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Regulation of expression of Connexin43 and other related genes by Wnt3a

Xiaoyu Liu[1], Xiaotian Li[2], Xen Liu[1], Xeili Xia[1], Ji Zuo[1]
[1]Department of Cellular and Genetic Medicine, [2]Department of gynaecology and obstetrics, Shanghai Medical College, Fudan University, Shanghai, China

To investigate the influence of Wnt3a protein on expression of Connexin43 and their involvement in cardiac development, we detect mRNA and protein expression of Connexin43 and other related genes by RT-PCR and Western blotting after transfecting pCDNA3.1/wnt-3a eukaryotic expression vector into rat cardiomyocyte cell line H9c2. Results showed that compared to those of the control group, Wnt3a overexpression results in increase of mRNA and protein expression of Connexin43 as well as increase of mRNA expression of cardiac-related genes such as GATA4 and Nkx2.5. These results mean that Wnt3a signaling pathway has influence on expression of Connexin43 and it regulates cardiac development.

***Keywords*:** wnt-3a; Connexin43 gene; cardiac development

Correspondence: Xiaoyu Liu
E-mail: 041101035@fudan.edu.cn

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Subcellular localization and function of *Oryza sativa* phosphate transporter in *Pichia pastoris*

Feng Ming, Wei Wang, Shanshan Zhang, Jianyao Xu, Daleng Sheng
Institute of Genetics, State Key Laboratory of Genetic Engineering, School of Life Science, Fudan University, Shanghai, China

OsPT6:1, a putative high-affinity phosphate (Pi) transporter gene in rice (*Oryza sativa*) we reported in our previous paper was introduced into pIC9K as pIC9K–OsPT6:1-GFP1 and expressed in *Pichia pastoris*. The Western blotting analysis confirmed the transformation at molecular level. A Hanes analysis of transformed yeast and control cells indicated the functional activity of the introduced OsPT6:1, further suggesting the success of yeast expression. An in-depth scrutiny through the complementation analysis lent credit to the fact that OsPT6:1 mediated in the Pi uptake process of *Pichia pastoris* at limited external Pi concentrations in *Pichia pastoris*, which was well consistent with the relative growth of transformed yeast cell. The GFP-tagged chimera made subcellular localization and real-time tracing of OsPT6:1 possible. OsPT6:1 seemed to have a greater positive effect on the growth of transformed yeast cells in high Pi concentration (HP for short hereafter) than in low Pi concentration (LP for short hereafter), which may implied a possible housekeeping function of this high-affinity transporter. Phylogenetic analysis indicated that OsPT6:1 may belong to a newly classified type subfamily of high-affinity phosphate transporter (type II), which characterizes low expression under high Pi concentration and inducibility by Pi deprivation. In summary, OsPT6:1 is the first identified and extensively studied type II high-affinity phosphate transporter in *Orzya sativa*.

***Keywords*:** *Oryza sativa* L.; real time; type II high-affinity phosphate transporter; *Pichia pastoris*; subcellular localization

Correspondence: Feng Ming
E-mail: fming@fudan.edu.cn

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siRNAs down regulate the expression of lZP3 in Xingjiang Lagurus oocytes result in reduced fertilization ability in IVF

Shuzhen Zhuang, Fuchun Zhang
Xinjiang Key Laboratory of Biological Resources and Genetic Engineering, Urumqi 830046, China

Species-specific binding of sperm to eggs is mediated by oligosaccharides linked to ZP3. Acrosome-intact sperm recognize and bind to O-linked oligosaccharides linked to Ser residues at the sperm combining-site of ZP3. Structural differences in oligosaccharides on ZP3 from different species may account for whether or not sperm are able to bind to the ZP. We report here that by microinjecting the siRNA interference vector pGenesil-ZP31 encoding an lZP3 hairpin dsRNA of 21 bp and the control vector pGenesil-HK encoding a non-related gene of human and rodent into the inmmatured oocytes of Xingjiang Lagurus lagurus respectively. After *in vitro* maturation (IVM), in vitro feitilization (IVF) and *in vitro* culture (IVC) for the oocytes of Xingjiang Lagurus lagurus, we got the results as follows: semi-quantitative RT-PCR analysis shows that the lZP3 mRNA in the oocytes injected with pGenesil-ZP31 is almost not expressed comparing to β-actin, whereas the lZP3 mRNA in the oocytes injected with pGenesil-HK is expressed insusceptibly. And real-time PCR identification gets the corresponding results of semi-quantitative RT-PCR. Moreover, detection with immunohistochemistry shows that the zona pellucida of the former is positive for the lZP3 antibody, and the latter is negative. After *in vitro* maturation the immature oocytes of Xingjiang Lagurus

lagurus and *in vitro* fertilization find that the fertilization ability of the transgenic oocytes carried pGenesil-ZP31 is decreased obviously (2.82%) compared to the oocytes carried pGenesil-HK (15.71%). It suggests that the transgenic RNAi-mediated silencing of lZP3 in Xingjiang Lagurus lagurus oocytes result in reduced fertility. These results prove that lZP3 is essential for the recognition between oocytes and sperms of Xingjiang Lagurus lagurus like other mammalians.

Keywords**:** ZP3; microinjection; *in vitro* maturation; *in vitro* feitilization

Correspondence: Shuzhen Zhuang
E-mail: zhuangshuzhen@126.com

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Transcriptional activity of androgen receptor is modulated by twin RNA splicing factors: PSF and p54nrb

Xuesen Dong[1, 2], John R G Challis[2], Joan Sweet[4], Theodore Brown[1, 3], Stephen J Lye[1, 2, 3]
[1]SLRI, Mount Sinai Hospital, [2]Department of Physiology, [3]Department of Obstetrics and Gynecology, University of Toronto, Toronto, Canada; [4]Laboratory Medicine and Pathology, University Health Network, Toronto, Canada

Androgen hormone acting through its receptor (AR) plays a vital role maintaining normal prostate epithelial cell proliferation and stimulating prostate tumor growth. The principle treatment for prostate cancer (PCa) depends upon androgen depletion therapy (ADT). Although initially effective, the disease almost invariably progresses to an ADT-failed androgen-independent state. Resistance to ADT has been postulated to diverse mechanisms such as changes of AR expression and functions (mutations in the AR sequence, and increased levels of AR), alterations of levels of nuclear receptor cofactors, activation of growth factor or kinase pathways. These studies indicate that despite the eventual failure of ADT, the AR remains a viable therapeutic target. In this study, we have demonstrated that two RNA splicing factors, PSF and p54nrb, inhibit transcriptional activity of AR. Both PSF and p54nrb form protein complex with AR in a ligand-independent fashion. PSF and AR are both presented and form complex on PSA promoter in the androgen response region. Overexpression of PSF inhibits while reduction of endogenous PSF expression by siRNA enhances AR-mediated MMTV promoter activity in several cellular contexts including the prostate (LNCaP and PC3) and 293T cells lines. In contrast to previously published data, we demonstrated that p54nrb possess intrinsic inhibitory domain and can inhibit but not activate AR function in both 293T and LNCaP cells. PSF does not affect AR protein stability as it does to progesterone receptor. However, PSF but not p54nrb can block AR/ARE interaction. We also observed that HDAC inhibitor could reverse the inhibitory effects of PSF and p54nrb on AR transactivation. Using prostate tissue array to perform immunohisto-chemistry, we observed PSF is expressed at luminal epithelium but not basal epithelium and stoma cells in normal/high grade PIN prostate. Thus, we conclude that PSF and p54nrb are corepressors of AR and may play as key modulators of androgen signaling in prostate epithelium. Targeting PSF would effectively modulate AR signaling in both ligand-dependent and independent contexts in prostate cancer, thus providing an alternative to current ADT failed cancer.

Keywords**:** androgen receptor; corepressor; RNA splicing factor; prostate cancer; transcription regulation

Correspondence: Xuesen Dong
E-mail: dong@mshri.on.ca

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Luman/CREB3 induces herp through transcriptional activation of an ERSE-II element in its promoter during the endoplasmic reticulum stress response

Genqing Liang[1], Timothy E Audas[1], Yu Li[1], Gregory P Cockram[1], J Doug Dean[1], Amanda C Martyn[1], Koichi Kokame[2], Rui Lu[1]
[1]Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada; [2]National Cardiovascular Centre Research Institute, Suita, Osaka, Japan

Luman/CREB3 (also called LZIP) is an endoplasmic reticulum (ER) membrane-bound transcription factor, which is believed to undergo regulated intramembrane proteolysis in response to cellular cues. We have previously found that Luman activates transcription from the unfolded protein response element. Here we report the identification of Herp, a gene involved in ER stress-associated protein degradation (ERAD), as a direct target of Luman. We found that Luman was transcriptionally induced and proteolytically activated by the ER-stress inducer, thaspsigargin. Overexpression of Luman activated transcription of cellular Herp via an ER stress response element-II (ERSE-II, ATTGG-N-CCACG) in the promoter region. Mutagenesis studies and chromatin-immunoprecipitation assays showed that Luman physically associates with the Herp promoter, specifically the second half site (CCACG) of the ERSE-II. Luman was also necessary for the full activation of Herp during the ER stress response, since Luman siRNA knockdown or functional repression by a dominant negative mutant attenuated Herp gene expression. Like Herp, overexpression of Luman protected cells against ER stress-induced apoptosis. With Luman structurally similar to ATF6 but resembling XBP1

in DNA-binding specificities, we propose that Luman is a novel factor that plays a role in ERAD, and a converging point for various signaling pathways channeling through the ER.

***Keywords*:** Luman/CREB3; endoplasmic reticulum stress response; unfolded protein response; regulated intramembrane proteolysis; transcriptional regulation

Correspondence: Rui Lu
E-mail: rlu@uoguelph.ca

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Curcumin inhibits HT-29 cell growth and suppresses the transcriptional expression of IGF-1R by a PPAR-gamma dependent mechanism

Yanqiu Xia[1], Yongping Xu[1, 2], Jiji Jin[1,2], Bin Zhang[1], Hongyu Xue[1]
[1]Department of Bioscience and Biotechnology, School of Environmental and Biological Science and Technology, [2]State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian, China

Curcumin, a natural phenolic compound isolated from turmeric, can exert chemopreventive effects in various cell lines and animal models. Numerous studies have revealed that curcumin mediates its anticancer effects by modulation of several important molecular targets, including transcription factors, enzymes, cell cycle proteins and receptors. The peroxisome proliferator-activated receptor –gamma (PPAR-gamma or PPAR-γ), a ligand-dependent transcription factor belonging to the family of nuclear receptors, has been implicated in the regulation of cell growth and differentiation. The insulin-like growth factor-1 receptor (IGF-1R), a tyrosine kinase-containing transmembrane protein, plays a critical role in transformation, growth, and survival of various malignant cells. High expression of IGF-1R is found in a variety of primary tumors, including colorectal cancer. IGF-1R has been identified as a general and promising target for anticancer therapy. The aim of this study was to elucidate the cellular and molecular mechanisms by which curcumin inhibits colon cancer cell growth. To explore the anticancer effects of curcumin, we have observed that treatment with curcumin resulted in a dose and time-dependent inhibition of cell proliferation and induction of apoptosis in HT-29 cells, a human colon cancer-derived cell line. To investigate the effects of curcumin on regulation of IGF-1R expression and the relevant transcription factors, we examined the expression of IGF-1R and two important transcription factors, Sp1 and p53, using real time RT-PCR. The data demonstrated that curcumin treatment resulted in reduction in the level of IGF-1R, Sp1 and p53 mRNA in HT-29 cells. We also determined the curcumin-induced PPAR-γ activation as measured by real time RT-PCR and Western blotting. The data showed that curcumin caused upregulation of PPAR-γ mRNA and de-phosphorylation/activation of PPAR-γ protein. Further studies examined the role of activation of PPAR-γ in cell growth inhibition and apoptogenic effect of curcumin. A PPAR-γ antagonist, GW9662 was used to block PPAR-γ before curcumin treatment. The studies demonstrated that GW9662 pretreatment abrogated cell death and prevented apoptosis induced by curcumin treatment. Finally we analyzed the role of activation of PPAR-γ in regulation of IGF-1R expression. The results demonstrated that curcumin-induced reduction of IGF-1R mRNA level was also prevented by GW9662. Taken together, our findings suggest that curcumin inhibits cell proliferation and induces cell apoptosis in a PPAR-γ dependent way, and that curcumin-induced suppression of the gene expression of IGF-1R may be mediated by the interaction of PPAR-γ with the transcription factors of IGF-1R gene. These results provide a novel insight into the roles and mechanisms of curcumin in inhibition of colon cancer cell growth and potential therapeutic strategies for treatment of colon cancer.

***Keywords*:** Curcumin; IGF-1R; PPAR-γ; colon cancer

Correspondence: Yongping Xu
E-mail: xyqdlut@yahoo.com.cn

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Expression pattern of some key genes that regulate tooth development in human

Dahe Lin, Yanding Zhang, Yide Huang, Mingfen Zhang
College of Life Sciences, Fujian Normal University, Fuzhou, China

It has been demonstrated that in the mouse odontogenesis is governed by interactions between the oral ectoderm and neural crest-derived mesenchyme. Members of several growth factor families, such as bone morphogenetic protein (BMP) and fibroblast growth factor (FGF), function to regulate epithelial–mesenchymal interactions in tooth development from the very beginning. The expression pattern of a number of genes encoding transcription factors have been revealed in the developing tooth, Prominent among them are homeobox-containing genes, including homeobox, msh-like 1 (*msx1*), paired box gene 9 (*pax9*), and paired-like homeodomain transcription factor 2 (*pitx2*), A common function of these transcription factors in the marine tooth development is to regulate the expression of growth factors. It was therefore suggested that transcription factors participate in epithelial-mesenchymal interactions through linking the signaling loops between tissue layers by responding to inductive signals and regulating the ex-

pression of other signaling molecules. However, how the human homologue behavior during tooth development is still unknown. In the study, we checked the expression pattern of two growth factors: Bmp4 and Fgf8, and three transcription factors: *msx1*, *pitx2*, and *pax9* at cap stage and bell stage of the developing milk teeth in human by *in situ* Hybridization. Our results demonstrate that Bmp4 is expressed in stratum intermedium and dental papilla at bell stage. In contrast, *bmp4* transcripts only appear in dental papilla at the same stage in the mouse. *fgf8* expression still maintain in dental epithelium at the cap and bell stage in human. However, in the mouse *fgf8* expresses in the dental epithelium at initial stage and function in conjunction with *bmp4* to define the tooth-forming region; at the bud stage transcripts appear in the distal part of the tooth and in the oral epithelium; after the bud stage no expression can be detected in the developing tooth. We further found the expression pattern of *msx1* and *pitx2* in human is almost the same as in the mouse. At the cap stage, *pitx2* is asymmetrically expressed in the buccal outer enamel epithelium, while buccal dental epithelium growth faster than lingual side. During the subsequent bell stage, signal of *pitx2* in lingual side become more intensive, at the same time lingual dental epithelium grow down quickly. These suggested that *pitx2* signaling may be required for asymmetric development of human tooth. We also found that expression pattern of human *pax9* is quite different from that of mouse *pax9*. *Pax9* transcripts can be detected in both dental epithelium and mesenchyme at the cap and bell stage, whereas they only appear in dental mesenchyme at the same developing stage in the mouse. Our results suggest that in developing tooth genes expression and regulating mechanism may differ between human and mouse.

***Keywords*:** gene expression pattern; odotogenesis; human

Correspondence: Yanding Zhang
E-mail: ydzhang@fjnu.edu.cn

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Functional characterization of the promoter of human kinetochoric protein Hec1: novel link between cell cycle and CREB family proteins

Liansheng Cheng, Liangwei Li, Xinxian Qiao, Jing Liu, Xuebiao Yao
School of Life Science, University of Science and Technology of China, Hefei, China

Hec1 (highly expressed in cancer), a protein that localizes to kinetochore in cell mitosis，plays an essential role in chromosome segregation for M phase progression. It is expressed most abundantly in the S and M phases of rapidly dividing cells but not in terminal differentiated cells. To clarify the mechanism of transcriptional regulation of the Hec1 gene, we identified a fragment of 2039 bp from its 5'-flanking region. Mapping of this region revealed a TATA-less promoter and several putative binding sites for cp2, SRY, Nkx-2, deltaE, Bm-2, ATF4, CREB and E2F. The results from HeLa cells transfected with a series of pGL3 luciferase reporter vectors containing different sections of the Hec1 5'-flanking region demonstrated that two sequences, one from -70 to -63 bp and the other from -155 to -144 bp to the translational start site contain CREB and ATF4 site respectively, and are critical for transcriptional activity. Gel shift and supershift assays also demonstrated specific binding of transcriptional factor CREB and ATF4 to their putative binding sites. Furthermore, we found that overexpression of CREB and ATF4 had a synergistic effect on the activation of the Hec1 promoter, and the expression of Hec1 mRNA was upregulated by overexpression of CREB, and downregulated by overexpression of either its Ser-133 to Ala mutant or treatment with LY294002, an inhibitor of PI3-K. Additionally, the cell cycle distribution was changed and a G2/M arrest was observed in cells overexpressing CREB mutan or treated with LY294002. These results suggested that a novel mechanism by which CREB family proteins promotes the Hec1-related cancer cell proliferation and how cell cycle is regulated through signal-induced phosphorylation arising from growth stimuli outside.

***Keywords*:** Hec1; kinetochore; promoter; ATF4; CREB

Correspondence: Liansheng Cheng
E-mail: charlsoncheng@ustc.edu

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Cloning and characterization of Beta-carotene hydroxylase gene promoter in *Haematococcus pluvialis*

Changying Teng[1], Li Zhang[1], Song Qin[2]
[1]Life Science College, Ludong University, Yantai, China; [2]Key Lab, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China

The unicellular green alga *Haematococcus pluvialis* can surprisingly accumulate ketocarotenoid, astaxanthin, up to 4% dry weight under various environmental stresses. It is known as the highest amount astaxanthin producer and a model organism for studying the mechanism of carotenoid metabolism and environmental adaptation. beta-carotene hydroxylase (CrtZ) and beta-carotene ketolase (BKT) play key roles in astaxanthin biosynthesis in *H. pluvialis* and their transcription levels have been proved to be closely related to astaxanthin biosynthesis. After studying *BKT* gene promoter, in the presented paper we cloned and characterized *crtZ* gene promoter in *H. pluvialis*. An approximate

302-bp 5'-flanking region of the *crtZ* gene, which represents a putative promoter, was cloned through walking upstream. The results of the sequence analysis showed that this *crtZ* 5'-flanking region might have some cis-acting elements related to environmental stress, such as ABA-responsible elements(ABRE3) and pyrimidine box (P-box) in gibberellin induced gene transcriptional regulatory region of *Oryza sativa*. The results of the beta-galactosidase assay and the transient expression of *lacZ* driven by this 302-bp region suggest that the 302-bp region is the promoter of *H. pluvialis crtZ* gene.

***Keywords*:** beta-carotene hydroxylase; promoter; cloning; *Haematococcus pluvialis*

Correspondence: Changying Teng
E-mail: lzhangt@sina.com

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DNA methylation and histone modification play a role in regulation of Rat Reverse Trascriptase (MTERT) gene expression

Haiquan Yu, Yongli Yue, Xia Wu, Junxia Li, Chunwei Liang, Shorgan Bao
Key Laboratory of Mammal Reproductive Biology and Biotechnology, Inner Mongolia University, Huhhot 010021, China

Telomerase is involved in the regulation of the replicative life span of cells by maintaining telomere length. Telomerase is activated in cells capable of extended replication, such as germ and stem cells, while repressed in normal somatic cells. The activity of telomerase closely correlates with the expression of its catalytic subunit, telomerase reverse transcriptase (TERT). Our primary studies show that rTert is widespread expression in adult tissues, while the expression level is different, higher in liver, and low in kidney, testis and heart. Then, the mechanism of epigenetic regulation of rTert expression was studied. The 5'-upstream region of rat TERT gene (rTert) harbors a large CpG island with a dense CG-rich, suggesting that DNA methylation may play a role in the regulation of rTert expression. By using bisulfite genomic sequencing method, we revealed that the methylation status of the 5' regulation region of rTert gene shows a region-specific methylation pattern. In a 644bp fragment upstream of the initiating ATG codon, 14 CpG sites were found to be under a cell type-specific DNA methylation, it was hypomethylated in liver and testis (with 13.6% and 13.8% of CpG sites were methylated, respectively), while hypermethylated in heart and kidney (69.0% and 67.9% methylated, respectively). These results showed that rTert regulation region harbors a tissue-dependent, differentially methylated region (T-DMR), which were hypomethylated in tissues with higher replicative or regenerative ability, while were hypermethylated in other differentiated tissues. In addition to DNA methylation, histone modifications also play a role in regulation of rTert expression. ChIP assay was conducted to study the role of histone acetylation and methylation on regulation of rTert expression. Results showed that the T-DMR was the core sequence in regulation of rTert expression, in this region histone modification is according with the rTert expression pattern, tissues with a higher rTert expression levels showed a lower H3K9 methylation status and a higher acetylation status of histone H3 and H4. In a conclusion, our results suggested that DNA methylation and histone modifications (methylation and acetylation) synergistically regulate the chromatin construction, resulting in a compress and inactive, or loosened and active chromatin, and decide the tissue-specific regulation of rTert expression.

***Keywords*:** rTert expression; epigenetic regulation; DNA methylation; histone modification

Correspondence: Haiquan Yu
E-mail: haiquan_yu@yahoo.com

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Mechanistic study of *Saccharomyces cerevisie* Est1 on telomerase activity

Mingliang Zhang, Wei Qian, Jinqiu Zhou
Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Telomerase is a specialized reverse transcriptase that extends one strand of the telomere templated by a short element embedded in its RNA component. In *Saccharomyces cerevisie*, Est2p (telomerase catalytic subunit) and Tlc1 (telomerase RNA template subunit) constitute the enzymatic core complex. Est1p and Est3p are essential for telomerase activity *in vivo*, and believed to be the subunits of telomerase holoenzyme. The biochemical mechanism by which Est1 affects the telomerase activity is still unclear yet. Here we reported that co-overexpression of Est1 with either Est2 or Tlc1 induces longer telomeres than overexpression of Est1 alone, while co-overexpression of Est1 with both Est2p and Tlc1 leads to even longer telomeres. We have purified recombinant Est1p, and study on effect of recombinant Est1p on telomerase activity/processivity is in progress.

***Keywords*:** telomere; telomerase; processivity; yeast; Est1

Correspondence: Mingliang Zhang
E-mail: zhangml@sibs.ac.cn

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Establishment of promoter trapping system in *Torenia fournieri* L

Hai T Long, Hong Q Li, Ling Li

College of Life Sciences,South China Normal University, Guangzhou 510631, China

Promoter is the most important cis-acting element that regulates gene expression temporally and spatially. Isolation and characterization of various specific promoters have been paid great attention to, and establishment of promoter trapping system has been developed for this issue. *Torenia* (*Torenia fournieri* L.) is an important annual flowing ornamental plant of tropical and subtropical areas and is regarded as a novel model plant for research of flower development and fertilization with respect to its short lifecycle and long florescence. In the present study, based on the optimization of genetic transformation of *Torenia* mediated by *Agrobacterium tumefaciens*, a binary vector, designated as pHAHCL, was constructed for promoter trapping in *Torenia*, and was introduced into *Torenia* to establish an efficient promoter trapping system in *Torenia fournieri* L. The main results were as follows: (1) The GUS gene was obtained by digestion of the pAHC27 plasmid with *Eco*R I and Xba I, and was ligated into the pCAMBIA1200 binary vector at the sites of *Eco*R I and *Xba* I, resulting in the pHAHCL construct harboring *GUS* gene with no promoter to drive in the T-DNA region. The pHAHCL construct was then introduced to *Agrobacterium* of EHA101 strain and used in following Torenia transformation. (2) Genetic transformation of Torenia was performed by using young leaflet as explants. After incubation in *Agrobacterium* suspension of OD 600 at 0.5 for 5-10 min, the explants were pre-cultivated on MS agar medium containing 400 mg/L acetosyringone for 4 d under dark. The explants were then transferred to the selection agar medium containing 12 mg/L hygromycin and 500 mg/L carbenicillin, and cultivated for 14 d to obtain the resistant calla. The resistant calla were transferred to the shoot induction agar medium containing 400 mg/L ceftomine. After 7 d, the calla were then transferred to shooting agar medium supplemented with 15 mg/L hygromycin and 400 mg/L ceftomine, and cultured for another 28 d to obtain resistant shoots. The 0.5 cm or taller resistant shoots were finally transferred to rooting agar medium containing 15 mg/L hygromycin and 400 mg/L ceftomine, and the rooted resistant plantlets were achieved after a 14-d cultivation. 82 hygromycin-resistant plants were confirmed to be positive transformants by PCR and southern blotting check. The transformation frequency was up to 12.5% overall. (3) GUS histochemical staining of the 82 transgenic plants showed that 12 of them were stained, suggesting GUS gene expression only in the 12 transformants. 10 out of the 12 transformants were stained only in veins of plants, and the other 2 transformants were stained in veins and stems. (4) TAIL-PCR was performed to amplify the flanking sequences of T-DNA insertion sites in the 12 transgenic plants. A 394-bp fragment was amplified and sequenced. The nucleotide sequence of this fragment shared 86% homology with the gene encoding carboxylic ester hydrolase in *Arabidopsis thaliana* and the deduced amino acid sequence from this fragment was of 57% similarity to the carboxylic ester hydrolase.

***Keywords*:** promoter; trapping system; *Torenia fournieri* L

Correspondence: Ling Li
E-mail: lilab@scnu.edu.cn

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IL-1β regulates the mouse Fas ligand expression in corneal endothelial cells

Jie Zhang[1], Ke Yang[1], Deyong Tan[1], Junying Zeng[1], Fine Alan[2]

[1]The Department of Biochemistry and Molecular Biology, School of Life Science, Yunnan University, Kunming, Yunnan 650091, China; [2]The Pulmonary Center and the Department of Biochemistry, Boston University, Boston, USA

Constitutively expressed Fas ligand in several distinct epithelial cell types appears to protect tissues by inducing apoptosis of Fas+ immune cells during inflammatory reactions. To study the relationship of Fas ligand and inflammation process in cornea, we examined the effects of inflammatory cytokine IL-1β on the FasL production, expression and cytotoxic function in corneal endothelial cells. In this paper, we demonstrate that IL-1β inhibits the FasL production and expression in corneal endothelial cells. The promoter activity of FasL in these cells is reduced by IL-1β in a dose-dependent manner. Finally, we also find that IL-1β blocks the cytotoxic effects of FasL derived from corneal endothelial cells to the Fas+ target cells. These data support the view that FasL derived from corneal endothelial cells modulates inflammation within cornea.

***Keywords*:** FasL; promoter; cornea; IL-1β; mouse

Correspondence: Ke Yang
E-mail: ykk_ykkk@126.com

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Effect on proliferation of SW480 cell line with p33ING1b gene transfection

Xiusheng He, Shuai Zhao, Qiao Luo, Chao Zeng, Min Deng, Qingchao Qiu, Bo Hu

Cancer Research Institute, Nanhua University, Hengyang

421001, China

Gene p33ING1b is a new coloned tumor suppressor gene recently, the genetic structure and expression's abnormality of which exists in human several types of tumors. This investigation is designed to select SW480 cells as target, the expression of p33ING1b of which is low, then to observe the effect of SW480 cells and analysis the possible molecule mechanism of p33ING1b in SW480 cells after transfecting p33ING1b gene into it. Methods pcDNA3.1(+)/p33ING1b expression vector was constructed and the recombinant plasmid was transfected into SW480 by liposome transfection method as well as pcDNA3.1 (+) plasmid. After transfection, the positive cell clones of pcDNA3.1(+)/p33ING1b/SW480 and pcDNA3.1(+)/SW480 were selected by G418 and identified by RT-PCR, Western blot and S-P immunohistochemical method. In order to elucidate the effect of expression of exogenous p33ING1b gene on the colorectal cancer cell SW480, the proliferation rate *in vitro* of cells including SW480, pcDNA3.1(+)/p33ING1b/SW480 and pcDNA3.1(+)/SW480 were analyzed by growth curves and colony formation assay in soft agar. At same time, the apoptotic rate of cell and the cell cycle analysis were also tested by the flow cytometry. At last, using western blot analysis, we detect the expression level of the protein p53, p21WAF1, Bax and Bcl-2 in pcDNA3.1(+)/p33ING1b/SW480, pcDNA3.1(+)/SW480 and SW480, which initially indicate the molecule mechanism of inducing apoptosis by gene p33ING1b. Results The results of restriction enzyme cutting and DNA sequencing demonstrate that the pcDNA3.1(+)/p33ING1b eukaryotic expression vector was successfully constructed. The pcDNA3.1(+)/p33ING1b and pcDNA3.1(+) were used to transfect SW480 cells using positive ion liposome method. After selection of G418, and identification of RT-PCR, SP immunocytochemistry and western blot assay, the pcDNA3.1(+)/p33ING1b/SW480 and pcDNA3.1(+)/SW480 were established successfully. The cell growth curve showed that the cell growth rate of SW480 transfected with pcDNA3.1 (+)/p33ING1b was slower than those transfected with pcDNA3.1(+) or untransfected(P<0.05). At last, in order to approach the mechanism of growth inhibition effect on SW480 cells after transfected with pcDNA3.1(+)/p33ING1b, western blot analysis was used to detect the expression of P53, P21WAF1, Bax and Bcl-2. The results showed that ectopic overexpression of p33INGlb up-regulated the expression of Bax remarkably and down-regulated the expression of Bcl-2 in SW480 cells (P<0.05). Conclusion The eukaryotic expression vector of pcDNA3.1(+)/p33ING1b were constructed successfully. The pcDNA3.1(+)/p33ING1b/SW480 which has stable overexpression of P33ING1b was established successfully. After over expression of exogenous p33ING1b protein in SW480 cell, the proliferation rate of it decreased and apoptotic rate of it increased, the mechanism of which is associated with up-regulated expression of Bax and down-regulated expression of Bcl-2.

***Keywords*:** colon cancer; SW480 cell line; p33ING1b gene; expression; transfection

Correspondence: Xiusheng He
E-mail: hexiusheng@hotmail.com

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Development and mutant analysis of *Agrobacterium tumefaciens*-mediated transformation system in *Setosphaeria turcica*

Shouqin Gu[1], Yongshan Fan[2], Jingao Dong[1], Jianguang Liu[1]
[1]Mycotoxin Lab, Agricultural University of Hebei, Baoding, China; [2]Department of Life Science, Tangshan Teacher's College, Tangshan, Chia

Although the *Agrobacterium tumefaciens*-mediated transformation system had been reported in *Setosphaeria turcica*, an important fungal pathogen of corn all over the world, its transformation efficiency is still necessary to be improved and its mutant analysis needs to be done. We have built our transformation system by optimization of the protoplast formation, vector condition, transformation time, mutant screening, and reconfirmation, in order to build a universal method to generate a large scale of mutants for functional genomic analysis of the fungus. In our transformation system, the binary vector pBHtl, carrying a bacterial hygromycin B phosphotransferase (*hph*) gene under the control of the Aspergillums nidulans trpC promoter, is employed to transform the conidia of S. turcica. The reason for the substitution of conidium for mycelium is that conidium culturing for 24-48h in Fries medium can germinate and generate germ tubes, which is more tender than young mycelium and easier to be infected by the bacterium. Our experiments confirmed that there is higher transformation efficiency for germinated conidium than 5-day-old mycelium. The proportion between binary vector and fungal conidium in coincubation period, the transformation temperature, and the composition of transformant's screening medium are also optimized. Under the optimized condition, averagely 300 transformants can be obtained from 106 conidial spores, and above 90% of transformants are confirmed to have the T-DNA insertion by PCR with primer pairs specific to the T-DNA flanks. About 30% of the confirmed transformants have a single T-DNA insert in the fungal genome through Southern blotting analysis. Within the transformants, 13 mutants in colony morphology, 16 mutants in mycelium growth rate, 5mutants in

sporulation, 9 mutants in conidial germination, 6 mutants in appressorium formation, 23 mutants in pathogenicity to susceptible corn leaves, 15 mutants in HT-toxin activity, are obtained by comparison with the wild types. The detailed cell biology in mutants, including the morphology change of mycelium, conidium, and appressorium, the toxicity and activity change of HT-toxin, and the T-DNA insertion sites in genomic DNA are documented at the same time. The above results have enlarged the resources of the analysis population of the pathogen, and will offer more information to deeply understand the mechanisms of extracellular signal transduction in regulation of the virulence of HT-toxin and the pathogenicity to corn leaves, the genes regulating growth rate, conidial germination, appressorium formation and infection, and the interaction between different dominant disease-resistant genes of corn and different races in *S. turcica*.

Keywords**:** *Setosphaeria turcica*; *Agrobacterium tumefaciens*-mediated transformation; development; mutant analysis

Correspondence: Shouqin Gu
E-mail: gushouqin@126.com

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Identification and analysis of four novel molecular markers linked to powdery mildew resistance gene *Pm21* in 6VS chromosome short arm of *Haynaldia villosa*

Zhenying Wang[1], Hongmei Zhao[1], Jingxin Hong[1], Liyuan Chen[1], Jie Zhu[1], Gang Li[1], Yongkang Peng[1], Chaojie Xie[2], Zhiyong Liu[2]
[1]Department of Biology, Tianjin Normal University, Tianjin 30074, China; [2]College of Agronomy and Biotechnology, China Agricultural University, Beijing 100094, China

In this study, wheat-Haynaldia Chromosome substitution line (6A/6V), translocation lines (6AL/6VS，6DL/6VS) were used as experiment materials for identification molecular markers which located in chromosome short arm 6VS by using RAPD, AFLP techniques. The purpose of investigation is to analyze the relativity between molecular markers located in 6VS and *Pm21*. RAPD test indicated that a *Haynaldia villosa* chromosome 6VS - specific polymorphic DNA segment OPK08910 was amplified in 6A/6V substitution line, 6AL/6VS, 6DL/6VS translocation line of wheat and *H. villosa*. AFLP analysis showed three H villosa chromosome 6VS - specific polymorphic DNA seqments (264bp, 218bp, 232bp) can be amplified by three AFLP primer pairs, P02/M05, P03/M08, P09/M09 in substitution line (6A/6V), translocation lines (6AL/6VS, 6DL/6VS) and H. villosa (VV). The AFLP markers of P02/M05-264, P03/M08-218 and P09/M09-232 derived from 6VS were found to be cosegregated with powdery mildew resistance gene *Pm21*. These molecular markers can be used as novel DNA markers for selecting powdery mildew resistance genes *Pm21* in wheat breeding program.

Keywords**:** wheat-powdery mildew; 6A/6V; 6AL/6VS; AFLP molecular marker; marker - assited selection

Correspondence: Yongkang Peng
E-mail: pykcell@yahoo.com.cn

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Differentially expressed genes related to human gastric cancer MGC803 cells induced by Diallyl disulfide

Na Xie, Qi Su, Hui Ling
Cancer Research Institute, Nanhua University, Hengyang, Hunan, China

To isolate and clone differentially expressed genes in human gastric cancer MGC803 cells induced by diallyl disulfide (DADS). Methods: Differentially expressed genes induced by DADS in MGC803 cells were determined by suppression subtractive hybridization (SSH), T/A cloning, sequencing and analyzing for homologs in the Genbank database with BLAST. Results: RNA isolation: Total RNA exhihited clear three bands (28S RNA , 18S RNA and 5S RNA) and protein were not obviously found. The quality of mRNA was also good and exhibited clear smear bands. The analysis of ligation showed that the band intensity of PCR products differs by less than four-fold, indicating that at least 25% of the Testers 1-1 and 1-2 cDNAs have adaptors on both ends. Subtraction efficient: Using specific primers of G3PDH gene as PCR primers and subtracted products as templates, no products were seen even cycled 25-30 times. However, when unsubtracted products were used as templates, obvious product of G3PDH gene was seen even only cycled 15 times. Nested PCR analysis: The subtracted products and unsubtracted products were totally different. The former appeared clear band, while the latter was smear. After T/A cloning, the positive clones (white clones) were amplified in LB liquid medium. Random analyses of clones with restriction digestion and PCR showed that 60 positive clones contained a 100 - 1000 bp insert. Sequencing results showed that there were 34 clones were identified. After comparing with public database (GenBank/NCBI), we found that there were 12 known genes in the two libraries. Data showed that those known genes represent a variety of groups on the basis of their cellular functions and have 98-100% identities in sequences. In the forward substraction library, we identified the cell cycle related genes p21WAF1 and ENO1, the antioxidation-related genes FTH1 and PRDX II, the cytoskeletal gene PFN1, and the metabolism-related gene MDH2, which were up-regulated

in MGC803 cells after DADS treatment. In the reversed substraction library, we identified the cell cycle related gene PTOV1, the cytoskeletal and migration related genes RAC1, ANXA2 and WDR1, the metabolism-related gene ALDOA, and the gene EWSR1, which were down-regulated after DADS induction in MGC803 cells. Conclusion: The differentially expressed genes between primarily cultured and DADS-treated MGC803 cells were screened by SSH and two substracted cDNA libraries were established. A total of 12 differentially expressed genes were identified in the two libraries. Genes from the forward substraction library include p21WAF1, ENO1, FTH1, PRDX II, PFN1 and MDH2; those from the reversed library include PTOV1, RAC1, ANXA2, WDR1, ALDOA and EWSR1. These genes represent a variety of functional groups, which may be involved in cell cycle, cytoskeletal organization, migration, antioxidation and metabolism, and *etc*.

***Keywords*:** diallyl disulfide; gastric cancer MGC803 cells; suppression subtractive hybridization; differentially expressed gene

Correspondence: Qi Su
E-mail: suqi1@hotmail.com

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Screening and cloning of differently expressed genes in human leukamia HL-60 cells treated by Dially disulfide

Ling Ge, Qi Su, Hui Tan, Jie He, Weiguo Huang
Cancer Research Institute, Nanhua University, Hengyang, Hunan, China

To screen and clone differentially expressed genes in human leukamia HL-60 cells. Methods: Differentially expressed genes in HL-60 cells treated by DADS were identified by suppression subtractive hybridization (SSH), T/A cloning, sequencing and analyzing for homologs in the Genbank database with BLAST. Results: Cellular size shrinked in HL-60 cells following treatment with DADS for 24h, as nuclear-to-cytoplasmic ratio was reduced, and coloring of nucleus fainted. Isolation of RNA: Total RNA isolated by TRizol reagent was detected by agrose gel electrophoresis. 28S RNA and 18S RNA displayed as two bands without trail. mRNA appeared as a distinct smear strap. Results of RsaI digestion: Undigested cDNAs manifested as a distinct strap of 0.5-1.0 kb; digested cDNAs manifested as a distinct strap of 0.1-2 kb. Analysis of ligation: Efficiency of adaptor-ligation with digested cDNAs exceeded 25%. Nest PCR analysis of SSH: Fragments of subtracted products were less than unsubtracted products, which had a size range of about 100-600 bp. PCR analysis of subtraction efficiency: Using G3PDH 3' primer and 5' primer G3PDH was amplified from the subtracted and unsubtracted products. The amplified product was detected after 28 cycles using both templates, with the higher amount for the unsubtracted template. It implies a high subtraction efficiency. T/A cloning: The ratio of white to blue colonies was about 2:1. Purified plasmids from the positive clones exceeded 3kb in length. Size of PCR products is about 200-300 bp. Sequencing results showed that 89 clones were identified. After comparing with public database (Genbank/EMBL) and Expressed Sequence Tag (EST), 7 known genes were found in the two libraries. Data showed that those known genes represent a variety of groups on the basis of their cellular functions and have 99-100% identities in sequences. In the forward substraction library, we identified PRDX2, CNOT8, PQBP1 and RPS7 that were up-regulated by DADS induction in HL-60 cells, while hRRM2, calcium/calmedulin-dependent protein kinase and Homo sapiens isolate C30 MK2 non-allergic IgE heavy chain were down-regulated in the reversed substraction library. These genes are related to cell metabolism, immunoreaction, antioxidation and drug resistance, respectively. Conclusion: The subtracted cDNA libraries constructed by SSH contains differentially expressed genes in human leukamia HL-60 cells treated by DADS. DADS up-regulates PRDX2, CNOT8, PQBP1 and RPS7 genes in HL-60 cells, while it declines the expression of hRRM2, CaMk and IGHV2-5.

***Keywords*:** dially disulfide; leukamia; HL-60 cells; subtracted suppression hybridization; differentially expressed genes

Correspondence: Qi Su
mail: suqi1@hotmail.com

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Isolation of differentially expressed genes related to growth inhibition induced by Diallyl disulfide on the colon cancer HT-29 cells

Yousheng Huang, Qi Su, Qianjin Liao, Jian Su,
Xiutian Zhou
Cancer Research Institute, Nanhua University, Hengyang, Hunan, China

To primarily screen and identify growth inhibition-associated genes induced by diallyl disulfide (DADS) in human colon cancer HT-29 cells and to illustrate the molecular mechanisms of the antiproliferative ability of DADS in HT-29 cells. Methods: The growth inhibition model was established by inducing with DADS in human colon cancer HT-29 cells, and was identified by MTT and FCM. Differentially expressed cDNA species induced by DADS (120 μmol/L) in human colon cancer HT-29 cell line were determined by using suppression subtractive hybridization (SSH). Then these cDNA species were directly inserted into

an T/A cloning vector, and amplification of the libraries was carried out by transforming into JM109. After being identified by PCR and restriction digestion, positive clones were sequenced and analyzed for homologs in the Genbank database with BLAST. Ten genes randomly chosen from the two libraries were analyzed by using semi-quantitative reverse transcription- polymerase chain reaction. Results: Subtractive libraries that contain growth inhibition-associated genes induced by DADS in human colon carcinoma HT-29 cells were set up successfully. There were about 500 positive clones in the amplified libraries. Random analyses of 100 clones with PCR and restriction digestion showed that all clones contained a 200-1300 bp insert. After comparing with public database (GenBank/EMBL) and Expressed Sequence Tag (EST) , we found that there were 38 known genes and a nove EST in the two libaries. The expression of 10 genes chosen randomly from the two libraries in the untreated and DADS- treated (120 μM) HT-29 cells, analyzed by semiquantative RT-PCR, showed a consistent pattern with that of the SSH. Conclusion: The differentially expressed genes before and after induction by DADS were screened by SSH and two substracted cDNA libraries were established successfully with high subtractive efficiency. A variety of functional genes that might be involved in the antitumorigenic effects of DADS have been identified. Further characterization of these genes may allow a better understanding of the protective roles of DADS.

Keywords**:** diallyl disulfide; colon cancer; HT-29 cells; subtracted suppression hybridization; differentially expressed genes

Correspondence: Qi Su
E-mail: suqi1@hotmail.com

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Traditional Chinese medicine baixingdan capsule could up-regulate the expression level of serotonin 1 A receptor and the viability of rat cerebral cortex neural cells

Qitao Zhao[1], Heng Liu[1], Lei Sun[1], Huiyun Zhang[2], Mingqi Qiao[1]

[1]Institute of Traditional Chinese Medicine theory, School of Basic Medicine, [2]Key Laboratory of Natural Drugs, Shandong University of Traditional Chinese Medicine, Jinan 250014, China

Premenstrual dysphoric syndrome (PMDD) is a common disorder affecting some women during their reproductive years. Our previous clinical and experimental studies testified that Chinese medicine Baixingdan capsule, composed of Radix Paeoniae Alba, Rhizoma Cyperi, Cortex Moutan, emerged as a very effective remedy for PMDD in China for years. To investigate the underlying mechanism by which Baixingdan cure PMDD patient, we studied the effect of this medicine on rat cerebral cortex neuron *in vitro* with serum pharmacological method. The serum concentration of paeoniflorin, the bioactive ingredient of this herbal medicinal compound, was measured by capillary zone electrophoresis. Rat cerebral cortex neurons were incubated with normal rat serum, PMDD model rat serum, or different concentrations of Baixiangdan medicated serum (serum isolated from PMDD model rats fed with Baixiangdan), respectively. The viability of neural cells was assayed by MTT, and the expression levels of serotonin 1A receptor (5-HT(1)AR) in this cells were evaluated by immunofluorescence techniques and Laser Scanning Confocal Microscope. Our results showed that Baixiangdan medicated serum could markedly up-regulate the viability and the 5-HT(1)AR expression of rat cerebral cortex neurons in a dose-dependent way. Our results first demonstrated that there was at least one important component target to 5-HT(1)AR in traditional Chinese Medicine Baixiangdan capsule; moreover, Baixiangdan might partially play its role in ameliorating PMDD symptom by up-regulating the expression of 5-HT(1)AR.

Keywords**:** traditional Chinese medicine Baixingdan; serotonin 1A receptor; neural cells; serum pharmacological study; paeoniflorin

Correspondence: Mingqi Qiao
E-mail: Mingqiqiao@yahoo.com.cn

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Molecular cloning and function analysis of a novel gene *STGC3* associated nasopharyngeal carcinoma on chromosome 3p21

Xiusheng He[1], Zhuchu Chen[2], Zhiqiang Xiao[2], Zhimin He[2], Yanhui Yu[2], Qiao Luo[1]

[1]Cancer Research Institute, Nanhua University, Hengyang 421001, China; [2]Cancer Research Institute, Central South University, Changsha 410078, China

STGC3, a putative human tumor suppressor gene on chromosome 3p21, has been isolated by our laboratory recently (GenBank Accession No. AY078383). The *STGC3* transcripts expressed ubiquitously in human tissues with different intensities. Its attenuated expression has been observed in both in carcinoma (NPC) and in sarcoma (lymphoma). In order to investigate the function of *STGC3*, subcellular localization of STGC3 protein and the effects of *STGC3*over-expression in CNE2 cell line were studied. The results showed that the fusion protein pEGFP-C2-STGC3 was located in the nucleus and cytoplasm. CNE2 cell line stably over-expressing *STGC3* had a reduced growth rate, lower levels of anchorage-independent growth, an increase

of apoptosis, and altered protein expression patterns in comparison with its control cell line. All the data suggest that STGC3 may play a role in tumor suppression in NPC.

Keywords**:** *STGC3*; nasopharyngeal neoplasm; gene expression; gene cloning; chromosome 3p21

Correspondence: Xiusheng He
E-mail: tcbl@public.cs.hn.cn

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Insulin-like growth factor-1 prevents vascular smooth muscle cell death induced by Apop-1 through activation of the PI3K/Akt pathway

Xin Sun[1], Xiaotao Zhu[2], Luping Xu[1], Zhiping Yang[1], Jinghui Cui[1], Osamu Yasuda[3], Keisuke Fukuo[4], Toshio Ogihara[3]

[1]Beihua University Graduate School of Medicine, Jilin, China; [2]Department of Surgery, Central Hospital of Jilin, Jilin, China; [3]Department of Geriatric Medicine, Osaka University Medical School, Suita, Japan; [4]Department of Food Science and Nutrition, School of Human Environmental Sciences, Mukogawa Woman's University, Hyogo, Japan

Apoptosis of vascular smooth muscle cells (VSMCs) has recently been identified as an important process in a variety of human vascular diseases, including atherosclerosis. Apop-1, a novel gene identified in cultured atherosclerotic smooth muscle cells of ApoE-deficient mouse and is known to induce apoptosis in several cells, including VSMC. Insulin-like growth factor (IGF-1) and platelet-derived growth factor (PDGF) are well characterized survival factors for VSMC. However, the interactions between the pro-apoptotic protein Apop-1 and survival factors IGF-1 and PDGF on mediation of apoptosis in VSMC are poorly understood. In this report we show that the signaling cascade involved in IGF-1 protects VSMC against Apop-1-induced apoptosis, while PDGF has no effect. In addition, pretreatment of Apop-1 transfected VSMCs with phosphatidylinositol-3-kinase inhibitor wortmannin, or infection with an adenoviral construct expressing the dominant negative *Akt* gene (Adeno-dnAkt) blocks the cytoprotective effect of IGF-1, whereas the MEK inhibitor PD98059 has no effect. Conversely, infection with an adenoviral construct expressing the constitutively active *Akt* (Adeno-MyrAkt) gene, protects VSMC from apoptosis induced by Apop-1 even in the absence of IGF-1, suggesting that IGF-1 prevents VSMC apoptosis induced by Apop-1 through activation of the PI3K/Akt pathway. Furthermore, IGF-1 elevates phospho-Akt expression in Apop-1 transfected VSMCs, and Apop-1 decreases phospho-Akt expression. Importantly, IGF-1 inhibits cytochrome C release from mitochondria and blocks activation of intrinsic initiator caspase-9 in Apop-1 transfected VSMCs. These findings suggest that inhibition of Apop-1-induced apoptosis by IGF-1 is via promotion of Akt activation through PI3K/Akt signaling pathway which may contribute to stabilize atherosclerotic plaque in patients with atherosclerosis.

Keywords**:** atherosclerosis; apoptosis; Apop-1; growth factors; PI3K/Akt

Correspondence: Xin Sun
E-mail: sxin1122@yahoo.com.cn

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B-class Mads box genes regulate orchid floral morphogenesis

Hong-Hwa Chen

Department of Life SCiences, and Institute of Biotechnology, National Cehng Kung University, Tainan

Orchids have profound diversity of specialized pollination and ecological strategies and provide a rich setting for studying evolutionary relationships and molecular biology. The sophisticated orchid flower morphology offers an opportunity to discover new variant genes and different levels of complexity in the morphogenesis of flower. For systemic study floral morphogenesis of orchid flower, a total of 5593 expressed sequence tags (ESTs) were established from flower buds of Phalaenopsis equestris, a native orchid species in Taiwan. Four B-class Phalaenopsis DEF-like MADS-box and one GLO-like genes were identified and characterized, including PeMADS2, PeMADS3, PeMADS4, PeMADS5 and PeMADS6. Comparing their expression patterns in wild-type and peloric mutant floral organs revealed that diverse functions of determining floral development of these 5 B-class genes. PeMADS2 and PeMADS6 control sepal formation, PeMADS3, PeMADS4 and PeMADS6 involved in lip development, and PeMADS3, PeMADS5 and PeMADS6 correlated to petal development. In addition, PeMADS6 not only involved in petaloid formation but also correlated with flower longevity and ovary/ovule development. We further examined the quantitative expression profile of these B class genes in various orchid floral organs by using real-time RT-PCR in wild type and peloric mutant of three Phalaenopsis orchids including *P. equestris*, *P. equestris* "King Car" and *P. var*. (A3947). We found that certain expression ratios among these five B-class MADS box genes were maintained for various orchid floral organ morphogenesis. In addition, PeMADS4 was the key factor of lip development and crucial for the altered primordial development from petal to lip in the peloric flower. Furtherrmore, formation of higher order complexes was detected among these B-class proteins for the determination of the orchid floral organ identity. These results will open the gate

for the understanding of the molecular basis of orchid floral organ morphogenesis.

***Keywords*:** MADS box; B-class; orchid; floral morphogenes; protein interaction

Correspondence: Hong-Hwa Chen
E-mail: hhchen@mail.ncku.edu.tw

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Effect of STGC3 gene induced by Tet-on system on CNE2 cell lines in nude mice

Qingchao Qiu, Bo Hu, Xiusheng He, Qiao Luo
Cancer Research Institute, Nan Hua University, Hengyang 421001, China

STGC3, a novel candidate tumor suppressor gene, can inhibit the proliferation of nasopharyngeal carcinoma in vitro. To explore its role in suppressing the proliferation of CNE2 cells in nude mice, the nude mice experiment was employed by using the CNE2/Tet/pTRE-STGC3 cell line induced by Tet-on system. RT-PCR and Western-Blot were applied to detect the mRNA and protein level of STGC3 in transplanted tumor tissues. Flow cytometry was deployed to analyze the apoptosis ratio of the tumor cells. The results indicated that a high level of STGC3 expression can inhibit the tumor formation of the CNE2 cells in nude mice. It grew more slowly with a later tumor formation time and smaller tumor size compared with the other three control groups with a statistical significance ($p<0.05$). Flow cytometry(FCM) indicated that STGC3 increased the percentage of apoptotic CNE2 cells in the nude mice tumor cells. The data collected indicated that STGC3 had a role as a tumor suppressor gene *in vivo*, which was in line with the results *in vitro*.

***Keywords*:** nasopharyngeal carcinoma; STGC3 gene; tumorigenicity in nude mice; Tet on regulating expression system

Correspondence: Qingchao Qiu
E-mail: hb20040202@yahoo.com.cn

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The effect of the β-estradiol to the STGC3 gene on the CNE2 cells *in vitro* and *in vivo*

Bo Hu, Qingchao Qiu, Xiusheng He, Qiao Luo
Cancer Research Institute, NanHua University, Hengyang 421001, China

To explore the effect of STGC3 and the β-Estradiol on the CNE2 cells in the nude mice. The recombinant pcDNA3.1 (+)/STGC3 plasmid was constructed and transfected into the CNE2 cell line by lipofectamine 2000. Cell clones stably expressing STGC3 were obtained and identified with RT-PCR method. The effect of β-Estradiol on the growth rate of the pcDNA3.1 (+)/STGC3/CNE2 cells was examined by cytometry. Tumoregenisity experiment was applied to find the effect of STGC3 on the CNE2 cells in the female and male nude mice. The expression of the STGC3 was detected by RT-PCR and Western Blot methods. The pathological examination was deployed to understand the differentiation condition of the transplanted tumor tissue. Flow cytonmetry was used to analyze the cell cycles. The results indicated that the growth rate of the pcDNA3.1(+)/STGC3/CNE2 decreased when treated with β-Estradiol. There is statistical significance ($P < 0.05$) between the groups with or without transfected STGC3 and between the male and female nude mice transfected with STGC3. Therefore, the growth rate of the female nude mice inoculated pcDNA3.1(+)/STGC3/CNE2 were the lowest. There is no significance between the untransfected STGC3 gene groups. The pcDNA3.1(+)/STGC3/CNE2 cell group indicated a high level of STGC3 expression and with more cells blocked in G0/G1 phase. The data above indicated that the β-Estradiol can strengthen the effect of STGC3 to inhibit the proliferation of CNE2 cells *in vivo* and *in vitro*.

***Keywords*:** nasopharyngeal carcinoma; STGC3 gene; β-estradiol; tumoregenicity; nude mouse

Correspondence: Bo Hu
E-mail: hb20040202@yahoo.com.cn

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cDNA cloning, gene organization and variant specific expression of HIF-1α in high altitude Yak (*Bos grunniens*)

MA Qadar Pasha[1], Karamjit S Dolt[1], Manoj K Mishra[1], Jayashree Karar[1], Zakwan Ahmed[2]
[1]Institute of Genomics and Integrative Biology, Delhi, India; [2]Field Research Laboratory, Leh, Ladakh, J&K, India

Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric basic-helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) transcription factor consisting of HIF-1α and HIF-1β subunits. HIF-1α is the oxygen-regulated subunit of HIF-1, which regulates the transcription of genes involved in oxygen homeostasis in response to hypoxia. Yak (*Bos grunniens*), a mammal native to high altitude (HA) region (~3500–5500 m), has successfully adapted over many generations to the chronic hypoxia of HA. In the present work, cDNA encoding HIF-1α has been cloned from the blood of yak. Tissue specific expression of the mRNA was analyzed in blood, heart, lung, liver and kidney by RT-PCR with primers from three different regions of cDNA. The HIF-1α expression was restricted to liver and blood cells. The HIF-1α mRNA contains 823 bp long 3' UTR that is AU-rich and has ten

AUUUA pentamers and two overlapping copies of the nonamer UUAUUUAUUUAUU. Three potential microRNAs, hsa-miR-107/mmu-miR-107/rno-miR-107, hsa-miR-18b and hsa-miR-135a/mmu-miR-135a/rno-miR-135a, targeting 3' UTR of yak HIF-1α, were identified by using target prediction software. The CDS of HIF-1α mRNA encodes 823 residues and showed 99%, 95%, 92%, 90% and 90% similarity to bovine, human, plateau pika, mouse and rat HIF-1α, respectively. Cloning and sequencing of the HIF-1α cDNA in the present work has revealed the evolutionary conservation through multiple sequence alignment. Liver and blood specific stability of HIF-1α mRNA appears miR-107 regulated.

***Keywords*:** adaptation; himalaya; hypoxia; yak; mRNA instability element

Correspondence: MA Qadar Pasha
E-mail: qpasha@igib.res.in

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The reciprocal regulation of lipid metabolism and inflammation by liver X receptor in ApoE knockout mice

Xiao Y Dai[1], Chao K Tang[1], Xiang Ou[1], Gui X Wang[2], Zhi S Jiang[1], Dong L Cao[1], Xin R Hao[1], Guang H Yi[1], Lu S Liu[1]

[1]Institute of Cardiovascular Disease, Nanhua University, Hengyang, China; [2]School of Bioengineering, Chongqing University, Chongqing, China

Liver X receptors (LXRs), which are members of nuclear receptor superfamily, have been found to induce expression of a number of genes involved in the regulation of cellular cholesterol efflux, intestinal cholesterol absorption and hepatic lipid synthesis. LXR also has some anti-inflammatory effects. Here, based on our previous studies and the research progress, we investigate the reciprocal regulation of lipid metabolism and inflammation by LXR by examining the effect of a synthetic LXR agonist T0901317 on the atherosclerotic lesions in apolipoprotein E knockout (apoE$^{-/-}$) mice. Methods: Male apoE$^{-/-}$ mice were randomly divided into three groups, vehicle group (n=7), prevention group (n=7) and treatment group (n=7). All of the mice were fed a high-fat/high-cholesterol diet for 14 weeks. The vehicle group was treated with vehicle for 14 weeks. The prevention group was treated with LXR agonist T0901317 for 14 weeks. The treatment group was treated with vehicle for 8 weeks, and then was treated with the agonist for additional 6 weeks. At the end of experiment, mouse blood and tissues were collected for further analysis. The extent of atherosclerosis in en face mouse aortic preparations was quantified by computer-assisted image analysis. Plasma total cholesterol and triglyceride levels were determined by colorimetric enzymatic assays. Plasma HDL cholesterol was determined by precipitating non–HDL cholesterol (Wako Diagnostic 278-67409) and then assaying the remaining HDL cholesterol by means of the Infinity Total Cholesterol Reagent. Transcript levels were analyzed by microarray analysis and real-time quantitative PCR. Protein levels were determined by Western-blotting. Results: LXR agonist T0901317 treatment resulted in a significant reduction of lesion area in prevention and treatment group compared with vehicle-treated controls. There was no significant difference between prevention group and treatment group. LXR agonist contributed to the reduction of macrophage content and the increase in collagen content of lesions. Plasma total triglyceride, total cholesterol, HDL-C, LDL-C and apoA-I concentrations were markedly increased in prevention group and treatment group. In T0901317-treated mice, significant increase of mRNA and protein expression levels of LXRα, LXRβand ATP-binding cassette transporter A1 (ABCA1) were found in liver and small intestine, furthermore markedly improved ABCG5 and ABCG8 gene expression levels were found in liver and small intestine. However, the agonist down-regulated the mRNA expression levels of a battery of inflammatory genes in atherosclerotic lesions, including monocyte chemoattractant protein 1(MCP-1), interleukin-6(IL-6) and IL-1β. Conclusion: The synthetic LXR agonist T0901317 has a strong preventive and therapeutic effect on the atherosclerotic lesions in apoE$^{-/-}$ mice. These beneficial effects may derive from the increased cellular cholesterol efflux, lipid metabolism and decreased some inflammatory genes expression mediated by LXR.

***Keywords*:** liver X receptor; atherosclerosis; nuclear receptors; lipid metabolism; inflammation

Correspondence: Xiaoyan Dai
E-mail: daixiaoyan263@126.com

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Preliminary analysis on interleukin-18 gene expression in the occurrence and development of kidney-deficiency diabetes through a genetically identical Chinese twin

Miqu Wang[1], Ling Pan[1], Jianguo Wang[2], Conge Tan[1], Liping Yang[1]

[1]Genetics Laboratory, Chengdu University of Traditional Chinese Medicine, Chengdu, China; [2]Centre for Protective Technology, National University of Singapore, Singapore

This paper reports experimental results and findings from cDNA microarray tests carried out for a genetically identical Chinese twin in order to explore the molecular mechanisms for occurrence and development of kidney-deficiency diabetes. This research includes four parts: Firstly, the

development of type 2 diabetes was observed for a genetically identical Chinese twin through accumulated scores for kidney-deficiency diabetes diagnosis scaling table, Liu Gang and blood GLUL index at different time series. The younger sister was identified as a type 2 diabetic patient and the elder sister as a normal but at critical state person. Secondly, their peripheral bloods were tested for differential gene expressions through cDNA microarray technology. Differentially expressed genes were obtained by comparing the diabetic sister's gene expressions to the normal one's. It was found that 19 differential gene expressions were saliently regulated, in which, 7 genes were up-regulated and 12 genes were down-regulated. Those genes for metabolism, cellular physiological processes, and responses to stimulus are all down-regulated, but those genes for cell communication are mixedly regulated. Thirdly, biological pathways for these differential expression genes were explored through KEGG database and BioCarta Map and only 9 genes were recoded. Among these 9 genes, only up-regulated genes, NM_0038555 (IL18R1) and NM_004086 (COCH), had pathways, while down-regulated important genes included NM_001670 (ARVCF) and NM_012483 (GNLY). Fourthly, literature reviews indicates that above 4 genes may play key roles in occurrence and development of this kidney-deficiency diabetic patent. Particularly, our findings on the high level expression of interleukin-18 (IL-18) are consistent with laboratory and clinical observations carried out by other researchers in following aspects: 1) high serum interleukin-18 concentrations were found in patients with coronary artery disease and type 2 diabetes mellitus. High level expression of interleukin-18 gene can accelerate cell communications and thus results in high concentration. This hypothesis was partially verified by a clinical outcome that an improvement occurred after initiation of sarpogrelate therapy, a significant decrease in IL-18 levels was observed after 2 months of therapy. 2) High level expression of IL-18 is also consistent with our clinical observations based on the above-mentioned accumulated scores. These twin sisters have identical gene structures but different gene expressions. Their unique difference on macro-behaviors is the extent of diabetic history. We can assure that the high level expression of IL-18 is associated with the occurrence and development of kidney-deficiency diabetes. Finally, it was observed that serum and urinary IL-18 levels were significantly elevated in Japanese patients with type 2 diabetes as compared with control subjects. Therefore, IL-18 level as a marker for type 2 diabetes can be checked from them.

Keywords**:** genetically identical twin; cDNA microarray; gene differential expression; interleukin-18; cell communication

Correspondence: Miqu Wang
E-mail: wangmiqu@yahoo.com.cn; cvewjg@nus.edu.sg

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Cloning and functional analysis of a new *CBF* gene in wheat

Zhenying Wang, Liyuan Chen, Shaoxia Wang, Zhijuan Xue, Xiaoying Liu
Department of Biology, Tianjin Normal University, Tianjin 300074, China

Drought, salt loading and freezing are stresses that cause adverse effect on the growth of plants and to the productivity of crops. The physiologic response to these stresses arises out of changes in cellular gene expression. Usually the products of gene inducible by these stresses can be classified into two groups. One is the protective ptoteins directly taken part in resisting environmental stress. The other is proteins function as regulator of gene expression and signal transduction factors, including transcriptional factors, proteinase and enzymes involved in phosphoinositide metabolism. DREB (dehydration responsive element binding protein) is one of the transcriptional factors in plant, which plays an important role in anti-cold, -drought and -salt reaction. Because DREB factor can active another 12 stress responsive genes expression, such as rd29A, cor15a, rd17, which depend on DRE cis-acting element.In this study, we reported a new DREB gene, which plays an important role in regulating gene expression in response to salt stress. According to sequences of 3 published rye DREB genes (gi/17148646, gi/17148648, gi/17148650), we designed a pair of specific primers as following: P1, 5'-TGC CTC AAC TTC GCC GAC TCC-3'; P2, 5'-CGA GCA TCC CCT GCG CCA AG-3'. Partial DNA fragment was amplified by PCR using total DNA isolated from wheat cultivar Jing411. We obtained a specific DNA band about 290bp in size. Cloning and sequencing the PCR product, it was total 292bp in size. Databases were searched for sequence similarities using BLASTn program of NCBI. It was 90% identical to barley DREB gene (gi/12658318). We amplified the 3'-end of the gene from the total RNA isolated from cold induced (4 °C for 2h) wheat Jing411 using upstream primer (P3) designed according to the 292bp fragment, and the universal primer (P4) as downstream primer. The 5'-end of the gene was obtained by using 5'-full RACE Core Set Kit (TakaRa). The final assembled full length cDNA is 936bp in size. The BLASTx analysis showed that this gene had a 5' UTR of 130bp in size and a 3' UTR of 89bp in size and an ORF of 717bp in size. Compared with the database BLASTn, it has 89% identify to barley CBF4D (C-repeat/DRE banding factor 4D) gene (AY785852.1). It implied that this gene might be a new member of CBF4D

family of wheat. It was named as CBF4D-a. The accession No. in GenBank is DQ286550. To examine the function of CBF4D-a, we constructed the ORF of CBF4D-a to plant expression vector pROKII and transfered to Arabidopsis, it shown that the transgenic plants could growth well in 1.5% NaCl and the control could not.

***Keywords*:** DRBE gene; wheat Jing411; RACE; salt; *Arabidopsis*

Correspondence: Zhenying Wang
E-mail: wzycell@yahoo.com.cn

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Isolation and preliminary characterization of NBS class of resistance gene from common wheat brock

Gang Li, Zhenying Wang
Department of Biology, Tianjin Normal University, Tianjin 300074, China

In this study, total RNA isolated from common wheat Brock leaf, which was inoculated with a local isolate of *E. graminis* f. sp. tritici race NO.15, was used as experiment material. The RT-PCR method was applied to isolate and identify the NBS class of resistance gene homolog in wheat. Total RNA was reverse transcripted into cDNA by using specific primer which was designed according to conserved domain of NBS class resistance gene homolog from wheat. About 300 bp cDNA fragment was amplified. Cloning and sequencing analysis of this fragment indicated that full length of this fragment consisted of 284bp. Sequence alignment showed that it was similar to conserved domain of NBS class resistance gene from wheat. It had a part of similarity with conserved domain of other NBS class resistance genes, which have been cloned. This experiment showed that this cloned cDNA fragment contained the part of coding region of nucleotide sequence of NBS class resistance gene. 2100 bp specific cDNA fragment of 3' end of NBS class resistance gene was obtained, by using specific primer designed according to the cloned cDNA fragment, through the 3' RACE method. This 2100 bp DNA fragment was cloned and sequenced, result indicated that 3' non-translated region and poly(A) tail were revealed. Part overlapping sequence was observed between this fragment and the 284 bp fragment, a complementary DNA fragment could be spliced.

***Keywords*:** NBS class resistance gene; conserved domain; sequence aligament; *E. graminis* f. sp. tritici; common wheat Brock

Correspondence: Zhenying Wang
E-mail: wzycell@yahoo.com.cn

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Comparison studies on the expression difference of wheat aquaporin PIP2 in various water conditions

Zixin Mu, Zongsuo Liang, Rang Cao, Yongjun Wu, Linsheng Zhang
Life science College, Northwest A&F University, Yangling, China

Plant water channels, aquaporins, a class of membrane proteins that facilitate not only water diffusion across cell membranes, but also root system water uptake, has been shown to be regulated in response to osmotic stress. Using a draught-tolerant wheat genotype, Shaanhe No.6, and a drought-sensitive genotype, Zhengyin No.1, we investigated the dynamic changes in expression of aquaporin gene PIP2 in the process from normal water conditions to water stress to water status resumed by a semi-quantative reverse transcription-PCR analysis. The results indicated that under normal water conditions, the transcript level of PIP2 is higher in Zhengyin No.1 than in Shaanhe No.6, while under water stress with -0.4 MPa PEG-8000, the expression pattern is reversed. It is also shown that temporal PIP2 expression occurs in the root system for both wheat genotypes. At the early stage of water deprivation (stressed for 12h), the transcript level of PIP2 is higher in drought treatment than in the control for Shaanhe No.6, and it then gradually decreases to close to the control level (stress for 60h). For Zhengyin No.1, the transcript level of PIP2 always decreases with the water stress advance, and the level is close to zero when the plant is subject to water stress for 60h. After re-watering, the transcript level of PIP2 resumes significantly for both genotypes, and the difference is that the resumed level is higher than its control in draught-tolerant genotype while it is not completely resumed in draught-sensitive genotype. In conclusion, this work shows that aquaporin gene PIP2 plays a key role in response of wheat plant to changed water environment, and that the different expression pattern among genotypes may be one of the mechanisms for draught stress toleration of wheat plant.

***Keywords*:** wheat; aquaporin; drought stress; semi-quantitative RT-PCR

Correspondence: Zixin Mu
E-mail: muzx810@126.com

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Silencing of the metastasis suppressor RECK by RAS oncogene is mediated by DNA methyltransferase 3b-induced promoter methylation

Wenchun Hung
Institute of Biomedical Sciences, National Sun Yat-Sen University

and NSYSU-KMU joint Research Center, Kaohsiung

RECK is a membrane-anchored glycoprotein, which may negatively regulate matrix metalloproteinase (MMP) activity to suppress tumor invasion and metastasis. Our previous study indicated that oncogenic RAS inhibited RECK expression via a histone deacetylation mechanism. In this study, we address whether DNA methyltransferases (DNMTs) participate in the inhibition of RECK by RAS. Induction of Ha-RASVal12 oncogene increased DNMT3b, but not DNMT1 and 3a, expression in 2-12 cells. In addition, induction of DNMT3b by RAS was through the ERK signaling pathway. Oncogenic RAS increased the binding of DNMT3b to the promoter of RECK gene and this binding induced promoter methylation, which could be reversed by 5'-azacytidine and DNMT3b siRNA. The MEK inhibitor U0126 also reversed RAS-induced DNMT3b binding and RECK promoter methylation. Treatment of 5'-azacytidine and DNMT3b siRNA restored RECK expression in 2-12 cells and potently suppressed RAS-stimulated cell invasion. In addition, the inhibitory effect of 5'-azacytidine on RAS-induced cell invasion was attenuated after knockdown of RECK by siRNA. Interestingly, human lung cancer cells harboring constitutively activated RAS exhibited lower RECK expression and higher promoter methylation of RECK gene. 5'-azacytidine and DNMT3b siRNA restored RECK expression in these cells and effectively suppressed invasiveness. Collectively, our results suggest that RAS oncogene induces RECK gene silencing through DNMT3b-mediated promoter methylation, and DNMT inhibitors may be useful for the treatment of RAS-induced metastasis.

***Keywords*:** RECK; RAS; DNA methyltransferase; methylation; ERK

Correspondence: Wenchun Hung
E-mail: hung1228@ms10.hinet.net

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Human Pif1 inhibits telomerase activity by dissociating its RNA subunit from telomeric DNA

Bo Zhou, Xiajing Tong, Denghong Zhang, Jinqiu Zhou
Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Telomeres can be maintained by a specialized ribonucleoprotein, called telomerase that is minimally composed of a highly conserved reverse transcriptase, TERT and an associated template RNA, TR. Extension of the telomere terminus results in the addition of a repeated array of TTAGGG. It has well-studied how this complex is recruited onto telomere. However, how it is removed from telomere remains elusive. Here, we identified a new human helicase, hPif1 that inhibits telomerase activity. The primary sequence and biochemical analysis suggest that hPif1 is a potential homologue of *Escherichia coli* RecD, an ATP-dependent 5' to 3' DNA helicase. Ectopic expression of wild-type, not ATPase/helicase deficient hPif1, causes telomere shortening in telomerase positive cells, but not in telomerase negative cells, indicating that hPif1 acts via telomerase pathway. *In vitro*, hPif1 inhibits human telomerase activity probably due to reducing the repeat addition processivity of telomerase. hPif1 could unwind a general DNA/RNA duplex and preferentially binds telomeric DNA *in vitro* and *in vivo*. However, helicase activity seems not sufficient for hPif1 to release the telomerase complex from telomeric oligonucleotide. We propose that the mechanism of hPif1's inhibition on telomerase involves unwinding of the DNA/RNA duplex formed by telomerase RNA and telomeric DNA and that the removal of telomerase complex from telomere may require other protein.

***Keywords*:** telomere; telomerase; telomerase processity; human Pif1; helicase

Correspondence: Bo Zhou
E-mail: zhoub@sibs.ac.cn

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Transcription factor DPZF is the master regulator for the postnatal repression of alpha-fetoprotein

Zhifang Xie[2], Hai Zhang[1], Ye Zhang[1], Xuetao Cao[3], Michael Grusby[4], Weiping Zhang[1]
[1]Department of Pathophysiology, [2]Department of Cell Biology, [3]Department of Immunology, Second Military Medical University, Shanghai, China; [4]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, USA

The alpha-fetoprotein (*AFP*) gene exhibits high tissue specificity and tight temporal regulation during the mammalian development. It's activated, together with the evolutionarily related albumin gene, in the yolk sac, fetal liver and fetal gut. Shortly after birth, it's rapidly repressed to extremely low but detectable levels in the liver and gut. Hence, AFP gene is regarded as an ideal model to investigate the developmental control of transcription. Nevertheless, AFP can be reactivated during liver regeneration and carcinogenesis. Up to now, the mechanisms underlying the postnatal repression of *AFP* gene is poorly defined. We identified a novel POZ zinc finger protein DPZF, and generated the DPZF-deficient mice by homologous recombination. DPZF knockout mice exhibit consistently high expression of *AFP* in adult livers through their life, somehow close to the levels of this gene in fetal liver. Overexpression of DPZF in hepatoma cells dramatically inhibits AFP reporter activ-

ity. The DPZF-responding cis-elements in *AFP* gene were carefully delineated. Our data indicate that transcription factor DPZF is the key regulator for the postnatal repression of AFP. This is the first report describing the in vivo function of DPZF and a transcription factor contributing to AFP postnatal repression.

***Keywords*:** transcription factor; transcriptional repression; hepatic development; alpha-fetoprotein; hepatocyte

Correspondence: Weiping Zhang
E-mail: wpzhang68@yahoo.com

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Effect of oxidized low density lipoprotein on Niemann-Pick Type C1 expression in THP-1 macrophages

Xiang Ou, Xiaoyan Dai, Xinrui Hao, Dongli Cao, Chaoke Tang
Institute of Cardiovascular Disease of Nanhua University, Hengyang, China

Niemann-Pick Type C1 (NPC1) is located in the late endosome and it controls cholesterol transport from the lysosome to the plasma membrane. Availability of cholesterol in the plasma membrane is an all-important step for cholesterol efflux. Our aim is to investigate the effect of oxidized low density lipoprotein (ox-LDL) on Niemann-Pick Type C1 expression in THP-1 macrophages. After exposing the cultured THP-1 macrophages to ox-LDL at the same concentration for different periods and to ox-LDL at the different concentrations for 24h, NPC1 mRNA and protein level were determined by reverse transcriptase-polymerase chain reaction and Western blot, respectively. The cholesterol level in the perinuclear region and the plasma membrane was measured by staining with filipin. Cholesterol efflux was determined by FJ-2107P type liquid scintillator. Ox-LDL elevated NPC1 at both protein and mRNA levels, and increased apolipoprotein (apo) A-I-mediated cholesterol efflux in a time- and dose-dependent manner. Ox-LDL gradually increased the filipin fluorescence in the plasma membrane and decreased the signal intensity in the perinuclear region. As a result, ox-LDL can up-regulate the expression of NPC1 in THP-1 macrophages, and NPC1 may play an important role in the process of preventing macrophages to form foam cells.

***Keywords*:** oxidized low density lipoprotein; Niemann-Pick Type C1; cholesterol

Correspondence: Xiang Ou
E-mail: xou168@163.com

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YY1 is the key regulator of the characterized murine p55 gene promoter

Mei He, Naizheng Ding, Jinsong Hu, Yuhui Sun, Jianguo Chen
Department of cell biology, College of Life Sciences, Peking University, Beijing, China

The murine p55 gene is highly conserved and ubiquitously expressed, its function remains unclear. To understand the mechanisms of p55 gene expression and regulation, we first mapped the major transcription start site at the nucleotide 79bp upstream of the ATG codon. Murine p55 2.1kb and human homology 645bp promoter regions were cloned and analyzed. Truncation analysis of promoters identified minimal 0.2 kb efficient TATA-less promoter regions of both genes. The substantive repeat sequence of the murine p55 gene promoter greatly inhibited its transcription activity. Testis *in vivo* electroporation confirmed the activity of the identified murine promoter. Electrophoretic mobility shift assays validated the specific interaction between YY1 and the predicted element in murine minimal region. Site mutagenesis and knockdown of YY1 indicated that YY1 was crucial to p55 promoter activity; while truncation analysis and overexpression of YY1 suggested that the function of YY1 was related to upstream elements. Furthermore, during embryogenesis, the expression patterns of p55 and YY1 were similar. Our data demonstrated that YY1 was a key regulator of the characterized murine p55 promoter.

***Keywords*:** P55; promoter; YY1; repeat sequence; *in vivo* electroporation

Correspondence: Jianguo Chen
E-mail: chenjg@pku.edu.cn

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Molecular cloning and characterization of a novel gene—Oocyte-G1

Yong Zhang, Ji Wu
School of Life Science and Technology, Shanghai Jiao Tong University, Shanghai, China

The oocyte is recognized as a source of regulatory molecules that influence follicular development through an array of actions on granulose cells. During efforts to clone oocyte development-related genes, we isolated a cDNA fragment by differential display reverse-transcription PCR (DDRT-PCR). To obtain its 5'-and 3'-end sequences, a mouse ovarian cDNA library (Stratagene) was screened. The library was constructed with poly(A)+ mRNA from mouse ovaries of day 15. Using both oligo(dT) and random hexamer primining with superscript reverse transcriptase

(Invitrogen), *Eco*RI-adapted cDNAs were ligated to lZAP Express vector arms and packaged with Gigapack III packaging extracts (Stratagene). In the end, an open reading frame of 2880 bp for the novel gene (Oocyte-G1), which encodes a putative 960-residue protein, was cloned. Northern blot analysis revealed the presence of the 2.9-kb Oocyte-G1 mRNA in ovary, lung, kidney and testis. Northern analysis of RNA from ovaries in vivo showed that Oocyte-G1 was expressed at moderate level at day10. Thereafter, at day 15, there was an increase in its expression, followed by a decline in ovaries at day 20 and in adults. However, Oocyte-G1 was undetected in ovaries at day 5. Furthermore, we studied production of Oocyte-G1 protein by using an antiserum recognizing a peptide sequence unique to this gene in Western blotting and in immunolocalization. Signal was detected in oocytes and most granulose cells. The antiserum recognized a single prominent band of approximately 110 kDa in immunoblots. Taken together, the results suggest that Oocyte-G1 might play a critical role in oocyte development.

***Keywords*:** Oocyte-G1; ovarian follicle; Oocyte development; granulose cell

Correspondence: Ji Wu
E-mail: jiwu@sjtu.edu.cn

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Construction and expression of *HBsAg* in alga

Jie Sun[1], Yahui Wei[1], Ning Zou[2]
[1]Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, Northwest University, Xi'an, China; [2]College of Life Science, Ludong University, Yantai, China

To express *HBsAg* gene in alga, the experiment compared different intimidate preliminary treatments and different electrofusion condition under eukaryotic cells mode, bacteria yeasts mode and cell fusion mode. We find that double simple cell alga *Dunaliella Salina* and *Chlorella* can show higher GUS expression after 800 pulse voltage, 0.05 s pulse duration and 99 total number of pulse under eukaryotic cells mode or even after 1200V~1600V electro oration under bacteria and yeast mode, salt intimidate and low temperature can increase the expression in *Chlorella*, but it's inapplicable in *Dunaliella Salina*. In the experiment, we electrofusion alga with higher plant express vector pCAMBIA1301/HB with contain HBsAg gene of HBV, then co-cultured with hygromycin, the antibiotic smaller than 25 mg/L there will be no choice between transgenic and common *Chlorella* alga, two or three days separateness before the co-culture is in need, this also inapplicable in *Dunaliella Salina*. With the improved CTAB technique, high quality transgenic alga DNA can be distilled and the hole of *HBsAg* gene can be cloned from it by PCR.

***Keywords*:** alga; *HBsAg*; electrofusion; plant expressive vector

Correspondence: Yahui Wei
E-mail: weiyahuicn@yahoo.com.cn

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Molecular cloning and construction of plant expressive vector of porcine interferon-α

Jie Sun[1], Yahui Wei[1], Ning Zou[2]
[1]Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, Northwest University, Xi'an, China; [2]College of Life Science, Ludong University, Yantai, China

Clone and sequence porcine interferon-α (INF-α) gene, construct efficient plant expressive vector of porcine interferon. Designed primers according to DQ248997 of NCBI. Amplified the target porcine INF-α gene by PCR protocol from the porcine genome DNA, then cloned into pBS-T Vector and sequenced. Constructed plant expressive vector of porcine INF-α. The gene cloned in this experiment is compared by Blastn, 98 % nucleic acids are the same , 98% proteins are the same, three no function amino acids differ from the sequence in GeneBank. Confered that it is a subunit model of porcine interferon-α. Both of the two plant expressive vectors could be digested by *Bam*H I/*Sac* I restriction enzymes and got the target fragment (570bp). Successfully cloned the porcine interferon-α gene, and constructed two efficient plant expressive vectors pBI121/INF and pCAMBIA1301/INF of porcine interferon-α.

***Keywords*:** interferon-α; molecular cloning; Kozak sequence; plant expressive vector

Correspondence: Yahui Wei
E-mail: weiyahuicn@yahoo.com.cn

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Study of cell proliferation regulation of endothelial cells and smooth muscle cells with simiao yongan decoction *in vitro*

Junping Zhang[1], Jun Wang[2]
[1]Tianjin Key Laboratory of Pharmacology of Traditional Chinese Medicine, [2]Tianjin University of Traditional Chinese Medicine, Tianjin, China

The ischemic diseases such as atherosclerosis of advanced stage and complications of diabetes mellitus are the serious threatens for human health as well as the main reason of the raising high mortality and high disability rate in our country. After the definition of "remedial angiogenesis" is

introduced, it has been widely used in the ischemic diseases. Theoretical mechanism of traditional Chinese medicine on ischemic diseases can be explained by the theory of "collateral disease". The study of Chinese herb inducing angiogenesis is supported by TCM theory of "activating blood to eliminate stasis" and "invigorating meridian and generating blood". Simiao yongan decoction (SMYA) was originally used for gangrene and we chose this decoction as the intervention to discover its targets and underlying molecular mechanisms. Both proliferation of ECV304 and inhibition of vascular smooth muscle cells (VSMC) are substantial courses during angiogenesis. Consequently, we selected the cell line—ECV304 and primarily cultured VSMC as cell models to investigate the regulatory effect on proliferation of SMYA and its compounds—ferulic acid and chlorogenic acid by observing different index. BrdU-ELISA was used for checkign DNA synthesis of ECV304 and VSMC when cultured in the DMEM/F12 medium that contained SMYA serum, ferulic acid, chlorogenic acid and in the DMEM/F12 medium that contained SMYA serum respectively. Then the cell cycles of ECV304 were analyzed by using flow cytometer(FCM). VEGF mRNA and Cyclin D mRNA were examined by reverse transcription polymerase chain reaction (RT-PCR). Compared with the normal control group, serum contained SMYA group stimulated the DNA synthesis of ECV304. The percentage of ECV304 cells in S phase for the drug group increased, while mRNA expression of cyclin D, PCNA and VEGF was not changed obviously. Compared with the normal control group, both ferulic acid group and chlorogenic acid group promoted the proliferation of ECV304 cells. Ferulic acid stimulated ECV304 proliferation by means of the VEGF mRNA enhancement, but it had no effect on mRNA expression of PCNA and Cyclin D. Chlorogenic acid stimulated ECV304 proliferation by means of Cyclin D mRNA enhancement without changes in mRNA expression of PCNA and VEGF. Compared with the normal control group, serum that contained 10% or 20% SMYA groups showed no inhibitory effect on VSMC proliferation.

Keywords**:** proliferation; endothelial; smooth muscle; simiao yongan decoction; regulation

Correspondence: Junping Zhang
E-mail: junpingzhang@hotmail.com

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Characterization of human cytoglobin gene promoter region

Xiumei Guo[1], Sjaak Philipsen[3], Kian-Cheng Tan-Un[1, 2]
[1]Department of Zoology, [2]Department of Zoology, School of Professional and Continuing Education, The University of Hong Kong, Hong Kong SAR, China; [3]Department of Cell Biology, Erasmus MC, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

Cytoglobin (CYGB) is a member of the vertebrate globin family together with hemoglobin, myoglobin and neuroglobin. Although the physiological function of CYGB is still unclear, spectroscopic studies show that CYGB contains a hexacordinated heme iron pocket similar to other pentacoordinated globin proteins. CYGB shares a common phylogenetic ancestry with vertebrate myoglobin from which it diverged by duplication before the appearance of jawed vertebrates. The objective of this study is to identify the regulatory and promoter region of the human CYGB gene as well as to unravel the underlying molecular mechanisms that regulate human CYGB expression. 5' unidirectional deletion constructs demonstrated that the proximal promoter elements of human CYGB gene are located between -1113 to -10 relative to the translation start site. Site-directed mutagenesis showed that mutation of a c-Ets-1 motif at -1008 and Sp1 motifs at -400, -230 and -210 remarkably decreased the promoter activity. Gel shift assays confirmed the binding of DNA-nuclear proteins to these motifs. All these results indicate that CYGB gene expression can be up-regulated by c-Ets-1 and Sp1 motifs. For hypoxia study, cells were cultured in a humidified 1% O2, 5% CO2, 94% N2 incubator for the indicated time. Total RNA samples were prepared from Hela and BEAS-2B cell lines exposure to hypoxia for 3hr, 6hr, 12 hr and 24 hr. During hypoxia, the expression of endogenous CYGB was up-regulated to a maximum of 1.73 and 1.65 folds in BEAS-2B and HeLa cells. We also analyzed the effects of hypoxia on CYGB promoter activity in transient transfection assays by site directed mutagenesis. Our results showed that the CYGB promoter activity increased 72.4% and 57.7% respectively upon exposure of the BEAS-2B and HeLa cells to hypoxia compared with normoxia. These results clearly indicate that under hypoxia conditions the regulation of CYGB expression is dependent on hypoxia response elements.

Keywords**:** cytoglobin (CYGB); promoter region; transcription factor; c-Ets-1; Sp1; HRE; hypoxia

Correspondence: Kian-Cheng Tan-Un
E-mail: kctanun@hkucc.hku.hk

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HDAC6 modulates cell motility by altering the acetylation level of cortactin

Xiaohong Zhang[1], Zhigang Yuan[1], Sarah Yong[1], John Koomen[1], J Thomas Parsons[2], Sharon R Dent[3], Tso-Pang Yao[4], William S Lane[5], Edward Seto[1]
[1]Molecular Oncology Program, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL 33612 USA; [2]Department of

Microbiology, Health Sciences Center, University of Virginia, Charlottesville, VA 22903 USA; [3]Department of Biochemistry and Molecular Biology, M.D. Anderson Cancer Center, Houston, TX 77030 USA; [4]Department of Pharmacology and Cancer Biology, Duke University, Durham, NC 27710 USA; [5]Microchemistry and Proteomics Analysis Facility, Harvard University, Cambridge, MA 02138 USA

Histone deacetylase 6 (HDAC6) is a tubulin-specific deacetylase that regulates microtubule-dependent cell movement. In this study, we identify the F-actin-binding protein, cortactin, as a novel HDAC6 substrate. We demonstrate that HDAC6 binds cortactin and that overexpression of HDAC6 leads to hypoacetylation of cortactin, while inhibition of HDAC6 activity leads to its hyperacetylation. By modulating a "charge patch" in its repeat region, HDAC6 alters the ability of cortactin to bind F-actin. Interestingly, the introduction of charge-preserving or charge-neutralizing mutations in this cortactin repeat region correlates with the gain or loss, respectively, of F-actin binding ability. In addition, cells expressing a charge-neutralizing cortactin mutant were less motile than control cells or cells expressing a charge-preserving mutant. Furthermore, the charge-neutralizing cortactin mutant protein did not translocate to membrane ruffles in cells expressing active Rac1. These findings suggest that, in addition to its role in microtubule-dependent cell motility, HDAC6 influences actin-dependent cell motility by altering the acetylation status of cortactin, which, in turn, changes the localization and F-actin binding activity of cortactin.

***Keywords*:** histone deacetylase; HDAC6; protein acetylation; cortactin

Correspondence: Edward Seto
E-mail: setoe@moffitt.usf.edu

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Catalase gene expression in rice (*Oryza sativa*, indica cv. Ratna)

Ardhendu Kumar Dash[1], Prosenjit Mondal[1],
Manoranjan Kar[2], Surendra Chandra Sabat[1]
[1]Plant Biotechlogy Division, Institute of Life Sciences, Bhubaneswar, India; [2]Department of Botany, Utkal University, Bhubaneswar, India

Catalase is a ubiquitous enzyme in aerobic organisms. In majority of plant species the enzyme is encoded by a small multigene family. In rice, so far three catalase genes, named as Cat A, Cat B and Cat C have been identified and shown to be expressed differentially during the ontogenic development of the plant. We have studied the relative expression of these three genes in embryonic stages of seedling, in one of the Indica rice cultivar (Ratna). The studies were performed to ascertain the effect of low intensity of light in their expression pattern. The study was further extended to identify the relative expression of these three genes in laboratory grown matured rice plant. Our experimental results indicated that while Cat A and Cat B transcript levels are abundantly expressed at embryonic stages, Cat C expression is more confined to the matured leaf and leaf-sheath in fully matured plant. The abundance of Cat C transcript accumulation was found to be enhanced with enhanced accumulation of chlorophyll pigments. The effective expression of Cat C transcript in matured leaf, including its enhanced transcript expression that parallels with the increased greening of the leaf, suggests that the isoforms of Cat C product may essentially regulate the level of photorespiratory evolved hydrogen peroxide. In addition, the Cat C translation product was found to bear a nuclear translocation signal (NLS), not reported in catalase cloned and sequenced from other plant sources.

***Keywords*:** catalase gene; hydrogen peroxide; light effect; rice; photorespiration

Correspondence: Surendra Chandra Sabat
E-mail: scsabat@yahoo.com

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Characterization and expression of amphiApoD encoding a apolipoprotein D from amphioxus Branchiostoma belcheri-tsingtauense

Lei Wang[1], Shicui Zhang[2], Yongjun Wang[2], Zhenhui Liu[2]
[1]College of life Sciences, Lu Dong University; [2]Department of Marine Biology, Ocean University of China

An amphioxus cDNA, AmphiApoD, encoding apolipoprotein D was isolated from the gut cDNA library of Branchiostoma belcheri tsingtauense. Phylogenetic analysis places AmphiApoD at the base of the phylogenetic tree, suggesting that AmphiApoD is the archetype of the vertebrate ApoD genes. *In situ* hybridization revealed that AmphiApoD was expressed only in the hind-gut, notochord and testis of adult amphioxus, not in the neural tissues. Semi-quantitative RT-PCR showed that the expression of AmphiApoD was regulated by steroid and sex hormone. The AmphiApoD mRNA level was down-regulated by exposure to β-estradiol, while its expression in the same tissue was up-regulated by incubating with methyltestosterone or dexamethasone. This suggests that AmphiApoD acts as a lipid- or steroid hormone-binding protein in amphioxus.

Keywords: AmphiApoD; Apolipoprotein D; amphioxus; expression; regulation

Correspondence: Lei Wang
E-mail: wanglei9909@163.com

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A liver-derived lipid activity induces *PEPCK* transcripts in primary hepatocytes

Guoxun Chen
Department of Nutrition, The University of Tennessee, Knoxville, Tennessee, USA

Hepatic expression of phosphoenolpyruvate carboxyl kinase (PEPCK) plays a key role in gluconeogenesis. Elevation of *PEPCK* gene transcripts has been observed in livers of obese and insulin resistant animals. Here I used quantitative Real-Time PCR (RT-PCR) to determine the effects of endogenous lipids from rat livers on levels of *PEPCK* gene transcripts in isolated rat hepatocytes. My data demonstrated that *PEPCK* gene transcripts were increased 5.7±3.3 fold by treatment with 80 mg/ml of lipid extracts in the absence of insulin. Although this induction was inhibited in the presence insulin (100nM), levels of PEPCK gene transcripts remained higher in lipid-treated hepatocytes (0.46±0.4) than in mock-treated hepatocytes (0.07±0.04, $P<0.03$). In addition, the fold induction of PEPCK gene transcripts by treatment with the lipid extracts remains comparable (6.6±2.6) regardless of insulin's presence or not. Insulin dosage curve showed that treating hepatocytes with 0.1 nM or 1 nM of insulin alone decreased PEPCK transcripts by 89% or 95%, respectively. The same treatment in the presence of lipid extracts only decreased PEPCK transcripts by 69% or 79%, respectively. This lipid activity can be further enriched by conventional lipid separation methods. I conclude that a bio-molecular derived from endogenous lipid of rat livers can induce PEPCK transcript levels and attenuates insulin-mediated reduction of PEPCK transcripts in primary.

***Keywords*:** hepatocyte; *PEPCK*; insulin; RT-PCR

Correspondence: Guoxun Chen
E-mail: guoxunc@yahoo.com

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Development of super host cells for newcastle disease viral vaccine production

Myung Hee Kim, Juno Jang, Ik Hwan Kim
College of Life Sciences and Biotechnology, Korea University, Seoul, Korea

Newcastle disease virus (NDV) is the etiological agent of Newcastle disease, which causes severe losses in domestic poultry production. To develop a process that produces NDV via animal cell culture, MDBK cell line was selected and modified genetically. Transformed animal cells convert large amounts of glucose to lactate through glycolysis. Only a small part of glucose-derived carbon enters the tricarboxylic acid (TCA) cycle. The cell culture medium is acidified by the lactate secretion and is not adequate for cell survival and production of NDV. To overcome this problem, the expression level of lactate dehydrogenase (LDH), converting pyruvate to lactate, was decreased by siRNA. The LDH knock down mutants were selected via western blotting and measuring their LDH activity. Viral infection generates ROS and induces oxidative stress. The oxidative stress can induce viral pathogenesis and apoptosis of the host cell. The overexpression of superoxide dismutase (SOD) is introduced to decrease the ROS level against the virus infection. The physiological properties and capability of NDV production of these mutants will be presented.

***Keywords*:** newcastle disease virus; lactate dehydrogenase; superoxide dismutase; viral vaccine ; super host cell

Correspondence: Ik Hwan Kim
E-mail: maenge7@korea.ac.kr

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Expression of actin and tubulin genes in differentiating C6 glioma cells

Ping Wang[1,2], Takeshi Onuma[2], Naoko Birukawa[2], Masashi Abe[2], Jicheng Li[1], Akihisa Urano[2]
[1]Institute of Cell Biology, Zhejiang University, Hangzhou 310031, China; [2]Division of Biological Science, Graduate School of Life Science, Hokkaido University, Sapporo 060-0810, Japan

Expression of genes encoding cytoskeletons is involved in the regulation of cell morphology and differentiation. However, little is known about the cytoskeletal gene expression during the morphogenesis of C6 glioma cell differentiation. Here we analysed changes of gene expression for actin and tubulin isoforms and cytoskeleton arrangements in C6 cells following cAMP-induced differentiation. Amounts of mRNAs for actins and tubulins were determined by quantitative real-time polymerase chain reaction (QRT-PCR) in C6 cells which treated with low concentration of fetus bovine serum (FBS) and 1mM dibutyryl cAMP (dbcAMP). These treatments induced morphological changes which were accompanied by massive and distinct rearrangements of actin filaments and microtubules. Whereas, expression of cytoskeletal genes (beta-actin, gamma-actin and alpha-tubulin) was suppressed during the morphological changes. Among cytoskeletal mRNAs, the amounts of gamma-actin mRNA significantly decreased at 30 min after the treatment, and then alpha-tubulin mRNA decreased at later stage of differentiation. The amount of beta-actin mRNA was gradually down-regulated during the process. Thus, expression of cytoskeletal genes assessed by the changes in mRNA amounts was down-regulated during cAMP-induced dif-

ferentiation of C6 glioma cells. Our present results suggest that multiple signaling pathways regulate molecular and morphological changes in differentiating glial cells in the nervous system.

***Keyword*:** dibutyryl cAMP; differentiation,; cytoarchitecture; gene expression; quantitative real-time PCR

Correspondence: Wang Ping
E-mail: pinoav@hotmail.com

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Coding region analysis of the RET, EDNRB, EDN3 genes and polymorphism analysis of SNPs in MCS+9.7 in Chinese patients with isolated Hirschsprung disease

Miao-Ni Zhou, Xian-Ning Zhang, Tao Guan, Shi-Ping Ding, Ji-Cheng Li
Department of Lymphology, Institute of Cell Biology, Medical College of Zhejiang University, Hangzhou 310058, China

Hirschsprung's disease (HSCR) is a frequent congenital malformation regarded as a multigenic neurocristopathy. To elucidate the molecular genetic mechanism of HSCR in Chinese population, the coding regions of RET, EDNRB and EDN3 were analysed in 80 sporadic non-syndromic HSCR patients by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and DNA direct sequencing. Otherwise, to determine if polymorphic loci of RET could act as low penetrance predisposing allele, we examined allelic frequencies at single nucleotide polymorphic loci within MCS+9.7 in intron 1 of RET, RET+3, rs2506005 and rs2506004, in 99 isolated Chinese HSCR patients by PCR-direct sequencing. Whether allele frequency differed from that in the control population was determined by either chi-squared analysis or Fisher's exact test. In the results, five patterns of variants, G15165A in exon 11, A18919G, T18888G and 18974insG in exon 13 and G20692A in exon 15 were identified in RET proto-oncogene, the frequency was 10% (8/80); two variants, G831A in exon 4 and G553A in exon 2 were found in EDNRB gene, the incidence was 17.5% (14/80); no genetic variations were observed in EDN3 gene. In addition, two HSCR families associating with RET were collected in this study and the genotypes of probands were 18974insG and T18888→G, respectively. As for the RET+3 locus, this polymorphic allele was over-represented among HSCR cases versus controls (P<0.0001). And rs2506005 and rs2506004 were complete linkage disequilibrium with RET+3. Our researches provide further support for an important role of RET and EDNRB genes in the HSCR phenotype and elucidate that polymorphisms in the RET appear to predispose to HSCR in a complex, low penetrance fashion and may also modify phenotypic expression.

***Keywords*:** Hirschsprung's disease; RET proto-oncogene; endothelin B receptor gene; endothelin-3 gene; polymorphism

Correspondence: Jicheng Li
E-mail: lijichen@zju.edu.cn

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Expression of scavenger receptor BI in J774 cells reduces transcription of some genes related to inflammation

Jingtao Feng, Zhongcheng Mo, Lin Sun, Dexing Zeng, Junhui Xia, Jing Wang, Guanghui Yi
Institute of Cardiovascular Disease, Nanhua University, Hengyang 421001, China

Previous studies have shown that scavenger receptor BI (SR-BI) deficiency led to a significant induction of genes implicated in adhesion and transendothelial migration of monocytes. In the present study, the impact of SR-BI on genes related to inflammation was investigated in J774 cells that express low basal levels of SR-BI. METHODS: J774 cells were transiently transfected to express a full-length human SR-BI cDNA in pcDNA 3.1(-), and the expression of an 82 kDa protein, human SR-BI, were detected by immunoblot analysis. The expression of genes related to inflammation such as monocyte chemoattractant protein MCP-1, matrix metalloproteinase MMP-9 and tumor necrosis factor (TNF) α in J774 cells transfected with human SR-BI or vector were analysed by RT-PCR. RESULTS: The genes expression of MCP-1, MMP-9 and TNF-α is down-regulated (P<0.05) in J774 cells transfected with human SR-BI relative with vector. CONCLUSION: Expression of human SR-BI in J774 cells can reduce the genes expression of MCP-1, MMP-9 and TNF-α. So, the protective role of SR-BI expressed in macrophages is not only related with its functions in cholesterol trafficking, but also with its effect to down-regulate expression of some genes related to inflammation.

Keywords: Scavenger receptor BI; Gene expression; Transfection

Correspondence: Guanghui Yi
E-mail: ghyi@126.com