROMT3syn produced pinostilbene as the major product besides a very small amount of pterostilbene. By contrast, very small amounts of methylated resveratrol derivatives were detected in E. coli expressing the VrROMTsyn protein. This suggests that the ShROMT3syn is more useful in the production of pinostilbene compounds than pterostilbene from resveratrol in E. coli.

Keywords: Metabolic engineering; Pinostilbene; Pterostilbene; Resveratrol O-Methyltransferase (ROMT); Stilbene

PMID: 24267561

Purification and functional characterization of the first stilbene glucoside-specific β-glucosidase isolated from Lactobacillus kimchi


This study aimed to develop viable enzymes for bioconversion of resveratrol-glucoside into resveratrol. Out of 13 bacterial strains tested, Lactobacillus kimchi JB301 could completely convert polydatin into resveratrol. The purified enzyme had an optimum temperature of 30-40°C and optimum pH of pH 5.0 against polydatin. This enzyme showed high substrate specificities towards different substrates in the following order: isorhapontic > polydatin > malbeiroside A > oxyresveratrol-3-O-glucoside. Additionally, it rarely hydrolyzed astringin and desoxyrhapontic. Based on these catalytic specificities, we suggest this enzyme be named stilbene glucoside-specific β-glucosidase. Furthermore, polydatin extracts from Polygonum cuspidatum were successfully converted to resveratrol with a high yield (of over 99%). Stilbene glucoside-specific β-glucosidase is the first enzyme isolated from lactic acid bacteria capable of bio-converting various stilbene glucosides into stilbene.

Keywords: β-Glucosidase; Bioconversion; Lactobacillus kimchi; Polydatin; Resveratrol

PMID: 25442950
Expression and characterization of a novel 2-deoxyribose-5-phosphate aldolase from *Haemophilus influenzae* Rd KW20


Woo MH, Kim MS, Chung N, Kim JS.

A co-corresponding: Joong-Su Kim josungsu@kribb.re.kr

A codon-optimized 2-deoxyribose-5-phosphate aldolase (DERA) gene from *Haemophilus influenzae* Rd KW20 was synthesized and expressed in *Escherichia coli*, and the biochemical properties of its product were investigated. DERA was purified using affinity chromatography and characterized using 2-deoxyribose-5-phosphate as the substrate. Specific activity of the recombinant DERA was 34.1 U/mg. The pH and temperature optima were 7.5 and 40°C, respectively. Additionally, the recombinant enzyme retained stability up to temperatures below 50°C. Maximal enzyme activity was observed in presence of 300 mM of acetaldehyde. The apparent $K_m$ and $V_{max}$ of purified enzyme towards 2-deoxyribose-5-phosphate were 0.14 mM and 70.42 µmol min$^{-1}$ mg$^{-1}$ and towards 2-deoxy-D-ribose were 24.77 mM and 1.94 µmol min$^{-1}$ mg$^{-1}$, respectively. For synthesis of statin intermediates, the bioconversion process for production of (3R, 5S)-6-chloro-2,4,6-trideoxy-erythro-hexose from chloroacetaldehyde and acetaldehyde using the recombinant DERA was studied and this process took 3 h for maximal conversion. This recombinant DERA could be potentially applied in the production of (3R, 5S)-6-chloro-2,4,6-trideoxy-erythro-hexose.

**Keywords**: 2-deoxyribose-5-phosphate aldolase (DERA); *Haemophilus influenzae*; Statin intermediates synthesis

Simultaneous quantitation and validation of triterpenoids and phytosteroids in *Phaseolus angularis* seeds

Molecules. 19(7):10309-19.


A reproducible analytical method using reverse-phase high liquid performance chromatography combined with UV detecting was developed for the quantitative determination of four compounds isolated from the ethanol extract of *Phaseolus angularis* seeds (PASE): oleanolic acid (1), oleanolic acid acetate (2), stigmasterol (3) and β-sitosterol (4). This method was fully validated in terms of linearity ($r^2 > 0.999$), accuracy (98.5%-100.8%), precision (<0.92%), LOD (<0.0035 mg/mL), and LOQ (<0.0115 mg/mL). The effects of the PASE and isolated compounds 1-4 on TLR4 activation were tested in THP1-Blue cells. Among the tested substances, compound 2 showed potent inhibitory activity with an IC$_{50}$ value of 3.89 ± 0.17 µM.

**Keywords**: HPLC-UV; *Phaseolus angularis* seeds (PASE); TLR4 activation; Validation

PMID: 25033058

![Diagram of compounds](image)
Ampelopsis brevipedunculata extract prevents bone loss by inhibiting osteoclastogenesis in vitro and in vivo

Molecules. 19(11):18465-78.

Kim JY, Park SH, Oh HM, Kwak SC, Baek JM, Lee MS, Rho MC*, Oh J.
*Corresponding: Mun-Chual Rho(rho-m@kribb.re.kr)

Osteoclasts play a critical role in bone resorbing disorders such as osteoporosis, periodontitis, and rheumatoid arthritis. Therefore, discovery of agents capable of suppressing osteoclast differentiation may aid the development of a therapeutic access for the treatment of pathological bone loss. Ampelopsis brevipedunculata has been used as herbal folk medicine to treat liver diseases and inflammation in Asia. However, its effects on osteoclast differentiation are unknown. We were aimed to investigate the anti-osteoclastogenic activity in vitro and in vivo and to elucidate the underlying mechanism of Ampelopsis brevipedunculata extract (ABE). In this study, ABE inhibited receptor activator of NF-κB ligand (RANKL)-induced osteoclast differentiation, the formation of filamentous actin rings and the bone resorbing activity of mature osteoclasts. ABE inhibited RANKL-induced p38 and IκB phosphorylation and IκB degradation. Also, ABE suppressed the mRNA and protein expression of nuclear factor of activated T cells c1 (NFATc1) and c-Fos, and the mRNA expression of genes required for cell fusion and bone resorption, such as osteoclast-associated receptor (OSCAR), tartrate resistant acid phosphatase (TRAP), cathepsin K, dendritic cell-specific transmembrane protein (DC-STAMP), β3-integrin and osteoclast stimulatory transmembrane protein (OC-STAMP). Furthermore, results of micro-CT and histologic analysis indicated that ABE remarkably prevented lipopolysaccharide (LPS)-induced bone erosion. These results demonstrate that ABE prevents LPS-induced bone erosion through inhibition of osteoclast differentiation and function, suggesting the promise of ABE as a potential cure for various osteoclast-associated bone diseases.

Keywords: Ampelopsis brevipedunculata extract (ABE); Bone loss; Bone resorption; Osteoclast differentiation

PMID: 25397737

Saikosaponin D isolated from Bupleurum falcatum inhibits selectin-mediated cell adhesion


Jang MJ, Kim YS, Bae EY, Oh TS, Choi HJ, Lee JH, Oh HM*, Lee SW.
*Corresponding: Hyun-Mee Oh(ohhm@kribb.re.kr)

Three saikosaponins were isolated from the MeOH extract of the roots of Bupleurum falcatum L.: saikosaponins B3 (1); B4 (2); and D (3). Of the three, compound 3 inhibited the interaction of selectins (E, L, and P) and THP-1 cells with IC50 values of 1.8, 3.0 and 4.3 μM, respectively. Also, the aglycone structure 4 of compound 3 showed moderate inhibitory activity on L-selectin-mediated cell adhesion. From these results, we suspect that compound 3 isolated from Bupleurum falcatum roots would be a good candidate for therapeutic strategies to treat inflammation.

Keywords: Anti-inflammatory agent; Bupleurum falcatum; Cell adhesion molecule; Saikosaponin; Selectin

PMID: 25486247
First thermostable endo-β-1,4-glucanase from newly isolated Xanthomonas sp. EC102

Woo MH, Chang YH, Lee HS, Pak PJ, Kim JS, Chung N. 'Co-corresponding: Joong-Su Kim(joongsu@kribb.re.kr)

A novel gene encoding thermostable endoglucanase was identified in Xanthomonas sp. EC102 from soil. The gene had 1,458 base pairs of open reading frame, which encode a 52-kDa protein of 486 amino acid residues. Sequence of the amino acid residues was similar with the endoglucanase from Xanthomonas campestris pv. campestris ATCC33913 (GenBank Accession No. NP_638867.1) (94 % identity). The endoglucanase was overexpressed in Escherichia coli BL21 and purified. Temperature for the highest enzymatic activity was 70 °C and pH optima was pH 5.5. The specific activity of the endoglucanase toward carboxymethylcellulose (CMC) was approximately 2 µmol min⁻¹ mg⁻¹, Vmax for CMC was 1.44 µmol mg⁻¹ min⁻¹, and Km values was 25.6 mg mL⁻¹. The EC102 endoglucanase was stable at temperatures up to 60 °C, and it was activated by 0.1 mM of Mn²⁺ and Co²⁺. This is the first report about thermostable endoglucanase from Xanthomonas sp.

**Keywords**: Characterization; Endoglucanase; Thermostability; Xanthomonas

PMID: 24399319

Anti-rotavirus effects by combination therapy of stevioside and Sophora flavescens extract


Anti-rotiral activities of Sophora flavescens extract (SFE) and stevioside (SV) from Stevia rebaudiana Bertoni either singly or in various combinations were examined in vitro and in vivo using a porcine rotavirus G5[P7] strain. Combination of SFE and SV inhibited in vitro virus replication more efficiently than each single treatment. In the piglet model, SV had no effect on rotavirus enteritis, whereas SFE improved but did not completely cure rotavirulent enteritis. Interestingly, combination therapy of SFE and SV alleviated diarrhea, and markedly improved small intestinal lesion score and fecal virus shedding. Acute toxicity tests including the piglet lethal dose 50, and body weight, organ weight and pathological changes for the combination therapy did not show any adverse effect on the piglets. These preliminary data suggest that the combination therapy of SV and SFE is a potential curative medication for rotaviral diarrhea in pigs. Determination of the efficacy of this combination therapy in other species including humans needs to be addressed in the future.

**Keywords**: Combination therapy; Enteritis; Rotavirus; Sophora flavescens extract (SFE); Stevioside; Synergistic effect

PMID: 24704033
**Vigna angularis** inhibits IL-6-induced cellular signalling and ameliorates collagen-induced arthritis

*Rheumatology.* 53(1):56-64.

*Corresponding: Mun-Chual Rho(rho-m@kribb.re.kr)

**OBJECTIVES:** The present study was conducted in order to assess whether extracts or isolated compounds from *Vigna angularis* were able to suppress IL-6 signalling and to show the therapeutic effect on collagen-induced arthritis (CIA) in mice.

**METHODS:** The effect of *V. angularis* on IL-6 signalling was studied by measuring Stat3-dependent luciferase activity, expression of inflammation-related genes, and phosphorylation of Janus kinase 2 (JAK2), signal transducer and activator of transcription 3 (STAT3) and extracellular signal-regulated kinase (ERK) induced by IL-6. CIA was induced by immunizing with bovine type II collagen. *V. angularis* extract (VAE) was administrated orally at 50 and 100 mg/kg from day 1 to day 28. Induction of arthritis was evaluated with a visual scoring system and histological analysis.

**RESULTS:** Extracts or two triterpenoid compounds from *V. angularis* showed potent inhibitory effects on pSTAT3-inducible luciferase activity, STAT3 tyrosine phosphorylation and the expression of inflammation-related genes induced by IL-6. Administration of VAE significantly suppressed the progression of CIA, accompanied by a reduced antibody response to type II collagen and protection from tissue damage in knee joints.

**CONCLUSION:** Administration of VAE has a therapeutic effect on CIA and this effect is associated with the inhibitory activity on IL-6/STAT3 signalling. These results suggest that extracts or compounds from *V. angularis* could be a useful treatment for diseases related to IL-6, including RA.

**Keywords**: Collagen-induced arthritis (CIA); IL-6; Rheumatoid arthritis (RA); STAT3; *Vigna angularis*

PMID: 24097134
Article 299
Genome sequence of the acrystalliferous Bacillus thuringiensis serovar israelensis strain 4Q7, widely used as a recombination host

Genome Announc. 2(2):e00231.

Jeong H*, Park SH, Choi SK*.
*Co-corresponding: Soo-Kean Choi (sookcun@kribb.re.kr), Haeyoung Jeong (hyjeong@kribb.re.kr)

PMID: 24699954

Article 302
Genome sequence of Bacillus amyloliquefaciens GB03, an active ingredient of the first commercial biological control product

Genome Announc. 2(5): e01092.

Choi SK, Jeong H, Kloepper JW, Ryu CM*.
*Corresponding: Choong-Min Ryu (cmryu@kribb.re.kr)

PMID: 25359911

Article 300
Complete genome sequences of noncoding regions of Korean equine H3N8 influenza virus

Genome Announc. 2(3):e00461.

Na W, Hong M, Yeom M, Kim S, Kim JK, Song D.*
*Co-corresponding: Daesub Song

PMID: 24831153

Article 303
Genome sequence of the plant endophyte Bacillus pumilus INR7, triggering induced systemic resistance in field crops

Genome Announc. 2(5):e01093.

Jeong H, Choi SK, Kloepper JW, Ryu CM*.
*Corresponding: Choong-Min Ryu (cmryu@kribb.re.kr)

PMID: 25359912

Article 301
Complete genome sequence of Bacillus anthracis HYU01, isolated from soil samples in the Korean peninsula

Genome Announc. 2(4):e00769

Kim SK, Chung WH, Kim SH, Jung KH, Kim N*, Chai YG.
*Co-corresponding: Namshin Kim (deepreds@kribb.re.kr)

PMID: 25103761
Indexes

- Author Index
- Journal Index
- Keyword Index
<table>
<thead>
<tr>
<th>Author</th>
<th>Article No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahn CY (Chi-Yong Ahn)</td>
<td>263, 267-9, 274</td>
</tr>
<tr>
<td>Ahn JS (Jong Seog Ahn)</td>
<td>234-5, 238, 241-2</td>
</tr>
<tr>
<td>Ahn KS (Kyung Seop Ahn)</td>
<td>224, 226, 229, 231-2</td>
</tr>
<tr>
<td>Bae KH (Kwang-Hee Bae)</td>
<td>41, 43, 48, 50</td>
</tr>
<tr>
<td>Bae KS (Kyung Sook Bae)</td>
<td>134, 140</td>
</tr>
<tr>
<td>Ban HS (Hyun Seung Ban)</td>
<td>12</td>
</tr>
<tr>
<td>Chang KT (Kyu Tae Chang)</td>
<td>161, 163-5, 167-71, 173-7</td>
</tr>
<tr>
<td>Chang YH (Young-Hyo Chang)</td>
<td>189-94</td>
</tr>
<tr>
<td>Chi SW (Seung-Wook Chi)</td>
<td>29-30, 32, 42</td>
</tr>
<tr>
<td>Cho EW (Eun Wie Cho)</td>
<td>24</td>
</tr>
<tr>
<td>Cho HS (Hye Sun Cho)</td>
<td>259, 262</td>
</tr>
<tr>
<td>Cho S (Sungchan Cho)</td>
<td>245-6, 249-52</td>
</tr>
<tr>
<td>Cho YS (Yee Sook Cho)</td>
<td>207-8</td>
</tr>
<tr>
<td>Choi ES (Eui Sung Choi)</td>
<td>196</td>
</tr>
<tr>
<td>Choi I (Inpyo Choi)</td>
<td>69, 72, 75-6</td>
</tr>
<tr>
<td>Choi SK (Soo Keun Choi)</td>
<td>299</td>
</tr>
<tr>
<td>Chu IS (In-Sun Chu)</td>
<td>210</td>
</tr>
<tr>
<td>Chung BH (Bong Hyun Chung)</td>
<td>84-7, 93</td>
</tr>
<tr>
<td>Chung IS (Im Sik Chung)</td>
<td>106</td>
</tr>
<tr>
<td>Chung KS (Kyung-Sook Chung)</td>
<td>2</td>
</tr>
<tr>
<td>Ha TH (Tae Hwan Ha)</td>
<td>103</td>
</tr>
<tr>
<td>Han KH (Kyoun Hoon Han)</td>
<td>104</td>
</tr>
<tr>
<td>Hong YS (Young-Soohong)</td>
<td>236-7, 239-40, 244</td>
</tr>
<tr>
<td>Jang JH (Jae-Hyuk Jang)</td>
<td>241</td>
</tr>
<tr>
<td>Jeon JH (Jae Heung Jeon)</td>
<td>253, 262</td>
</tr>
<tr>
<td>Jeong H (Haeyoung Jeong)</td>
<td>101, 299</td>
</tr>
<tr>
<td>Jeong JY (Jin Young Jeong)</td>
<td>92</td>
</tr>
<tr>
<td>Jeong SC (Soon-Chun Jeong)</td>
<td>178, 186</td>
</tr>
<tr>
<td>Jeong TS (Tae-Sook Jeong)</td>
<td>130</td>
</tr>
<tr>
<td>Jeong WJ (Won Jong Jeong)</td>
<td>256, 260</td>
</tr>
<tr>
<td>Jin TE (Tae-Eun Jin)</td>
<td>195</td>
</tr>
<tr>
<td>Kang JS (Jong Soon Kang)</td>
<td>181-2, 187</td>
</tr>
<tr>
<td>Kang YK (Yong Kook Kang)</td>
<td>23</td>
</tr>
<tr>
<td>Kim BC (Byoung Chan Kim)</td>
<td>141</td>
</tr>
<tr>
<td>Kim BY (Bo Yeon Kim)</td>
<td>235, 238, 241-2, 248</td>
</tr>
<tr>
<td>Kim CG (Chang-Gi Kim)</td>
<td>179-80</td>
</tr>
<tr>
<td>Kim CH (Chul Ho Kim)</td>
<td>278-81</td>
</tr>
<tr>
<td>Kim CJ (Chang-Jin Kim)</td>
<td>142, 145</td>
</tr>
<tr>
<td>Kim CY (Cha Young Kim)</td>
<td>287</td>
</tr>
<tr>
<td>Kim DU (Dong Uk Kim)</td>
<td>57</td>
</tr>
<tr>
<td>Kim H (Haseong Kim)</td>
<td>80</td>
</tr>
<tr>
<td>Kim H (HyeRan Kim)</td>
<td>265-6, 275, 277</td>
</tr>
<tr>
<td>Kim HC (Hyoung-Chin Kim)</td>
<td>148, 151, 158</td>
</tr>
<tr>
<td>Kim HS (Hee-Sik Kim)</td>
<td>254-5, 257, 261</td>
</tr>
<tr>
<td>Kim HS (Hyun Soon Kim)</td>
<td>253, 255, 258</td>
</tr>
<tr>
<td>Kim J (Janghwan Kim)</td>
<td>206</td>
</tr>
<tr>
<td>Kim JJ (Jeong Hoon Kim)</td>
<td>37-9, 45</td>
</tr>
<tr>
<td>Kim JS (Jang Seong Kim)</td>
<td>47, 49, 298</td>
</tr>
<tr>
<td>Kim JS (Joong-Su Kim)</td>
<td>289, 293</td>
</tr>
<tr>
<td>Kim JW (Jae Wha Kim)</td>
<td>107</td>
</tr>
<tr>
<td>Kim KM (Kyung Mo Kim)</td>
<td>137, 146</td>
</tr>
<tr>
<td>Kim KS (Kyoung-Shim Kim)</td>
<td>150, 156</td>
</tr>
<tr>
<td>Kim M (Moonil Kim)</td>
<td>89</td>
</tr>
<tr>
<td>Kim MH (Myung Hee Kim)</td>
<td>66</td>
</tr>
<tr>
<td>Kim N (Namshin Kim)</td>
<td>19, 25, 178, 301</td>
</tr>
<tr>
<td>Kim NS (Nam-Soon Kim)</td>
<td>160</td>
</tr>
<tr>
<td>Kim S (Semi Kim)</td>
<td>67, 70-1, 73</td>
</tr>
<tr>
<td>Kim S (Seonghun Kim)</td>
<td>282</td>
</tr>
<tr>
<td>Kim S (Sunhong Kim)</td>
<td>247</td>
</tr>
<tr>
<td>Kim SG (Song-Gun Kim)</td>
<td>139</td>
</tr>
<tr>
<td>Kim SH (Seok Ho Kim)</td>
<td>54-5</td>
</tr>
<tr>
<td>Kim SJ (Seung Jun Kim)</td>
<td>26, 34, 40, 44</td>
</tr>
<tr>
<td>Kim SU (Sung Uk Kim)</td>
<td>128, 132</td>
</tr>
<tr>
<td>Kim SU (Sun-Uk Kim)</td>
<td>164, 176-7</td>
</tr>
<tr>
<td>Kim SW (Suk Weon Kim)</td>
<td>138, 144, 147</td>
</tr>
<tr>
<td>Kim SY (Seon-Young Kim)</td>
<td>5-6, 9-10, 15, 20-1, 51</td>
</tr>
<tr>
<td>Kim TD (Tae-Dom Kim)</td>
<td>75-6</td>
</tr>
<tr>
<td>Kim WG (Won Gon Kim)</td>
<td>95-6</td>
</tr>
<tr>
<td>Kim YS (Yong Sam Kim)</td>
<td>52</td>
</tr>
<tr>
<td>Kim YS (Yong Sung Kim)</td>
<td>9, 17-8, 20-2</td>
</tr>
<tr>
<td>Ko G (Gunhwan Ko)</td>
<td>213</td>
</tr>
<tr>
<td>Ko SK (Sung-Kyun Ko)</td>
<td>243</td>
</tr>
<tr>
<td>Kwak SS (Sang Soo Kwak)</td>
<td>113, 116-7, 121-2</td>
</tr>
<tr>
<td>Kwon BM (Byoung-Mog Kwon)</td>
<td>4, 7</td>
</tr>
<tr>
<td>Kwon ES (Eun Soo Kwon)</td>
<td>58</td>
</tr>
<tr>
<td>Kwon KS (Ki Sun Kwon)</td>
<td>51, 56, 59-60</td>
</tr>
<tr>
<td>Kwon O (Ohsuk Kwon)</td>
<td>81, 83</td>
</tr>
<tr>
<td>Kwon SY (Suk Yoon Kwon)</td>
<td>110-2, 114, 120, 123-5</td>
</tr>
<tr>
<td>Lee B (Byungwook Lee)</td>
<td>211</td>
</tr>
<tr>
<td>Lee C (Chul Lee)</td>
<td>286</td>
</tr>
<tr>
<td>Lee CH (Chul Ho Lee)</td>
<td>149, 152, 155, 157</td>
</tr>
<tr>
<td>Lee DH (Dae-Hee Lee)</td>
<td>78</td>
</tr>
<tr>
<td>Lee DY (Da Yong Lee)</td>
<td>205</td>
</tr>
<tr>
<td>Lee EG (Eun Gyo Lee)</td>
<td>197</td>
</tr>
<tr>
<td>Lee HG (Hee Gu Lee)</td>
<td>13-4</td>
</tr>
</tbody>
</table>
Lee HS (Haeng-Soon Lee)  116-7  Yeom YI (Young Il Yeom)  8
Lee J (Jeongyeo Lee)  276  Yoon SR (Suk Ran Yoon)  74
Lee J (Jinhae Lee)  20, 214-5, 217-9  Yoon SW (Sun Woo Yoon)  200
Lee J (Joongku Lee)  159  Yu DY (Dae Yeul Yu)  61-2
Lee JS (Jung-Sook Lee)  133, 135, 143  Yu K (Kweon Yu)  88
Lee JW (Jeong Woong Lee)  28, 33  Yun J (Ji Eun Yun)  183
Lee SC (Sang Chul Lee)  41, 43, 48
Lee SG (Seung Goo Lee)  77, 82
Lee SJ (Sang Jun Lee)  63, 65
Lee SJ (Seon-Jin Lee)  1
Lee SR (Sang-Rae Lee)  166, 168
Lee WS (Woo Song Lee)  285, 288, 294
Lee YI (Young Ik Lee)  129, 131
Lim EK (Eun Kyung Lim)  91
Liu JR (Jang Ryol Liu)  115
Min JK (Jeong Ki Min)  46
Moon JS (Jae Sun Moon)  118-9
Oh DB (Doo-Byoung Oh)  79
Oh HM (Hee-Mock Oh)  263-4, 267-70, 272-3
Oh HM (Hyun-Mee Oh)  292
Oh SJ (Soo Jin Oh)  185, 188
Oh SR (Sei Ryang Oh)  220, 225, 227-8, 230, 233
Pan JG (Jae Gu Pan)  97, 102
Park BC (Byoung Chul Park)  37
Park DS (Doo-Sang Park)  136
Park HY (Ho-Yong Park)  126-7
Park KC (Kyung Chan Park)  8
Park SG (Sung Goo Park)  35, 38-9, 45
Park SH (Seung-Hwan Park)  99
Park SS (Sung Sup Park)  53
Poo H (Haryoung Poo)  64
Rho MC (Mun Chual Rho)  284, 290-1, 295
Ryu CM (Choong-Min Ryu)  94, 98, 100, 302-3
Ryu HW (Hyung Won Ryu)  221, 227
Ryu YB (Young Bae Ryu)  283
Seo JW (Jeong-Woo Seo)  279-80
Shin YB (Yong Beom Shin)  105, 108-9
Sim BW (Bo-Woong Sim)  162
Son KH (Kwang-Hee Son)  126-7
Song BS (Bong-Seok Song)  172
Sung BH (Bong Hyun Sung)  271
Won M (Misun Won)  2-3, 11-2
Won YS (Young Suk Won)  153-4
Woo EJ (Eui-Jeon Woo)  27, 31, 36
Yang JO (Jin Ok Yang)  211

* Retired authors were not included in the index.
<table>
<thead>
<tr>
<th>Journal</th>
<th>Article No</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS Synth Biol</td>
<td>77</td>
</tr>
<tr>
<td>Acta Crystallogr D Biol Crystal</td>
<td>26-7, 283</td>
</tr>
<tr>
<td>Acta Vet Hung</td>
<td>198</td>
</tr>
<tr>
<td>Aging</td>
<td>51</td>
</tr>
<tr>
<td>Am J Chin Med</td>
<td>284</td>
</tr>
<tr>
<td>Anaerobe</td>
<td>189</td>
</tr>
<tr>
<td>Anal Chem</td>
<td>86</td>
</tr>
<tr>
<td>Analyst</td>
<td>84-5, 103</td>
</tr>
<tr>
<td>Andrologia</td>
<td>148</td>
</tr>
<tr>
<td>Anim Reprod Sci</td>
<td>163</td>
</tr>
<tr>
<td>Anim Cells Syst</td>
<td>161-2</td>
</tr>
<tr>
<td>Ann Microbiol</td>
<td>190</td>
</tr>
<tr>
<td>Antioxid Redox Signal</td>
<td>1</td>
</tr>
<tr>
<td>Antonie Van Leeuwenhoek</td>
<td>133-6, 191-3, 263</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>2</td>
</tr>
<tr>
<td>Appl Microbiol Biotechnol</td>
<td>78, 253, 296</td>
</tr>
<tr>
<td>Arch Pharm Res</td>
<td>220</td>
</tr>
<tr>
<td>Bangladesh J Plant Taxon</td>
<td>159</td>
</tr>
<tr>
<td>Biochm Biophys Res Commun</td>
<td>28-31, 52, 67-8, 94, 205, 297</td>
</tr>
<tr>
<td>Biochem Pharmacol</td>
<td>4</td>
</tr>
<tr>
<td>Biochem Syst Ecol</td>
<td>234</td>
</tr>
<tr>
<td>Biochim Biophys Acta</td>
<td>3, 104, 245</td>
</tr>
<tr>
<td>Biochimie</td>
<td>137</td>
</tr>
<tr>
<td>Bioinformatics</td>
<td>5</td>
</tr>
<tr>
<td>Biol Blood Marrow Transplant</td>
<td>69</td>
</tr>
<tr>
<td>Biol Pharm Bull</td>
<td>95, 246</td>
</tr>
<tr>
<td>Biol Reprod</td>
<td>164</td>
</tr>
<tr>
<td>Biomass Bioenerg</td>
<td>254</td>
</tr>
<tr>
<td>Biomed Res Int</td>
<td>165, 255, 264</td>
</tr>
<tr>
<td>Biomol Ther</td>
<td>221</td>
</tr>
<tr>
<td>Bioorg Med Chem Lett</td>
<td>96, 235</td>
</tr>
<tr>
<td>Bioorg Med Chem</td>
<td>285</td>
</tr>
<tr>
<td>Biotech Bioproc Eng</td>
<td>105</td>
</tr>
<tr>
<td>Biotech Lett</td>
<td>79, 278</td>
</tr>
<tr>
<td>BMB Rep</td>
<td>32, 149, 247</td>
</tr>
<tr>
<td>BMC Biotechnol</td>
<td>236</td>
</tr>
<tr>
<td>BMC Genomics</td>
<td>6, 256, 265</td>
</tr>
<tr>
<td>Bone</td>
<td>33</td>
</tr>
<tr>
<td>Brain Res</td>
<td>53, 150</td>
</tr>
<tr>
<td>Bull Kor Chem Soc</td>
<td>34-5, 286</td>
</tr>
<tr>
<td>Can J Plant Sci</td>
<td>110-1</td>
</tr>
<tr>
<td>Cancer Lett</td>
<td>7, 17</td>
</tr>
<tr>
<td>Cancer Prev Res</td>
<td>248</td>
</tr>
<tr>
<td>Cancer Res</td>
<td>54</td>
</tr>
<tr>
<td>Cancer Sci</td>
<td>237</td>
</tr>
<tr>
<td>Carcinogenesis</td>
<td>8-9, 70</td>
</tr>
<tr>
<td>Cell Res</td>
<td>206</td>
</tr>
<tr>
<td>Cell Signal</td>
<td>71</td>
</tr>
<tr>
<td>Chem Commun</td>
<td>87</td>
</tr>
<tr>
<td>Chem Phys Lett</td>
<td>209</td>
</tr>
<tr>
<td>Clin Cancer Res</td>
<td>210, 298</td>
</tr>
<tr>
<td>Clin Exp Vaccine Res</td>
<td>199</td>
</tr>
<tr>
<td>Curr Opin Hematol</td>
<td>72</td>
</tr>
<tr>
<td>Curr Top Microbiol Immunol</td>
<td>200</td>
</tr>
<tr>
<td>Cytotherapy</td>
<td>55</td>
</tr>
<tr>
<td>DNA Res</td>
<td>178</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>10</td>
</tr>
<tr>
<td>Emerg Infect Dis</td>
<td>201</td>
</tr>
<tr>
<td>Enzyme Microb Technol</td>
<td>287-8</td>
</tr>
<tr>
<td>Epidemiol Infect</td>
<td>202</td>
</tr>
<tr>
<td>Eur J Soil Biol</td>
<td>179</td>
</tr>
<tr>
<td>Exp Neurobiol</td>
<td>166</td>
</tr>
<tr>
<td>FEBS J</td>
<td>249</td>
</tr>
<tr>
<td>FEBS Lett</td>
<td>266</td>
</tr>
<tr>
<td>FEMS Microbiol Lett</td>
<td>97</td>
</tr>
<tr>
<td>Food Chem Toxicol</td>
<td>151, 222-3</td>
</tr>
<tr>
<td>Food Chem</td>
<td>180</td>
</tr>
<tr>
<td>Forensic Sci Int Genet</td>
<td>18</td>
</tr>
<tr>
<td>Free Radic Biol Med</td>
<td>56, 152</td>
</tr>
<tr>
<td>Front Microbiol</td>
<td>63</td>
</tr>
<tr>
<td>G3</td>
<td>88</td>
</tr>
<tr>
<td>Gene</td>
<td>19</td>
</tr>
<tr>
<td>Genes Genom</td>
<td>112</td>
</tr>
<tr>
<td>Genome Announc</td>
<td>299-303</td>
</tr>
<tr>
<td>Genomics Inform</td>
<td>73, 211</td>
</tr>
<tr>
<td>Hepatology</td>
<td>153-4</td>
</tr>
<tr>
<td>Hortic Environ Biotech</td>
<td>138</td>
</tr>
<tr>
<td>Hum Mol Genet</td>
<td>20, 207</td>
</tr>
<tr>
<td>Hum Reprod</td>
<td>74</td>
</tr>
<tr>
<td>IEEE Sens J</td>
<td>89</td>
</tr>
<tr>
<td>Immunobiology</td>
<td>224</td>
</tr>
<tr>
<td>Int Immunopharm</td>
<td>225-6</td>
</tr>
<tr>
<td>Int J Adv Intell Parad</td>
<td>80</td>
</tr>
<tr>
<td>Int J Agricult Biol</td>
<td>194</td>
</tr>
<tr>
<td>Int J Cancer</td>
<td>11</td>
</tr>
<tr>
<td>Journal Name</td>
<td>Volume, Range</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Int J Cell Biol</td>
<td>212</td>
</tr>
<tr>
<td>Int J Mol Sci</td>
<td>36, 167</td>
</tr>
<tr>
<td>Int J Syst Oncol</td>
<td>21</td>
</tr>
<tr>
<td>Int J Agricul Food Evol Microbiol</td>
<td>139-43, 267-70</td>
</tr>
<tr>
<td>J Allergy Clin Immunol</td>
<td>75</td>
</tr>
<tr>
<td>J Alzheimers Dis</td>
<td>168</td>
</tr>
<tr>
<td>J Antibiot</td>
<td>238</td>
</tr>
<tr>
<td>J Biotechnol</td>
<td>196-7, 271</td>
</tr>
<tr>
<td>J Chem Ecol</td>
<td>98</td>
</tr>
<tr>
<td>J Food Biochem</td>
<td>181</td>
</tr>
<tr>
<td>J Genet Genomics</td>
<td>195</td>
</tr>
<tr>
<td>J Ginseng Res</td>
<td>144, 228, 250</td>
</tr>
<tr>
<td>J Hypertens</td>
<td>155</td>
</tr>
<tr>
<td>J Ind Microbiol Biotechnol</td>
<td>99, 279-80</td>
</tr>
<tr>
<td>J Invest Dermatol</td>
<td>64</td>
</tr>
<tr>
<td>J Kor Soc Appl Biol Chem</td>
<td>289</td>
</tr>
<tr>
<td>J Med Chem</td>
<td>12</td>
</tr>
<tr>
<td>J Microbiol Biotechnol</td>
<td>37-9, 81, 100, 127-8, 239-40, 257, 273-4</td>
</tr>
<tr>
<td>J Microbiol Methods</td>
<td>57</td>
</tr>
<tr>
<td>J Microbiol</td>
<td>126, 145, 203, 272</td>
</tr>
<tr>
<td>J Mol Evol</td>
<td>146</td>
</tr>
<tr>
<td>J Nat Prod</td>
<td>241</td>
</tr>
<tr>
<td>J Neurosci</td>
<td>156</td>
</tr>
<tr>
<td>J Pineal Res</td>
<td>169-71, 229</td>
</tr>
<tr>
<td>J Plant Biochem Biot</td>
<td>113</td>
</tr>
<tr>
<td>J Plant Physiol</td>
<td>114</td>
</tr>
<tr>
<td>J Reprod Dev</td>
<td>172</td>
</tr>
<tr>
<td>Kor J Horticul Sci</td>
<td>258</td>
</tr>
<tr>
<td>Lect Notes Elect Eng</td>
<td>213</td>
</tr>
<tr>
<td>Life Sci</td>
<td>174</td>
</tr>
<tr>
<td>Longev Healthspan</td>
<td>58</td>
</tr>
<tr>
<td>Macromolecules</td>
<td>106</td>
</tr>
<tr>
<td>Magn Reson Med</td>
<td>157</td>
</tr>
<tr>
<td>Mar Drugs</td>
<td>182</td>
</tr>
<tr>
<td>MBio</td>
<td>65</td>
</tr>
<tr>
<td>Med Chem Res</td>
<td>40</td>
</tr>
<tr>
<td>Metabolomics</td>
<td>230</td>
</tr>
<tr>
<td>Mol Biol Rep</td>
<td>41, 115-7, 275</td>
</tr>
<tr>
<td>Mol Biosyst</td>
<td>90</td>
</tr>
<tr>
<td>Mol Breeding</td>
<td>147</td>
</tr>
<tr>
<td>Mol Cells</td>
<td>42-3, 233</td>
</tr>
<tr>
<td>Mol Med Rep</td>
<td>129, 231-2</td>
</tr>
<tr>
<td>Mol Oncol</td>
<td>22</td>
</tr>
<tr>
<td>Molecules</td>
<td>130, 242, 290-2</td>
</tr>
<tr>
<td>Nanotechnology</td>
<td>91</td>
</tr>
<tr>
<td>Nat Chem</td>
<td>243</td>
</tr>
<tr>
<td>Nat Prod Sci</td>
<td>131</td>
</tr>
<tr>
<td>Nat Struct Mol Biol</td>
<td>44</td>
</tr>
<tr>
<td>Nat Commun</td>
<td>66</td>
</tr>
<tr>
<td>Nucleic Acids Res</td>
<td>23</td>
</tr>
<tr>
<td>Oncol Lett</td>
<td>183</td>
</tr>
<tr>
<td>Oncol Rep</td>
<td>160, 244</td>
</tr>
<tr>
<td>Oncotarget</td>
<td>13-5, 24, 45-6, 251</td>
</tr>
<tr>
<td>Photochem Photobiol</td>
<td>92</td>
</tr>
<tr>
<td>Physiol Entomol</td>
<td>184</td>
</tr>
<tr>
<td>Physiol Plantarum</td>
<td>259</td>
</tr>
<tr>
<td>Phytother Res</td>
<td>16, 158, 185</td>
</tr>
<tr>
<td>Plant Biotech Rep</td>
<td>260, 276</td>
</tr>
<tr>
<td>Plant Genet Resour</td>
<td>186</td>
</tr>
<tr>
<td>Plant Pathol J</td>
<td>118-20</td>
</tr>
<tr>
<td>Plant Physiol Biochem</td>
<td>121-2</td>
</tr>
<tr>
<td>Plant Signal Behav</td>
<td>123</td>
</tr>
<tr>
<td>PLoS One</td>
<td>25, 47-8, 76, 82-3, 101-2, 107, 124-5, 132, 175, 214-5, 252, 261-2</td>
</tr>
<tr>
<td>Res Vet Sci</td>
<td>294</td>
</tr>
<tr>
<td>Rheumatology</td>
<td>295</td>
</tr>
<tr>
<td>Sci Signal</td>
<td>60</td>
</tr>
<tr>
<td>Sensor Actuat B-Chem</td>
<td>109</td>
</tr>
<tr>
<td>Small</td>
<td>93</td>
</tr>
<tr>
<td>Stem Cell Res</td>
<td>208</td>
</tr>
<tr>
<td>Stem Cells</td>
<td>61</td>
</tr>
<tr>
<td>Toxicol Lett</td>
<td>188</td>
</tr>
<tr>
<td>Toxicol Res</td>
<td>187</td>
</tr>
<tr>
<td>Transl Oncol</td>
<td>49</td>
</tr>
<tr>
<td>Virol J</td>
<td>204</td>
</tr>
<tr>
<td>World J Hepatol</td>
<td>62</td>
</tr>
<tr>
<td>World J Stem Cells</td>
<td>50</td>
</tr>
</tbody>
</table>

2014 KRIBB Article Abstracts | 165 |
### Keyword Index

<table>
<thead>
<tr>
<th>Keyword</th>
<th>Article No</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-1,4-Transfase activity</td>
<td>31</td>
</tr>
<tr>
<td>α-1,6-Glucoisidase activity</td>
<td>31</td>
</tr>
<tr>
<td>α-chlorohydrin</td>
<td>158</td>
</tr>
<tr>
<td>α-Glucoisidase</td>
<td>219</td>
</tr>
<tr>
<td>β-1,4-xylanase (XylU)</td>
<td>126</td>
</tr>
<tr>
<td>β-amyloid (Aβ)</td>
<td>258</td>
</tr>
<tr>
<td>β-galactosidase</td>
<td>97</td>
</tr>
<tr>
<td>β-Glucoisidase</td>
<td>288</td>
</tr>
<tr>
<td>β-glycerophosphate</td>
<td>28</td>
</tr>
<tr>
<td>β-Lapachone</td>
<td>152</td>
</tr>
<tr>
<td>β-sophorosyl ent-16-α-hydroxykauran</td>
<td>234</td>
</tr>
<tr>
<td>1,3-propanediol</td>
<td>279</td>
</tr>
<tr>
<td>16S rRNA gene sequence</td>
<td>263, 267-70</td>
</tr>
<tr>
<td>17-demethoxy-reblastatin</td>
<td>240</td>
</tr>
<tr>
<td>2'-Benzoyloxycinnamaldehyde</td>
<td>7</td>
</tr>
<tr>
<td>2-Butanol/isobutanol</td>
<td>278</td>
</tr>
<tr>
<td>2-Cys peroxiredoxins</td>
<td>212</td>
</tr>
<tr>
<td>3-deoxyribose-5-phosphate aldolase</td>
<td>289</td>
</tr>
<tr>
<td>3-dioxygenase</td>
<td>238</td>
</tr>
<tr>
<td>3-Pentanol</td>
<td>98</td>
</tr>
<tr>
<td>4-O-methylhonokiol</td>
<td>185</td>
</tr>
<tr>
<td>5 hmC</td>
<td>20</td>
</tr>
<tr>
<td>5Aβ-FcK</td>
<td>258</td>
</tr>
<tr>
<td>5-FU</td>
<td>21</td>
</tr>
<tr>
<td>A/California/04/09 (CA04, H1N1)</td>
<td>199</td>
</tr>
<tr>
<td>Abiotic stress</td>
<td>111, 116</td>
</tr>
<tr>
<td>Abnormality detection</td>
<td>80</td>
</tr>
<tr>
<td>Acetogenic glycoside</td>
<td>286</td>
</tr>
<tr>
<td>Acetolactate synthase</td>
<td>279</td>
</tr>
<tr>
<td>Acidophilic bacterium</td>
<td>145</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>135</td>
</tr>
<tr>
<td>Actinobacterium</td>
<td>270</td>
</tr>
<tr>
<td>Activating transcription factor 3</td>
<td>28</td>
</tr>
<tr>
<td>Active form</td>
<td>39</td>
</tr>
<tr>
<td>Acute ischemic stroke</td>
<td>165</td>
</tr>
<tr>
<td>Acute myocardial infarction</td>
<td>36</td>
</tr>
<tr>
<td>Adenylyl cyclase</td>
<td>156</td>
</tr>
<tr>
<td>Adipogenesis</td>
<td>50, 241</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>48, 131</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>199</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td>22</td>
</tr>
<tr>
<td>ADNSHL</td>
<td>25</td>
</tr>
<tr>
<td>ADRB2</td>
<td>233</td>
</tr>
<tr>
<td>Adult fibroblast</td>
<td>206</td>
</tr>
<tr>
<td>Adult stem cell</td>
<td>161</td>
</tr>
<tr>
<td>Adverse intracellular condition</td>
<td>65</td>
</tr>
<tr>
<td>Aeration condition</td>
<td>272</td>
</tr>
<tr>
<td>Affinity binding</td>
<td>105</td>
</tr>
<tr>
<td>Agar plates</td>
<td>261</td>
</tr>
<tr>
<td>Aged black garlic</td>
<td>187</td>
</tr>
<tr>
<td>Agelasine D</td>
<td>182</td>
</tr>
<tr>
<td>Aggregation</td>
<td>162</td>
</tr>
<tr>
<td>Aging</td>
<td>18, 51, 58-9</td>
</tr>
<tr>
<td>Airway inflammation</td>
<td>224</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>63</td>
</tr>
<tr>
<td>Alcoholic liver</td>
<td>154</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase 2 (ALDH2)</td>
<td>154</td>
</tr>
<tr>
<td>ALDH1</td>
<td>218</td>
</tr>
<tr>
<td>ALDH2 deficiency</td>
<td>154</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>121-2</td>
</tr>
<tr>
<td>Algal consortium</td>
<td>257</td>
</tr>
<tr>
<td>Allicyclobacillus tengchongensis</td>
<td>145</td>
</tr>
<tr>
<td>Alkali pretreatment</td>
<td>282</td>
</tr>
<tr>
<td>Alkanes</td>
<td>271</td>
</tr>
<tr>
<td>Allergic asthma</td>
<td>224, 232</td>
</tr>
<tr>
<td>ALP</td>
<td>28</td>
</tr>
<tr>
<td>Alphaproteobacterium</td>
<td>142</td>
</tr>
<tr>
<td>Alternaria brassicicola</td>
<td>242</td>
</tr>
<tr>
<td>Alternative splicing</td>
<td>252</td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>166, 168, 258</td>
</tr>
<tr>
<td>Ameliorating renal injury</td>
<td>152</td>
</tr>
<tr>
<td>Ampelopsis brevipedunculata extract</td>
<td>291</td>
</tr>
<tr>
<td>AMPK</td>
<td>16, 155</td>
</tr>
<tr>
<td>Anaerobic cocci</td>
<td>189</td>
</tr>
<tr>
<td>Anaerobic condition</td>
<td>63</td>
</tr>
<tr>
<td>Anaerobic fermentation</td>
<td>281</td>
</tr>
<tr>
<td>Analysis workflow</td>
<td>5</td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>264</td>
</tr>
<tr>
<td>Aneurinibacillus soli</td>
<td>143</td>
</tr>
<tr>
<td>Animal biosensor</td>
<td>89</td>
</tr>
<tr>
<td>Animal proteomics</td>
<td>59</td>
</tr>
<tr>
<td>Anion transporter</td>
<td>243</td>
</tr>
<tr>
<td>Antartcobacter jejuniens</td>
<td>136</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>277</td>
</tr>
<tr>
<td>Anthracycles</td>
<td>251</td>
</tr>
<tr>
<td>Antiangiogenic therapy</td>
<td>49</td>
</tr>
<tr>
<td>Antipapoptotic gene API5</td>
<td>54</td>
</tr>
<tr>
<td>Anti-apoptotic protein</td>
<td>11</td>
</tr>
<tr>
<td>Antibacterial activity</td>
<td>96</td>
</tr>
<tr>
<td>Antibacterial drug</td>
<td>95</td>
</tr>
<tr>
<td>Antibiotic biosynthesis</td>
<td>83</td>
</tr>
<tr>
<td>Antibiotic resistance</td>
<td>191</td>
</tr>
<tr>
<td>Anticancer effect</td>
<td>62, 240</td>
</tr>
<tr>
<td>Anticancer therapy</td>
<td>32</td>
</tr>
<tr>
<td>Antifungal activity</td>
<td>128</td>
</tr>
<tr>
<td>Antifungal agent</td>
<td>132</td>
</tr>
<tr>
<td>Antifungal drug</td>
<td>132</td>
</tr>
<tr>
<td>Antifungal indolyl compounds</td>
<td>242</td>
</tr>
<tr>
<td>Antigene RNA</td>
<td>23</td>
</tr>
<tr>
<td>Anti-inflammatory agent</td>
<td>292</td>
</tr>
<tr>
<td>Anti-inflammatory effect</td>
<td>221</td>
</tr>
<tr>
<td>Antimicrobial activity</td>
<td>99, 241</td>
</tr>
<tr>
<td>Antineoplastic agents</td>
<td>2</td>
</tr>
<tr>
<td>Antioxidant enzyme</td>
<td>61, 212</td>
</tr>
<tr>
<td>Anti-tumor efficacy</td>
<td>68</td>
</tr>
<tr>
<td>Antiviral activity</td>
<td>250</td>
</tr>
<tr>
<td>Antiviral sialidase</td>
<td>283</td>
</tr>
<tr>
<td>Term</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Apolipoproteins A</td>
<td>47, 49</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>2-4, 8, 11, 16, 32, 38-9, 42, 45, 47, 54, 62, 160, 170, 172, 176-7</td>
</tr>
<tr>
<td>Apoptosis regulatory proteins</td>
<td>1</td>
</tr>
<tr>
<td>Apoptotic control</td>
<td>52</td>
</tr>
<tr>
<td>Apoptotic pathway</td>
<td>243</td>
</tr>
<tr>
<td>AR promoter</td>
<td>23</td>
</tr>
<tr>
<td>Arabidopsis</td>
<td>110, 112</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>115</td>
</tr>
<tr>
<td>Arabis tinctoria (AT)</td>
<td>231</td>
</tr>
<tr>
<td>Arginine kinase</td>
<td>217</td>
</tr>
<tr>
<td>Argonaute family protein (Ago2)</td>
<td>215</td>
</tr>
<tr>
<td>Argonaute-2 (AGO2)</td>
<td>23</td>
</tr>
<tr>
<td>Aromatic amino acids</td>
<td>275</td>
</tr>
<tr>
<td>Aromatic polyimides (PIs)</td>
<td>106</td>
</tr>
<tr>
<td>Artificial biosynthesis</td>
<td>239</td>
</tr>
<tr>
<td>Artificial biosynthetic pathway</td>
<td>236</td>
</tr>
<tr>
<td>Artificial sequence linker</td>
<td>57</td>
</tr>
<tr>
<td>Assembled transcriptome</td>
<td>262</td>
</tr>
<tr>
<td>Asthma</td>
<td>220, 225-6, 229, 233, 284</td>
</tr>
<tr>
<td>Astrocytes</td>
<td>205</td>
</tr>
<tr>
<td>AtnDPPK2</td>
<td>121</td>
</tr>
<tr>
<td>Atopic dermatitis (AD)</td>
<td>64, 284</td>
</tr>
<tr>
<td>Attached growth</td>
<td>274</td>
</tr>
<tr>
<td>Autophagy</td>
<td>1, 170</td>
</tr>
<tr>
<td>Autophagy marker</td>
<td>177</td>
</tr>
<tr>
<td>Auxin</td>
<td>94</td>
</tr>
<tr>
<td>AV/Rbh2</td>
<td>266</td>
</tr>
<tr>
<td>AV/Rbh2 effector</td>
<td>120</td>
</tr>
<tr>
<td>AXL inhibition</td>
<td>207</td>
</tr>
<tr>
<td>BACH2 hypermethylation</td>
<td>17</td>
</tr>
<tr>
<td>Bacillus pakistaniensis</td>
<td>192</td>
</tr>
<tr>
<td>Bacillus solimanogrovi</td>
<td>141</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>97</td>
</tr>
<tr>
<td>Bacillus thuringiensis</td>
<td>272</td>
</tr>
<tr>
<td>Bacterial flagellin (flg22)</td>
<td>124</td>
</tr>
<tr>
<td>Bacterial neuraminidase</td>
<td>285</td>
</tr>
<tr>
<td>Bacterial persistor</td>
<td>102</td>
</tr>
<tr>
<td>Bacterial sialidase inhibitor</td>
<td>283</td>
</tr>
<tr>
<td>Bacterioidetes</td>
<td>263</td>
</tr>
<tr>
<td>Bcl-2 family protein</td>
<td>29-30, 32, 42</td>
</tr>
<tr>
<td>Behavioral response</td>
<td>89</td>
</tr>
<tr>
<td>Beige-brite adipocytes</td>
<td>50</td>
</tr>
<tr>
<td>Benzoate trimer</td>
<td>238</td>
</tr>
<tr>
<td>Betaine supplementation</td>
<td>181</td>
</tr>
<tr>
<td>Beta-sitosterol</td>
<td>16</td>
</tr>
<tr>
<td>Bi-functional</td>
<td>31</td>
</tr>
<tr>
<td>Binding ability</td>
<td>126</td>
</tr>
<tr>
<td>Binding mechanism</td>
<td>42</td>
</tr>
<tr>
<td>Bioconversion</td>
<td>288</td>
</tr>
<tr>
<td>Biodiesel</td>
<td>274</td>
</tr>
<tr>
<td>Biofilm</td>
<td>198</td>
</tr>
<tr>
<td>Biofilm reactor</td>
<td>270</td>
</tr>
</tbody>
</table>
Caulobacter profunda 267
CBD 1Bs 82
CD326 43
Cdc2-like kinases (Clks) 252
cDNA library 138
Cell adhesion molecule 292
Cell attachment 105
Cell death 114
Cell growth 14
Cell hepatitis 153
Cell proliferation 1, 15, 17
Cell SELEX 48
Cell therapy 166
Cell viability 105
Cell wall 79
Cell wall peptidoglycans type A4a 194
Cell-based HRE-luciferase assay 12
Cell-surface marker 48
Cellular chloride anion concentration 243
Cellular compartment 212
Cellular dynamics 87
Cellular regulation 66
Cellulose binding domains (CBDs) 82
Cell-wall peptidoglycans 190
Centriole duplication 44
Cep152 peptide 44
c-Fos 150, 182
Chamoe 275
Characterization 293
Chemical cocktail treatment 206
Chemical composition 180
Chemical cue 184
Chemoresistance 21
Chromotaxonomy 234
Chimeric expression 271
Chinese cabbage 147
Chionanthus 159
Chlamydomonas reinhardtii 260-1
Chlorella sp. 257
Chlorella vulgaris 273
Chlorellas 256
Chloroplast 273
Chloroplast genome 19
Chloroplast luminal immunophilin 259
Chloroplast targeting 276
ChlR1 protein 35
CHO cell 105, 197
Cholesterol 181
Cholineresterase 227
CHOP 169
Chronic glutamate toxicity 53
Chronic inflammatory 64
Chronic obstructive pulmonary disease 229, 233
Chronic rhinosinusitis 189
Chronological genome 265
eLAPs 45
c-Jun 3

CK2-independent manner 252
Classification 211
Clinicopathologic analyses 13
Cloning 162
Clostridium perfringens 283
Clostridium vulturis 193
Cloud 213
Clusterin 195
CNS 216
Co2+ 219
codA 122
Collagen-induced arthritis (CIA) 295
Colon cancer 7
Colorectal cancer 12-4, 22, 47, 70
Combination therapy 49, 294
Comparative genomics 100
Comparative pathogenesis 204
Composite gene-set analysis 6
Compost 190
Comprehensive analysis 26
Concanavalin A (Con A) 105
Concerted methylation 17
Coniochaeta sp. 238
Consolidated bioprocessing 282
Contact inhibition 52
Conventional chemotherapy 47
Coprinus atramentarius 241
Corpus luteum 174
Corydalis tartschaninovii 285
Coumarin analogue 90
Coumarin derivative 90
cox1 intron 256
CpG DNA methylation 15
CpG site 18
Cp-Nan1 283
CRC aggressiveness 22
C-reactive protein (CRP) 109
Crude glycerol 278
Cryptic polo box (CPB) 44
Cryptococcus neoformans 128, 132
CsPti1-L 114
CTHRCl promoter 13
Cucumber 111
Cucumis melo 275
Cultivar 277
Cultivation 274
Cultivation age 144
Cumulus-oocyte complex 163
CVB3 250
CX-4945 252
Cyanobacteria 264, 269
Cyclooxygenase (COX) 53
Cyclophosphamide 151, 223
Cynomolgus monkey 168
CYP1A2 188
CYP2E1 188
Cysteine residues 26
Cytochrome P450 151
Cytochrome P450 3A1 222
Cytokine 220, 225-6
Cytolytic activity 74
Cytoplasmic protein kinase 114
Cytosolic NELL2 205
Cytosolic protein 82
Cytotoxicity 55, 74, 235, 243
DAF-16/FOXO 58
Dam MTase activity 85
Daphne genkwa 107
Dataset 5
Defense priming 98
Defense responses 114
Delayed mutation 88
Deletion cassette 57
Dephosphorylating Mst1 52
Depression 150
Desaturase 260
Development 177
Developmental competence 176
Developmental toxicity 223
D-galactosamine 187
d-galactose pathway 65
Diabetes 157
Diaclyglycerol acyltransferase 2 246, 249
Diagnostic marker 14-5
Diallyl disulfide 151, 222-3
Dihydrofolate reductase (DHFR) 95
Dilated acid pretreatment 282
Dimerization 45
Diplacone 283
Direct lineage reprogramming 208
Discrimination 147
Disease recurrence 210
Dispiro skeleton 96
Dissociation processes 84
Disulphide-switching 66
DNA copy number variation 211
DNA damage 11
DNA layer 80
DNA methylation 20, 23
DNA methylation marker 18
DNA methyltransferase (MTase) 85
DNA primer 8
DNA scaffold 271
Dna2 35
DNA-binding domain 42
DNA-DNA hybridization 194
Docking 40
Docking simulation 34, 217-8
Domestication 178
Donor natural killer cell 69
Dopaminergic neural differentiation 161
Dopaminergic neuronal progenitor 208
Dormant state 102
DraR/DraK 83
Drosophila germline mutant 88
Drought stress 117, 121-2
Drought tolerance 180
Drug design 34
Drug-drug interaction (DDI) 188
Dual specificity protein phosphatase 4 34
Dual-specificity protein phosphatases 26
Dual-targeting 30
DUSPs 26
Dyskinesia 156
E. coli 39
E3 ligase 249
E3 ubiquitin ligase 45
Early development 164
Early growth response protein-1 7
EC-18 225
EDS1 120
EGFR-TKIs 244
Embryo culture 164
Embryonic stem cell 61
Endangered species 234
Endogenous neural stem/progenitor 167
Endoglucanase 293
Endometriosis 74
Endonuclease activity 35
Endoplasmic reticulum 28
Endoplasmic reticulum (ER) stress 164
Endothelial cell 47
Endothelial nitric oxide synthase 155
Endo-β-1,3-1,4-glucanase 81
Endo-β-1,4-xylanase 127
Enrichment culture 281
ENT domain 25
Enteritis 294
Enzyme activity 26
Enzyme assay 39
Enzyme binding affinity 227
Enzyme library 77
Enzyme screening 77
EpCAM 43
Epigenetics 18
Epithelial cell 149
Epithelial-mesenchymal transition 7, 70, 73
Equine influenza virus 202
ER stress 174
ER-associated degradation (ERAD) 249
Ericaceae 234
ERK-dependent induction 13
ERKs 60
ESC stemness 61
Ethambutol 188
Ethnic group 211
Euphausia superba 217
EV71 250
Evolution 137, 146
Exercise 171
Exo-type hydrolase 27
<table>
<thead>
<tr>
<th>Term</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exploiting mechanism</td>
<td>65</td>
</tr>
<tr>
<td>Expressed sequence tag</td>
<td>138</td>
</tr>
<tr>
<td>Eyedrop vaccination</td>
<td>175</td>
</tr>
<tr>
<td>Fab1-selective inhibition</td>
<td>96</td>
</tr>
<tr>
<td>Fast evolving</td>
<td>265</td>
</tr>
<tr>
<td>Fatigue</td>
<td>56</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>260</td>
</tr>
<tr>
<td>Fen1</td>
<td>35</td>
</tr>
<tr>
<td>Ferruginibacter profundus</td>
<td>263</td>
</tr>
<tr>
<td>Fertilization</td>
<td>163</td>
</tr>
<tr>
<td>FGF2 signaling</td>
<td>54</td>
</tr>
<tr>
<td>Flg22</td>
<td>123</td>
</tr>
<tr>
<td>Flg22-triggered oxidative burst</td>
<td>123</td>
</tr>
<tr>
<td>Flg22-triggered response</td>
<td>124</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>77, 82</td>
</tr>
<tr>
<td>FLS2</td>
<td>123</td>
</tr>
<tr>
<td>Fluorescence acceptor</td>
<td>90</td>
</tr>
<tr>
<td>Fluorescence change</td>
<td>86</td>
</tr>
<tr>
<td>Fluorescence imaging</td>
<td>82</td>
</tr>
<tr>
<td>Fluorescence quenching</td>
<td>227</td>
</tr>
<tr>
<td>Fluorescent assay</td>
<td>85</td>
</tr>
<tr>
<td>Fluorescent protein variant</td>
<td>87</td>
</tr>
<tr>
<td>Fn14</td>
<td>21</td>
</tr>
<tr>
<td>Focal cerebral ischemia</td>
<td>173</td>
</tr>
<tr>
<td>Fold superfamily</td>
<td>137</td>
</tr>
<tr>
<td>Food safety assessment</td>
<td>180</td>
</tr>
<tr>
<td>Forensic</td>
<td>10, 18</td>
</tr>
<tr>
<td>Forkhead transcription factors</td>
<td>2</td>
</tr>
<tr>
<td>Fourier transform IR</td>
<td>144</td>
</tr>
<tr>
<td>FOXM1-CCNB1 activation</td>
<td>210</td>
</tr>
<tr>
<td>FoxO3a</td>
<td>2</td>
</tr>
<tr>
<td>Free radical</td>
<td>56, 152</td>
</tr>
<tr>
<td>Freezing tolerance</td>
<td>276</td>
</tr>
<tr>
<td>Freshwater sediment</td>
<td>263, 267</td>
</tr>
<tr>
<td>FRK1</td>
<td>123</td>
</tr>
<tr>
<td>Fructose transporter</td>
<td>78</td>
</tr>
<tr>
<td>Fructose-rich feedstock</td>
<td>78</td>
</tr>
<tr>
<td>FT-IR</td>
<td>147</td>
</tr>
<tr>
<td>Fullerene nanoparticle</td>
<td>92</td>
</tr>
<tr>
<td>Fumaricicene</td>
<td>285</td>
</tr>
<tr>
<td>Functional induced DPs (iDPs)</td>
<td>208</td>
</tr>
<tr>
<td>Functional recovery</td>
<td>167</td>
</tr>
<tr>
<td>Function-based screening</td>
<td>81</td>
</tr>
<tr>
<td>Functionomic data</td>
<td>146</td>
</tr>
<tr>
<td>Fungal pathogen</td>
<td>132</td>
</tr>
<tr>
<td>Fusaricidin</td>
<td>99</td>
</tr>
<tr>
<td>Fusion protein</td>
<td>258</td>
</tr>
<tr>
<td>Gadolinium</td>
<td>91</td>
</tr>
<tr>
<td>Gangliosides</td>
<td>161</td>
</tr>
<tr>
<td>Garcinia mangostana</td>
<td>227</td>
</tr>
<tr>
<td>Gardenia jasminoides</td>
<td>131</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>9, 15, 17, 21</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>237</td>
</tr>
<tr>
<td>Gefitinib resistance</td>
<td>244</td>
</tr>
<tr>
<td>Gelidium amansii</td>
<td>281</td>
</tr>
<tr>
<td>Gene deletion</td>
<td>57</td>
</tr>
<tr>
<td>Gene dosage</td>
<td>280</td>
</tr>
<tr>
<td>Gene expression</td>
<td>197, 277</td>
</tr>
<tr>
<td>Gene expression regulation</td>
<td>2</td>
</tr>
<tr>
<td>Gene ontology</td>
<td>6, 138, 146, 262</td>
</tr>
<tr>
<td>Gene regulatory network</td>
<td>80</td>
</tr>
<tr>
<td>Gene synthesis</td>
<td>57</td>
</tr>
<tr>
<td>Genetic circuit</td>
<td>77</td>
</tr>
<tr>
<td>Genetic engineering</td>
<td>278</td>
</tr>
<tr>
<td>Genetic etiology</td>
<td>25</td>
</tr>
<tr>
<td>Genetic regulatory</td>
<td>65</td>
</tr>
<tr>
<td>Genetically modified tree</td>
<td>179</td>
</tr>
<tr>
<td>Genkwadaphnin (GD-1)</td>
<td>107</td>
</tr>
<tr>
<td>Genome analysis</td>
<td>101</td>
</tr>
<tr>
<td>Genomic DNA</td>
<td>147</td>
</tr>
<tr>
<td>Genomic deletion</td>
<td>201</td>
</tr>
<tr>
<td>Genomic DNA sequence</td>
<td>256</td>
</tr>
<tr>
<td>Genomic variation</td>
<td>213</td>
</tr>
<tr>
<td>Genotype</td>
<td>211</td>
</tr>
<tr>
<td>Genotype-phenotype</td>
<td>25</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>148</td>
</tr>
<tr>
<td>Geraniol dehydrogenation</td>
<td>196</td>
</tr>
<tr>
<td>Geranyl diphosphate synthase</td>
<td>196</td>
</tr>
<tr>
<td>Geranylflavonoid</td>
<td>283</td>
</tr>
<tr>
<td>Germplasm</td>
<td>186</td>
</tr>
<tr>
<td>GH10 domain</td>
<td>126</td>
</tr>
<tr>
<td>GH10 enzyme</td>
<td>127</td>
</tr>
<tr>
<td>Ginseng cultivars</td>
<td>144</td>
</tr>
<tr>
<td>Ginseng leaves</td>
<td>144</td>
</tr>
<tr>
<td>Ginsenoside</td>
<td>228, 250</td>
</tr>
<tr>
<td>Glaciibabitans tibetensis</td>
<td>139</td>
</tr>
<tr>
<td>Glycan trimming</td>
<td>79</td>
</tr>
<tr>
<td>Glycerol</td>
<td>279</td>
</tr>
<tr>
<td>Glycine max</td>
<td>130, 186</td>
</tr>
<tr>
<td>Glycinebaine</td>
<td>122</td>
</tr>
<tr>
<td>Glycogen debranching enzyme (GDE)</td>
<td>31</td>
</tr>
<tr>
<td>Glycoprotein</td>
<td>79</td>
</tr>
<tr>
<td>Glycoside hydrolyase family 57</td>
<td>27</td>
</tr>
<tr>
<td>Glycosyl hydrolyase family 8</td>
<td>81</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>239</td>
</tr>
<tr>
<td>Glycosylphosphatidylinositol-anchored</td>
<td>79</td>
</tr>
<tr>
<td>G-network</td>
<td>80</td>
</tr>
<tr>
<td>Gold nanodot array (GNA)</td>
<td>108</td>
</tr>
<tr>
<td>Gordonia bacteri faecihominis</td>
<td>135</td>
</tr>
<tr>
<td>gp78</td>
<td>249</td>
</tr>
<tr>
<td>Gram-positive</td>
<td>189</td>
</tr>
<tr>
<td>Grain stacking</td>
<td>276</td>
</tr>
<tr>
<td>Graphene oxide (GO)</td>
<td>86</td>
</tr>
<tr>
<td>Grass soil</td>
<td>268</td>
</tr>
<tr>
<td>Green algae</td>
<td>254</td>
</tr>
<tr>
<td>GRNs</td>
<td>80</td>
</tr>
<tr>
<td>Growth stage</td>
<td>230</td>
</tr>
<tr>
<td>GRP78</td>
<td>47</td>
</tr>
</tbody>
</table>
Isoflavone 230
Isoform 58
Isoquinoline alkaloids 285
ISR 98
JAK3 4
Jerusalem artichoke 262, 282
JNK 16, 60-1
Kaempferol glycone 230
Kaposi's sarcoma-associated herpesvirus 251
KEGG 6
Keratinocytes 284
Klebsiella pneumoniae 278-80
Kluyveromyces marxianus 282
Knee joint instability 169
Korean 204
KR-72 132
KSHV infection 251
L-2,3-BD dehydrogenase 280
L-2,3-butanediol 280
Label-free 109
Label-free assay 85
Label-free measurement 105
Lactobacillus kimchi 288
Large-scale oligonucleotide chip 118
LCST behavior 106
L-DOPA 156
let-7 microRNA 245
Lettuce 119
Leukemia progression 69
Lichenase 81
LID 156
Ligase chain reaction 57
Light treatment 277
Lin28B 245
Linkage disequilibrium (LD) 178
Lipid metabolism 129
Lipogenic enzyme FAS 129
Lipogenic gene 131
Lipoxygenase (LOX) 111
Live imaging 87
Liver 1
Liver cancer 62
Liver disease 181
Liver injury 187
Liver lipotoxicity 129
Liver metastasis 47
Living biosensor 89
LMV 119
Localized surface plasmon resonance 108
Long retention 265
Longevity 58
Lower critical solution temperature 106
LSON chip 118
Luminal IMMs 259
Lung 1
Lung adenocarcinoma 24
Lung carcinoma 24
Luteal phase 174
Luteimicrobium xylanilyticum 140
LW6 12
LWE treatment 181
Lyceum chinense 181
Lycopene b-cyclase 117
Lysinibacillus compositi 190
Lysinibacillus pakistanensis 194
Lytic induction 251
Magnetic resonance image 91
Magnetic resonance imaging 157
Malignancy 251
Malignant transformation 248
Malignant tumor 46
Maltose-forming α-amylase (PSMA) 27
MAMP response 124
Mangosteneone F 221
MAPK 221, 229, 231
Marker 22, 273
Markers gene 161
Massicus raddei 140
Matrix metalloproteinase-9 220
MCF-7 cell 41
MD simulation 104
MDH2 enzyme assay 12
MDM2 29
MDM2-inhibiting peptide 30
Melatonin 148, 167, 169-71, 224, 229
Membrane protein 73
Mesenchymal stem cells 50
Metabolic disease 246
Metabolic disorder 247
Metabolic engineering 117, 279, 287
Metabolic network 65
Metabolic pathway 101
Metabolism 185, 272
Metabolomics 228
Metal clad waveguide 109
Metalloproteinase-9 (MMP-9) activity 224
Metallothiionen 116
Metastasis 7, 22, 68, 70, 73, 75
Methylated resveratrol compound 236
Methylation-resistant endonuclease 85
Methylobacterium oryzae 101
Methylosulfonylmethane (MSM) 62
Mevalonate pathway 196
Microalgae 274
Microarray 6
Microarray screening 10
Microbiaceae 139
Microbacterium trichothecenolyticum 127
Microbial adaptation 63
Microbial cell 102
Microbial metagenome 77
Micrococcineae 140
Microcystins 264
<table>
<thead>
<tr>
<th>Term</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPR1</td>
<td>123</td>
</tr>
<tr>
<td>NQO1</td>
<td>152</td>
</tr>
<tr>
<td>NQO1 activation</td>
<td>155</td>
</tr>
<tr>
<td>NQO1 knockout mice</td>
<td>149</td>
</tr>
<tr>
<td>Nrf-2</td>
<td>151</td>
</tr>
<tr>
<td>NS1 truncation</td>
<td>202</td>
</tr>
<tr>
<td>NSC26188</td>
<td>2</td>
</tr>
<tr>
<td>Nuclear factor E2-related factor 2</td>
<td>222</td>
</tr>
<tr>
<td>Nuclear factor kappaB</td>
<td>222</td>
</tr>
<tr>
<td>Nuclear transformation</td>
<td>261</td>
</tr>
<tr>
<td>O157 LPS antigen</td>
<td>175</td>
</tr>
<tr>
<td>Obesity</td>
<td>48, 241</td>
</tr>
<tr>
<td>Ochratoxin A (OTA)</td>
<td>103</td>
</tr>
<tr>
<td>OCT4-mediated direct reprogramming</td>
<td>206</td>
</tr>
<tr>
<td>Odor discrimination</td>
<td>89</td>
</tr>
<tr>
<td>Okazaki fragment processing</td>
<td>35</td>
</tr>
<tr>
<td>Oleaceae</td>
<td>159</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>260</td>
</tr>
<tr>
<td>Olfactory sensor</td>
<td>89</td>
</tr>
<tr>
<td>Oligo chip</td>
<td>118</td>
</tr>
<tr>
<td>Oncogene protein</td>
<td>8</td>
</tr>
<tr>
<td>OPLS-DA analysis</td>
<td>228</td>
</tr>
<tr>
<td>Optical biosensor</td>
<td>109</td>
</tr>
<tr>
<td>Optical transition</td>
<td>92</td>
</tr>
<tr>
<td>Optimal spacer</td>
<td>103</td>
</tr>
<tr>
<td>Oryza sativa L.</td>
<td>180</td>
</tr>
<tr>
<td>Osmolytes</td>
<td>217</td>
</tr>
<tr>
<td>Osteoblast</td>
<td>33</td>
</tr>
<tr>
<td>Osteoblast differentiation</td>
<td>28</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>33</td>
</tr>
<tr>
<td>Osteoclast differentiation</td>
<td>291</td>
</tr>
<tr>
<td>Osteoclastogenesis</td>
<td>182</td>
</tr>
<tr>
<td>OTA aptasensor</td>
<td>103</td>
</tr>
<tr>
<td>Ovalbumin (OVA)</td>
<td>224</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>49</td>
</tr>
<tr>
<td>Oxidative burst</td>
<td>124</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>56, 72, 121-2, 148, 151, 158, 223</td>
</tr>
<tr>
<td>p21-Activated kinases</td>
<td>8</td>
</tr>
<tr>
<td>p300</td>
<td>3</td>
</tr>
<tr>
<td>p53</td>
<td>32, 42</td>
</tr>
<tr>
<td>p53 Peptide analogue</td>
<td>29</td>
</tr>
<tr>
<td>p53 transactivation domain</td>
<td>30</td>
</tr>
<tr>
<td>p53DBD</td>
<td>42</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>49</td>
</tr>
<tr>
<td>Paenibacillus polymyxa</td>
<td>99</td>
</tr>
<tr>
<td>PAK4</td>
<td>8</td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>144</td>
</tr>
<tr>
<td>Panax ginseng Meyer</td>
<td>228</td>
</tr>
<tr>
<td>Pancreas</td>
<td>130</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma up-regulated factor (PAUF)</td>
<td>68</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>68</td>
</tr>
<tr>
<td>Pancreatic ductal adenocarcinoma</td>
<td>68</td>
</tr>
<tr>
<td>Pandemic</td>
<td>203</td>
</tr>
<tr>
<td>Pandemic 2009 H1N1</td>
<td>200</td>
</tr>
<tr>
<td>Parkinson's disease (PD)</td>
<td>150, 156</td>
</tr>
<tr>
<td>PARP-1</td>
<td>3</td>
</tr>
<tr>
<td>Pathology</td>
<td>204</td>
</tr>
<tr>
<td>pBYR2fp viral vector</td>
<td>253</td>
</tr>
<tr>
<td>pDI</td>
<td>29</td>
</tr>
<tr>
<td>PEDV variant</td>
<td>201</td>
</tr>
<tr>
<td>Penicillium verruculosum F375</td>
<td>96</td>
</tr>
<tr>
<td>Pepper</td>
<td>98</td>
</tr>
<tr>
<td>Peptoniphilus</td>
<td>189</td>
</tr>
<tr>
<td>Perforin-1 (Prf1)</td>
<td>75</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>113</td>
</tr>
<tr>
<td>Peroxiredoxin (Prx)</td>
<td>61</td>
</tr>
<tr>
<td>Peroxiredoxin 3 (Prx3)</td>
<td>56</td>
</tr>
<tr>
<td>pflB gene</td>
<td>63</td>
</tr>
<tr>
<td>pgm mutation</td>
<td>99</td>
</tr>
<tr>
<td>PGPR</td>
<td>98</td>
</tr>
<tr>
<td>pH regulation</td>
<td>83</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>1</td>
</tr>
<tr>
<td>Pharmaceutical glycoprotein</td>
<td>255</td>
</tr>
<tr>
<td>Pharmacokinetics</td>
<td>185</td>
</tr>
<tr>
<td>Phaseolus angularis seeds (PASE)</td>
<td>290</td>
</tr>
<tr>
<td>PHLPP1</td>
<td>52</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>99</td>
</tr>
<tr>
<td>Phospholipase A2 (PLA2)</td>
<td>53</td>
</tr>
<tr>
<td>Photophysical property</td>
<td>92</td>
</tr>
<tr>
<td>Photosynthetic stress</td>
<td>259</td>
</tr>
<tr>
<td>Phototoxic drug</td>
<td>86</td>
</tr>
<tr>
<td>Phycosphere bacteria</td>
<td>254</td>
</tr>
<tr>
<td>Phyllosphere</td>
<td>101</td>
</tr>
<tr>
<td>Phylogenetics</td>
<td>137</td>
</tr>
<tr>
<td>Phylogenomics</td>
<td>146</td>
</tr>
<tr>
<td>Phytoalexin detoxification</td>
<td>242</td>
</tr>
<tr>
<td>Phytohormone</td>
<td>179</td>
</tr>
<tr>
<td>Phytophthora infestans</td>
<td>266</td>
</tr>
<tr>
<td>Phytoestrogen</td>
<td>116</td>
</tr>
<tr>
<td>Phytostimulation</td>
<td>101</td>
</tr>
<tr>
<td>Piceid</td>
<td>239</td>
</tr>
<tr>
<td>Phytosideroxides (PQ)</td>
<td>232</td>
</tr>
<tr>
<td>Pig</td>
<td>201, 203</td>
</tr>
<tr>
<td>Pig SPAM1</td>
<td>163</td>
</tr>
<tr>
<td>Pinostilbene</td>
<td>287</td>
</tr>
<tr>
<td>Piperazines</td>
<td>2</td>
</tr>
<tr>
<td>Pitr3 mice</td>
<td>150</td>
</tr>
<tr>
<td>PKCβ1</td>
<td>205</td>
</tr>
<tr>
<td>PKD1</td>
<td>107</td>
</tr>
<tr>
<td>Placenta</td>
<td>162</td>
</tr>
<tr>
<td>Plant</td>
<td>255</td>
</tr>
<tr>
<td>Plant growth</td>
<td>101</td>
</tr>
<tr>
<td>Plant metabolites</td>
<td>236</td>
</tr>
<tr>
<td>Plant virus</td>
<td>118, 253</td>
</tr>
<tr>
<td>Plants</td>
<td>254</td>
</tr>
<tr>
<td>Plasmam ferric nanowire interstice (PNI)</td>
<td>93</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>183</td>
</tr>
<tr>
<td>PLFA (phospholipid fatty acid)</td>
<td>179</td>
</tr>
<tr>
<td>Pluripotency factor</td>
<td>208</td>
</tr>
<tr>
<td>PMAb83</td>
<td>68</td>
</tr>
<tr>
<td>PMEI</td>
<td>110</td>
</tr>
<tr>
<td>PMI</td>
<td>29</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>204</td>
</tr>
<tr>
<td>Term</td>
<td>Page</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Pollen</td>
<td>112</td>
</tr>
<tr>
<td>Pollen-specific gene</td>
<td>110</td>
</tr>
<tr>
<td>Polo-like kinase 4 (Plk4)</td>
<td>44</td>
</tr>
<tr>
<td>Polydatin</td>
<td>288</td>
</tr>
<tr>
<td>Polyhydroxylated fullerene</td>
<td>92</td>
</tr>
<tr>
<td>Polymorphism</td>
<td>273</td>
</tr>
<tr>
<td>Polyphasic taxonomy</td>
<td>193, 263</td>
</tr>
<tr>
<td>Poly-γ-glutamate (γ-PGA)</td>
<td>64</td>
</tr>
<tr>
<td>Porcine epidemic diarrhea virus</td>
<td>201</td>
</tr>
<tr>
<td>Porcine oocytes</td>
<td>177</td>
</tr>
<tr>
<td>Post-transcriptional regulation</td>
<td>75</td>
</tr>
<tr>
<td>Potyvirus</td>
<td>119</td>
</tr>
<tr>
<td>Powdery mildew</td>
<td>111</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>153</td>
</tr>
<tr>
<td>Premature antibody</td>
<td>36</td>
</tr>
<tr>
<td>PreSmO (Pre-Structured Motif)</td>
<td>104</td>
</tr>
<tr>
<td>Processing</td>
<td>67</td>
</tr>
<tr>
<td>Progeny</td>
<td>147</td>
</tr>
<tr>
<td>Progesterone production</td>
<td>174</td>
</tr>
<tr>
<td>Prognosis</td>
<td>22</td>
</tr>
<tr>
<td>Prognostic signature</td>
<td>5</td>
</tr>
<tr>
<td>Promoter</td>
<td>20, 112</td>
</tr>
<tr>
<td>Prostatic neoplasms</td>
<td>2</td>
</tr>
<tr>
<td>Protection</td>
<td>148</td>
</tr>
<tr>
<td>Protective effect</td>
<td>158</td>
</tr>
<tr>
<td>Protein expression data</td>
<td>80</td>
</tr>
<tr>
<td>Protein layer</td>
<td>80</td>
</tr>
<tr>
<td>Protein transport</td>
<td>2</td>
</tr>
<tr>
<td>Protein tyrosine phosphatase sigma</td>
<td>40</td>
</tr>
<tr>
<td>Protein tyrosine phosphatases</td>
<td>26</td>
</tr>
<tr>
<td>Protein-protein interaction</td>
<td>82</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>133-4</td>
</tr>
<tr>
<td>Proteolytic function</td>
<td>67</td>
</tr>
<tr>
<td>Proteomic analysis</td>
<td>37-8, 41, 59</td>
</tr>
<tr>
<td>Proto-oncogene proteins c-akt</td>
<td>2</td>
</tr>
<tr>
<td>PsAL stability</td>
<td>259</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>198</td>
</tr>
<tr>
<td>Pseudomonas syringae pv. syringae</td>
<td>100</td>
</tr>
<tr>
<td>Pseudouracil</td>
<td>134</td>
</tr>
<tr>
<td>Pseudosubstrate domain</td>
<td>205</td>
</tr>
<tr>
<td>P-solubilization</td>
<td>191</td>
</tr>
<tr>
<td>Pseudomonas sp. ATCC 19310</td>
<td>100</td>
</tr>
<tr>
<td>Psychrotolerant bacterium</td>
<td>139</td>
</tr>
<tr>
<td>pta gene</td>
<td>63</td>
</tr>
<tr>
<td>PTEN</td>
<td>7</td>
</tr>
<tr>
<td>Pterocarps</td>
<td>130, 230</td>
</tr>
<tr>
<td>Pterostilbene</td>
<td>287</td>
</tr>
<tr>
<td>Punctures</td>
<td>1</td>
</tr>
<tr>
<td>Pure neuron culture</td>
<td>53</td>
</tr>
<tr>
<td>Pycnogenol®</td>
<td>158</td>
</tr>
<tr>
<td>Py-Gd nanoparticle</td>
<td>91</td>
</tr>
<tr>
<td>Pyrococccus sp. ST04</td>
<td>27</td>
</tr>
<tr>
<td>Pyrrolobenzodiazepine</td>
<td>235</td>
</tr>
<tr>
<td>RACE</td>
<td>217</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>24</td>
</tr>
<tr>
<td>Rainout shelter</td>
<td>180</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>177</td>
</tr>
<tr>
<td>Raphanus sativus</td>
<td>19</td>
</tr>
<tr>
<td>Reactive oxygen species (ROS)</td>
<td>56, 60-1, 72, 116, 164</td>
</tr>
<tr>
<td>Receptor tyrosine kinases (RTKs)</td>
<td>207</td>
</tr>
<tr>
<td>Recombinant protein</td>
<td>81, 163</td>
</tr>
<tr>
<td>Red algae</td>
<td>281</td>
</tr>
<tr>
<td>Redox balance</td>
<td>63</td>
</tr>
<tr>
<td>Redox signaling</td>
<td>212</td>
</tr>
<tr>
<td>Reductive activity</td>
<td>280</td>
</tr>
<tr>
<td>Reference gene</td>
<td>183</td>
</tr>
<tr>
<td>Regulatory network</td>
<td>76</td>
</tr>
<tr>
<td>Reprogramming</td>
<td>176</td>
</tr>
<tr>
<td>Reseratrol</td>
<td>239, 288</td>
</tr>
<tr>
<td>Retinoblastoma cell</td>
<td>46</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>41</td>
</tr>
<tr>
<td>RGEN</td>
<td>88</td>
</tr>
<tr>
<td>RGP</td>
<td>276</td>
</tr>
<tr>
<td>Rheumatoid arthritis (RA)</td>
<td>37, 295</td>
</tr>
<tr>
<td>Rhizobium sp.</td>
<td>254</td>
</tr>
<tr>
<td>Rhizosphere</td>
<td>179</td>
</tr>
<tr>
<td>rhLK8</td>
<td>47, 49</td>
</tr>
<tr>
<td>RhoB</td>
<td>3</td>
</tr>
<tr>
<td>RhoB GTP-binding protein</td>
<td>2</td>
</tr>
<tr>
<td>Rhodobacteraceae</td>
<td>136</td>
</tr>
<tr>
<td>Rhododendron brachycarpum</td>
<td>234</td>
</tr>
<tr>
<td>Rhopalosiphum padi</td>
<td>184</td>
</tr>
<tr>
<td>RLGS</td>
<td>17</td>
</tr>
<tr>
<td>RNA duplex</td>
<td>215</td>
</tr>
<tr>
<td>RNA interference (RNAi)</td>
<td>215</td>
</tr>
<tr>
<td>RNAi</td>
<td>117</td>
</tr>
<tr>
<td>Rodent model</td>
<td>173</td>
</tr>
<tr>
<td>Root biomass</td>
<td>94</td>
</tr>
<tr>
<td>ROS</td>
<td>16</td>
</tr>
<tr>
<td>Roseivivax roseus</td>
<td>142</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>294</td>
</tr>
<tr>
<td>Rpi-blb2</td>
<td>120, 266</td>
</tr>
<tr>
<td>Rubi Fructus extract (RFex)</td>
<td>129</td>
</tr>
<tr>
<td>Runx2</td>
<td>33</td>
</tr>
<tr>
<td>S. plebeia extract (SPE)</td>
<td>284</td>
</tr>
<tr>
<td>SA</td>
<td>123</td>
</tr>
<tr>
<td>Saframomys cerevisiae</td>
<td>31</td>
</tr>
<tr>
<td>sAD</td>
<td>168</td>
</tr>
<tr>
<td>Saikosaponin</td>
<td>292</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>266</td>
</tr>
<tr>
<td>Salt mines</td>
<td>192</td>
</tr>
<tr>
<td>Salt stress</td>
<td>117, 121-2</td>
</tr>
<tr>
<td>Salt stress tolerance</td>
<td>115</td>
</tr>
<tr>
<td>Salt tolerance</td>
<td>114</td>
</tr>
<tr>
<td>Salvia plebeia</td>
<td>284</td>
</tr>
<tr>
<td>SA-mediated priming</td>
<td>123</td>
</tr>
<tr>
<td>Scenedesmus sp.</td>
<td>257</td>
</tr>
<tr>
<td>Scent detection</td>
<td>89</td>
</tr>
<tr>
<td>Schizosaccharomyces pombe</td>
<td>57</td>
</tr>
<tr>
<td>Screen</td>
<td>86</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Sea water bacteria</td>
<td>136</td>
</tr>
<tr>
<td>Seasonal morphs</td>
<td>184</td>
</tr>
<tr>
<td>Secondary metabolite</td>
<td>83</td>
</tr>
<tr>
<td>Sediment</td>
<td>269</td>
</tr>
<tr>
<td>Selectin</td>
<td>292</td>
</tr>
<tr>
<td>SELEX</td>
<td>43</td>
</tr>
<tr>
<td>Self-assembly</td>
<td>253</td>
</tr>
<tr>
<td>Senescence</td>
<td>170</td>
</tr>
<tr>
<td>Sensor domain</td>
<td>83</td>
</tr>
<tr>
<td>Sepsis</td>
<td>1</td>
</tr>
<tr>
<td>Sequence repeat</td>
<td>273</td>
</tr>
<tr>
<td>Sequencing assembly</td>
<td>125</td>
</tr>
<tr>
<td>Serine/arginine-rich (SR)</td>
<td>252</td>
</tr>
<tr>
<td>Serum</td>
<td>37</td>
</tr>
<tr>
<td>Serum TESC level</td>
<td>14</td>
</tr>
<tr>
<td>SGF</td>
<td>120, 266</td>
</tr>
<tr>
<td>SHH</td>
<td>8</td>
</tr>
<tr>
<td>ShiKinate pathway</td>
<td>275</td>
</tr>
<tr>
<td>Short chain fatty acids (SCFAs)</td>
<td>247</td>
</tr>
<tr>
<td>SHP-2</td>
<td>74</td>
</tr>
<tr>
<td>SID2</td>
<td>123</td>
</tr>
<tr>
<td>Siegesbeckia glabrescens</td>
<td>226</td>
</tr>
<tr>
<td>Signaling network</td>
<td>212</td>
</tr>
<tr>
<td>Signaling pathway</td>
<td>124, 128</td>
</tr>
<tr>
<td>Simple sequence repeat</td>
<td>19</td>
</tr>
<tr>
<td>Single nucleotide polymorphism</td>
<td>211</td>
</tr>
<tr>
<td>Single-chain variable fragment (scFv)</td>
<td>36</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>51, 56, 59</td>
</tr>
<tr>
<td>Skin cancer</td>
<td>248</td>
</tr>
<tr>
<td>Skin inflammation</td>
<td>64</td>
</tr>
<tr>
<td>Skin inflammatory</td>
<td>284</td>
</tr>
<tr>
<td>SICRK1</td>
<td>112</td>
</tr>
<tr>
<td>SPPMEI promoter</td>
<td>110</td>
</tr>
<tr>
<td>Small molecule inhibitor</td>
<td>246</td>
</tr>
<tr>
<td>SMILE</td>
<td>33</td>
</tr>
<tr>
<td>Sodium butyrate</td>
<td>197</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>113</td>
</tr>
<tr>
<td>Soil</td>
<td>133, 141-3</td>
</tr>
<tr>
<td>Soil metagenome</td>
<td>81</td>
</tr>
<tr>
<td>Soil microorganisms</td>
<td>179</td>
</tr>
<tr>
<td>Solvation</td>
<td>40</td>
</tr>
<tr>
<td>Somatic cell nuclear transfer (SCNT)</td>
<td>172</td>
</tr>
<tr>
<td>Sophora flavescens extract (SFE)</td>
<td>294</td>
</tr>
<tr>
<td>South Korea</td>
<td>201-2</td>
</tr>
<tr>
<td>SoxB1</td>
<td>216</td>
</tr>
<tr>
<td>SoxD</td>
<td>216</td>
</tr>
<tr>
<td>Soybean</td>
<td>130</td>
</tr>
<tr>
<td>Soybean leaf</td>
<td>178</td>
</tr>
<tr>
<td>Soybean mosaic virus (SMV)</td>
<td>186</td>
</tr>
<tr>
<td>Sp1 protein stability</td>
<td>70</td>
</tr>
<tr>
<td>Species description</td>
<td>194</td>
</tr>
<tr>
<td>Sperm hyalinidase</td>
<td>163</td>
</tr>
<tr>
<td>Spermatoxicity</td>
<td>158</td>
</tr>
<tr>
<td>Sperm-egg interaction</td>
<td>163</td>
</tr>
<tr>
<td>Sphingobacterium</td>
<td>191</td>
</tr>
<tr>
<td>Therapeutic target</td>
<td>41</td>
</tr>
<tr>
<td>Term</td>
<td>Page</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Thermal denaturation</td>
<td>217</td>
</tr>
<tr>
<td>Thermogenesis</td>
<td>50</td>
</tr>
<tr>
<td>Thermoresponsive rigid polymer</td>
<td>106</td>
</tr>
<tr>
<td>Thermoactivity</td>
<td>293</td>
</tr>
<tr>
<td>Thielaquin</td>
<td>238</td>
</tr>
<tr>
<td>Thioredoxin (TRX)</td>
<td>66, 72</td>
</tr>
<tr>
<td>Thioredoxin-interacting protein</td>
<td>66, 72</td>
</tr>
<tr>
<td>Tight junction</td>
<td>149</td>
</tr>
<tr>
<td>TIPRL</td>
<td>160</td>
</tr>
<tr>
<td>Tissue-specific gene</td>
<td>112</td>
</tr>
<tr>
<td>TLR4 activation</td>
<td>290</td>
</tr>
<tr>
<td>TM4SF4</td>
<td>24</td>
</tr>
<tr>
<td>TM4SF5</td>
<td>73</td>
</tr>
<tr>
<td>Tmprss4</td>
<td>67, 71, 73</td>
</tr>
<tr>
<td>TNF-α receptor</td>
<td>8</td>
</tr>
<tr>
<td>Tomato</td>
<td>110, 112</td>
</tr>
<tr>
<td>Toxin production</td>
<td>264</td>
</tr>
<tr>
<td>TRAIL resistance</td>
<td>160</td>
</tr>
<tr>
<td>Transcription</td>
<td>58, 149</td>
</tr>
<tr>
<td>Transcription factor</td>
<td>46, 71</td>
</tr>
<tr>
<td>Transcription network</td>
<td>6</td>
</tr>
<tr>
<td>Transcriptional gene silencing (TGS)</td>
<td>23</td>
</tr>
<tr>
<td>Transcriptome</td>
<td>94, 138</td>
</tr>
<tr>
<td>Transcriptome analysis</td>
<td>125</td>
</tr>
<tr>
<td>Transgenic crop</td>
<td>180</td>
</tr>
<tr>
<td>Transgenic mice</td>
<td>62</td>
</tr>
<tr>
<td>Transgenic plant</td>
<td>115</td>
</tr>
<tr>
<td>Transgenic potato</td>
<td>258</td>
</tr>
<tr>
<td>Transmembrane glycoprotein</td>
<td>43</td>
</tr>
<tr>
<td>Transmembrane serine protease</td>
<td>67, 71</td>
</tr>
<tr>
<td>Transplantation</td>
<td>166</td>
</tr>
<tr>
<td>Transplanted islet</td>
<td>157</td>
</tr>
<tr>
<td>Transporter</td>
<td>94</td>
</tr>
<tr>
<td>Treadmill exercise</td>
<td>169</td>
</tr>
<tr>
<td>Tree of life</td>
<td>137, 146</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>246, 249</td>
</tr>
<tr>
<td>Trim71</td>
<td>245</td>
</tr>
<tr>
<td>Triple-negative breast cancer (TNBC)</td>
<td>240</td>
</tr>
<tr>
<td>TRX-TXNIP</td>
<td>66</td>
</tr>
<tr>
<td>Tumor</td>
<td>71</td>
</tr>
<tr>
<td>Tumor growth</td>
<td>14, 75</td>
</tr>
<tr>
<td>Tumor progression</td>
<td>70</td>
</tr>
<tr>
<td>Tumor suppressor</td>
<td>15</td>
</tr>
<tr>
<td>Tumorigenic activity</td>
<td>24</td>
</tr>
<tr>
<td>Tunicamycin</td>
<td>28</td>
</tr>
<tr>
<td>Tussilago farfara</td>
<td>160</td>
</tr>
<tr>
<td>Two-component system</td>
<td>128</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>130</td>
</tr>
<tr>
<td>Type II collagen</td>
<td>171</td>
</tr>
<tr>
<td>Type III effector</td>
<td>100</td>
</tr>
<tr>
<td>Type strain</td>
<td>100</td>
</tr>
<tr>
<td>Type three secretion system (TTSS)</td>
<td>198</td>
</tr>
<tr>
<td>U266</td>
<td>16</td>
</tr>
<tr>
<td>U6 snRNA</td>
<td>183</td>
</tr>
<tr>
<td>Ubiquitination</td>
<td>245</td>
</tr>
<tr>
<td>Ultra-specific zeptomole</td>
<td>93</td>
</tr>
<tr>
<td>Unfolded protein response</td>
<td>174</td>
</tr>
<tr>
<td>Unfolding</td>
<td>219</td>
</tr>
<tr>
<td>UPLC-PDA-QTOF-MS</td>
<td>227</td>
</tr>
<tr>
<td>UPLC-QTOF-MS</td>
<td>230</td>
</tr>
<tr>
<td>Urokinase-type plasminogen activator</td>
<td>67, 71</td>
</tr>
<tr>
<td>Urototoxicity</td>
<td>151</td>
</tr>
<tr>
<td>UV</td>
<td>11</td>
</tr>
<tr>
<td>UV irradiation</td>
<td>86</td>
</tr>
<tr>
<td>Vaccine platform</td>
<td>199, 255</td>
</tr>
<tr>
<td>Validation</td>
<td>290</td>
</tr>
<tr>
<td>Variation</td>
<td>178</td>
</tr>
<tr>
<td>Varibacter gotjawalensis</td>
<td>133</td>
</tr>
<tr>
<td>VEGF</td>
<td>49</td>
</tr>
<tr>
<td>Verruactone C</td>
<td>96</td>
</tr>
<tr>
<td>Vietnam</td>
<td>159</td>
</tr>
<tr>
<td>Vigna angularis</td>
<td>295</td>
</tr>
<tr>
<td>Vigna mungo</td>
<td>191</td>
</tr>
<tr>
<td>Virinoparae</td>
<td>184</td>
</tr>
<tr>
<td>Virtual screening</td>
<td>34, 40</td>
</tr>
<tr>
<td>Virus diagnosis</td>
<td>118</td>
</tr>
<tr>
<td>Virus particle release</td>
<td>209</td>
</tr>
<tr>
<td>Virus-like particle</td>
<td>253</td>
</tr>
<tr>
<td>Virus-like particles (VLPs)</td>
<td>255</td>
</tr>
<tr>
<td>Viscum album</td>
<td>138</td>
</tr>
<tr>
<td>Volatile aromatic compound</td>
<td>275</td>
</tr>
<tr>
<td>Volatile organic compound</td>
<td>98</td>
</tr>
<tr>
<td>Vpu</td>
<td>209</td>
</tr>
<tr>
<td>Vulture intestine</td>
<td>193</td>
</tr>
<tr>
<td>Wastewater</td>
<td>274</td>
</tr>
<tr>
<td>Water-soluble fullerene derivative</td>
<td>92</td>
</tr>
<tr>
<td>White adipocytes</td>
<td>50</td>
</tr>
<tr>
<td>White ginseng</td>
<td>228</td>
</tr>
<tr>
<td>Whitefly</td>
<td>94</td>
</tr>
<tr>
<td>Whole plant utilization</td>
<td>282</td>
</tr>
<tr>
<td>Whole-exome sequencing</td>
<td>25</td>
</tr>
<tr>
<td>Wild aquatic bird</td>
<td>200</td>
</tr>
<tr>
<td>WK88-1</td>
<td>237, 244</td>
</tr>
<tr>
<td>WRKY29</td>
<td>123</td>
</tr>
<tr>
<td>Xanthomonas</td>
<td>293</td>
</tr>
<tr>
<td>YAP1</td>
<td>9</td>
</tr>
<tr>
<td>Yeast surface display</td>
<td>79</td>
</tr>
<tr>
<td>ZEB2-induced invasion</td>
<td>70</td>
</tr>
<tr>
<td>Zebrabrand</td>
<td>195</td>
</tr>
<tr>
<td>Zipper proteins (LZs)</td>
<td>82</td>
</tr>
</tbody>
</table>