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Inhibition of the mitotic kinesin Eg5 induces mitotic arrest and apoptosis, and upregulates Hsp70 through the PI3K/Akt pathway in multiple myeloma cells

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The microtubule-dependent motor protein Eg5 plays a critical role in spindle assembly and maintenance in mitosis. Herein we show that suppression of Eg5 by a specific inhibitor arrests mitosis, induces apoptosis, and upregulates Hsp70 in human multiple myeloma cells. Mechanistically, Hsp70 induction occurs at the transcriptional level via a cis-regulatory DNA element in Hsp70 promoter and is mediated by the PI3K/Akt pathway. Eg5 inhibitor-mediated Hsp70 upregulation is cytoprotective, because blocking Hsp70 induction directly by antisense or small interfering RNA or indirectly by inhibiting the PI3K/Akt pathway significantly increases Eg5 inhibitor-induced apoptosis. Furthermore, a farnesyltransferase inhibitor interacts synergistically with the Eg5 inhibitor in inducing apoptosis through disrupting the Akt/Hsp70 signaling axis. These findings provide the first evidence for Eg5 inhibitor activity in hematologic malignancy and identify Hsp70 upregulation as a critical mechanism responsible for modulating myeloma cell sensitivity to Eg5 inhibitors. In addition, these findings suggest that combination of Eg5 inhibitors with agents abrogating Hsp70 induction is more useful for myeloma therapy in the clinic.

***Keywords*:** Eg5; mitosis; apoptosis; Hsp70; PI3K/Akt

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APOCB2006-04-002

Effect of hypotonic solution on the mitochondrial membrane potential and apoptosis in human ssteoblast-like MG63

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To investigate the involvement of mitochondrial membrane potential(ΔΨm) in the early response of human osteoblast-like MG63 to mechanical strain , and study the proliferation response to strain, ALP and apoptosis rate under different hypotonic solution in vitro. Methods: Mechanical strain was applied by swelling osteoblat-like cells in varied hypotonic solutions, such as 300mOsm, 277mOsm, 240mOsm and 163mOsm..Mitochondrial membrane potential(ΔΨm), cell apoptosis ratio, S stage percent, ALP and Ca^{2+} (concentration of endocellular calcium)were used to assay pre-apoptosis, and cell proliferation in following periods, such as 30 min, 2 h, 4 h, 6h, 12 h and 24 h. Results: Stretching MG63 osteoblast-like by swelling in hypotonic solution could rapidly alter mitochondria membrane potential, while the cell proliferation showed an distinct changes. But under the intense stretching , the mitochondrial membrane potential (ΔΨm) began to depress as well as the enhancement of apoptosis ratio. Laser Confocal Microscope imaging of mitochondria stained with JC-1, a trans-membrane potential-sensitive vital dye, showed that ratio of red/green decreased under 163mOsm and 240 mOsm after exposed to stretching for 2 h and 4 h respectively. The change of S stage percent was consistent with the changed ratio of red/green. With the prolongation of stretching time, the changes of ALP and Ca^{2+} just showed different values in varied condition. Conclusion: (1) The proper stretching may promote differentiation and proliferation of osteoblast cell, and ALP shows an upward trend; (2) The overstress inhibits proliferation of osteoblasts and induces apoptosis of osteoblast-like; (3) The effect of hypotonic solution on human osteoblast-like is similar to that of the dynamic mechanical loading on bone cells reported; (4) The mitochondrial membrane potential (ΔΨm) may be an early sensitivity index of response to mechanical strain, however, ALP and Ca^{2+} just only correspond to alter during the later period of stretching.

***Keywords*:** human osteoblast-like cell; hypotonic solution; mitochondrial membrane potential; proliferation; apoptosis

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Murine mesenchymal stem cells isolated by low density primary culture system keep differentiation potential up to passage 10

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Murine mesenchymal stem cells (mMSCs) and the difficult

task of isolation and purification of them have been the subject of rather extensive investigation. The present study sought to isolate these cells from two different strains one outbred and the other inbred mouse, primarily through a relatively simple but novel approach, the most important feature of which was the low density primary culture of bone marrow cells. For this purpose, mononuclear cells from either NMRI or Balb/c bone marrow were plated at about 500 cells per well of 24-well plates and incubated for 7 d. At this point, the fibroblastic clones that had emerged were pooled together and expanded through several subcultures. To investigate the mesenchymal nature, we differentiated the cells into the osteoblastic, chondrocytic and adipocytic lineages in different subcultures up to passage 10. In present investigation, the best culture condition for maximum proliferation and also the expression of certain surface marker on isolated cells were examined.Furthermore; the colonogenic potential of the cells was tested by Colony Forming Unit- Fibroblast Assay (CFU-F). According to the results, one week after culture initiation, several clones each comprising several fibroblastic cells appeared in each plate. The cells from different passages were capable of differentiating into corresponding skeletal tissues. The cell had maximum proliferation when being cultured at density of 100 cell/cm^2 in a DMEM medium containing 15% fetal calf serum (FCS).FACS analysis indicated that more than 90% of the cells are CD44+. Sca-1 was expressed in about 20% of the cells and C-kit, VCAM and CD34 were not detected. Almost 75 colon each consisting of 20-70 cells was formed per each 100 cell from either strain plated in 25 cm^2-flasks. Two murine strains showed some differences in term of their growth and surface antigens. Taken together, low density primary culture system seems to be an appropriate and simple way for the isolation and purification of fibroblastic cells from the heterogeneous mixture of bone marrow cells. The fact that the fibroblastic cells isolated through this approach were not only able to differentiate into osteoblasts, chondrocytes and adipocytes but also able to maintain this property even after being subjected to several rounds of subcultures added weight to the assumption that they were, indeed, the mesenchymal stem cells described elsewhere.

Keywords**:** low density primary culture; mesenchymal stem cells; proliferation; differentiation; Balb/c and NMRI strains

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Accumulation of p27kip1 is associated with RA-induced growth arrest and neuronal differentiation of human neural progenitor vells

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Neural transplantation is a promising strategy for of CNS injuries and neurodegenerative disorders. Human neural progenitor cells (hNPCs) are currently believed to have important potential for clinical application. However,the underlying molecular mechanism involved in cell cycle withdrawal and neuronal differentiation remains poorly defined. hNPCs can differentiate into neuronal phenotype following all-trans Retinoic acid (RA) treatment. In the present study, we demonstrate a functional link between RA and one of the Kip/Cip proteins p27Kip1 in the control of neuronal differentiation in hNPCs. Methods: The hNPCs were derived from embryonic ventral telencephlon and cultured in serum-free medium supplied with EGF and bFGF. When the cells are 80% confluent, hNPCs were exposed to RA (1 μM) for 1, 3, 5, 7 days respectively. The properties of hNPCs were determined by using flow cytometry analysis (FACS), immunocytochemistry, RT-PCR and Western Blot. Results: Cell cycle analysis performed by FACS showed that G0-G1 rate and apoptotic rate of hNPCs increased significantly after exposure to RA for 3 d. The result of immunocytochemistry, RT-PCR and Western Blot showed that the expression of p27Kip1 increased significantly following RA treatment, with a peak at 5 d, while the expression of p21Cip1 and skp2 decreased significantly following RA treatment. The expression of CDK2 and cyclin E didn't change significantly before and after RA treatment. Conclusion: Accumulation of p27kip1 is associated with RA-induced growth arrest and neuronal differentiation of human neural progenitor cells. Studies have demonstrated that p27Kip1 plays a key role during neuronal differentiation. Moreover, high levels of p27Kip1 are regulated via double mechanisms: (1) increased mRNA expression (2) inhibited degradation through the ubiquition-proteasome machinery.

Keywords**:** human neural progenitor cells; neuronal differentiation; p27kip1; RA

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Overexpression of *HmgD* sffected the hemocyte proliferation pathway in *Drosophila*

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The gene *HmgD* encodes the Drosophila homologue of high mobility group proteins, HMGD. This protein is similar to the HMGB subfamily of mammals structurally and functionally, both having the HMG-box motif and thought to be architectural factors in assembling chromatin. Some scientists indicated that HMGD induces a less compact chromatin structure in the very early embryo when histone H1 is absent, possibly facilitating the very quick cycling of nuclear divisions at that time. While the others suggested that HMGD might exert functions other than that of a specific early embryonic substitute of H1, since HMGD is expressed not only in adult females and early embryos but also during later developmental stages. Furthermore, the comparison of the DNA-binding capacity and specificity in recognition of DNA clearly showed that HMGD does not share the binding properties with histone H1. These implicate that HMGD may have other unknown functions than only play a general mitosis-related role during early embryogenesis. In order to further analyse the function of *HmgD* on the development, we have followed a gain-of-function research strategy using UAS-Gal4 system and generated UASPHmgD transgenic flies via embryonic microinjection. Our results showed that overexpression of *HmgD* under the control of a ubiquitous Gal4 protein (Act-Gal4) caused serious lethality. Especially, four copies of transgenic flies in this expression system resulted in 100% lethality of the progeny at the late third instar larval stage. Some of these mutant larvae died with the melanotic tumours. The investigation of larval blood cell indicated that the hemocyte number was increased dramatically in hemolymph isolated from the mutant larvae with respect to wild types. RT-PCR analysis demonstrated that Ras-MAPK pathway involved in the hemocyte proliferation was abnormally activated in the mutant flies. These results suggest that HMGD may not be essential for the overall organization of chromatin but be necessary for controlling proper transcriptional regulations.

***Keywords*:** *HmgD*; overexpression; hemocyte; *Drosophila*

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FAK-KLF8 signaling axis in normal and malignant cell growth

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Focal adhesion kinase (FAK) is an important component of cell adhesion and mitogenic receptors mediated signaling that regulates cell cycle, survival, motility, *etc*. FAK also plays a critical role in malignant transformation and tumor formation, invasion and metastasis which is well correlated with its aberrant elevation in many types of cancers. We have recently identified Kruppel-like factor 8 (KLF8), a member of the family of KLF transcription factors, as a novel FAK downstream effector. We demonstrate that KLF8 mediates cell cycle progression by directly regulating the transcription of the key G1 checkpoint regulators such as cyclin D1. Furthermore, we show that KLF8 expression is tightly regulated at both the message and protein levels. Finally, we provide strong evidence suggesting a potentially significant role of KLF8 in oncogenic transformation and tumor progression.

***Keywords*:** FAK; KLF8; cell cycle; transformation; cancer

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Mammalian NudC-like protein, a novel dynein regulator, is essential for cell cycle progression

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Cytoplasmic dynein, a minus-end-directed microtubule motor, has been implicated in many cellular fundamental processes; however, little is known about the underlying molecular machinery that regulates its stability. In Aspergillus nidulans, nuclear distribution gene C (*nudC*) has been implicated in the regulation of dynein-mediated nuclear migration. Here, we characterize a novel mammalian NudC-like protein (NudCL). The expression and phosphorylation of NudCL are increased during mitosis. Depletion of NudCL by RNA interference in randomly growing or well-synchronized HeLa cells inhibits cell growth and induces mitotic arrest and multiple mitotic defects, which subsequently result in cell death. Unexpectedly, the majority of NudCL depletioninduced mitotic defects may result from loss of dynein function, which was

supported by the failure to recruit sufficient gamma-tubulin to spindle poles and the mislocalization of dynein complex at kinetochores, spindle microtubules and spindle poles during mitosis. Depletion of NudCL also results in the aggregation of dynein intermediate chain throughout the cytoplasm during mitosis. NudCL was shown to bind to the dynein complex, and its depletion induces the degradation of dynein intermediate chain. Furthermore, the degradation of dynein intermediate chain is blocked by MG132, a proteasome inhibitor. Taken together, these data suggest that a novel mechanism that NudCL appears to influence the dynein's stabilization.

***Keywords*:** dynein; cell cycle; mitosis; *NudC*; cell migration

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A novel butyrolactone derivative inhibited apoptosis and depressed integrin β4 expression in vascular endothelial cells

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To understand the effects of a novel butyrolactone derivative, 3-benzyl-5- ((2-nitrophenoxy) methyl)-dihydrofuran-2(3H)-one (3BDO), on the apoptosis of vascular endothelial cells (VEC), we exposed 3BDO (20-60 μg/ml) to VECs deprived of serum and FGF-2 for 24 h and 48 h respectively. The results showed that 3BDO (20-60 μg/ml) increased VEC viability and inhibited VEC apoptosis induced by deprivation of serum and FGF-2 in a dose-dependent manner. During this process, integrin β4 expression was depressed, but the level of reactive oxygen species (ROS) was not changed. The data suggested that 3BDO (20-60 μg/ml) could inhibit VEC apoptosis and suppress integrin β4 expression, but it could not depress the ROS level induced by deprivation of serum and FGF-2.

***Keywords*:** 3-benzyl-5-((2-nitrophenoxy) methyl)-dihydrofuran-2(3H)-one ; apoptosis; vascular endothelial cell; integrin β4; reactive oxygen species

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Functional genomic screens identified novel cell cycle regulators required for chromosome segregation and cytokinesis

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We have developed efficient functional screens for novel mitotic regulators by combining information on the gene expression profiling, cellular localization and loss-of-function studies. Our genomic analysis was based on two predictions. First, we predicted that a subset of genes induced in G2/M is likely to function in mitosis and cytokinesis. Second, we predicted that expression of genes in the core cell cycle machinery tends to co-vary during tumorigenesis, as these regulators should function as one module during tumor proliferation. Through transcriptional profiling, we identified a core group of novel cell cycle regulators using their co-variation pattern with known cell cycle genes in hundreds of tumor tissues and further defined their functional specificity to mitosis/cytokinesis based on their induction in G2/M. The physiological activities of these novel genes were analyzed as follow. First, when fused to GFP, candidate proteins with localization on mitotic and cytokinesis structures were selected for further analysis. Second, RNA interference experiments were performed for candidate genes to assay for mitotic/cytokinesis defects in knockdown cells. This approach has been applied to 40 candidate genes with best G2/M induction profiles and has led to the identification of multiple novel spindle-associated proteins essential for mitosis and cytokinesis. I will present our data on the function of these mitotic regulators. For example, we found that one of the identified proteins, hepatoma up-regulated protein (HURP), is required for chromosome congression and alignment. In HURP-depleted metaphase cells, the persistence of unaligned chromosomes and the reduction of tension across sister kinetochores resulted in the activation of the spindle checkpoint. Although these defects transiently delayed mitotic progression, HeLa cells initiated anaphase without resolution of these deficiencies. Furthermore, we showed that HURP is not a checkpoint protein and that this checkpoint bypass is an intrinsic property of tumor cells independent of HURP. Thus, this bypass of the checkpoint arrest provides a mechanism for aneuploidy and genomic instability in tumor cells. The lack of HURP in knockdown cells generates unaligned chromosomes which leads to tumor-specific chromosome mis-segregation due to this bypass mechanism in tumor cells. At the cellular level, HURP co-localized with the mitotic spindle in a concentration gradient increasing toward the chromosomes. HURP binds directly to MTs in

vitro and enhances their polymerization. *In vivo*, HURP stabilizes mitotic MTs, promotes MT polymerization and bipolar spindle formation, and decreases the turnover rate of the mitotic spindle. Thus, HURP controls spindle stability and dynamics to achieve efficient kinetochore capture at prometaphase, timely chromosome congression to the metaphase plate, and proper inter-kinetochore tension for anaphase initiation.

***Keywords*:** aneuploidy; functional genomics; genomic instability; HURP; mitosis

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Drosophila initiator caspase DRONC activates JNK signaling to induce compensatory proliferation

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Many animal tissues are capable of regeneration. Such tissues can maintain a proper size by "compensatory proliferation", even afer the tissue is damaged and many of the cells are lost by apoptosis. The genetically tractable *Drosophila* imaginal disc is a good model system to study the molecular mechanisms underlying this process. Previous studies have shown that, in the *Drosophila* imaginal disc, it is the apoptotic cells themselves that induce compensatory proliferation. Apoptotic cells secrete mitogens, such as Decapentaplegic (DPP), before they are cleared by macrophages. Here we provide genetic evidence that the initiator caspase DRONC and the JNK signaling pathway are required for compensatory proliferation. Genetic epistasis analysis places JNK signaling downstream of DRONC. Our results demonstrate that the apoptotic pathway bifurcates at DRONC, one branch leading to apoptosis of the cell, the other branch leading to non cell-autonomous induction of compensatory proliferation through activation of JNK signaling.

***Keywords*:** apoptosis; caspase; JNK; *Drosophila*

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NEK2A regulates the interaction of Hec1 with CENP-H and the attachment of spindle microtubules to the kinetochore

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Chromosome segregation in mitosis is orchestrated by the interaction of the kinetochore with spindle microtubules. Our recent study shows that NEK2A interacts with MAD1 at the kinetochore and possibly functions as a novel integrator of the spindle checkpoint signaling. However, it is unclear how NEK2A regulates kinetochore-microtubule attachment in mitosis. Here, we report that NEK2A modulates the interaction of Hec1 with CENP-H and the attachment of spindle microtubules to the kinetochore during mitosis. Hec1 interacts with CENP-H *in vitro* and *in vivo* via extended coiled-coil domains located in the middle of the two molecules, respectively. Our immunofluorescence study revealed that Hec1 is localized at the metaphase kinetochore exterior to CENP-H with the globular domain oriented toward microtubule plus end. NEK2A phosphorylates Hec1 at Ser165 during mitosis while such phosphorylation regulates Hec1-CENP-H interaction *in vitro* and *in vivo*. Interestingly, phosphorylation of Ser165 is not essential for assembly of Hec1 to kinetochore as non-phosphorylatable mutant Hec1S165A is localized to the kinetochore and remains associated with kinetochore microtubules. However, there was a significant increase in attachment errors, including syntelic attachment and monotelic attachment. In addition, these Hec1S165A–overexpressing cells display a chromosome-bridge phenotype with sister chromatids inter-connected. These findings reveal a key role for the NEK2A-mediated phosphorylation of Hec1 in governing proper kinetochore-microtubule attachment and support a notion by which NEK2A integrates kinetochore-microtubule attachment into spindle checkpoint signaling.

***Keywords*:** mitosis; kinetochore; spindle; CENP-H; Hec1

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Molecular dissection of mammalian kinetochore proteome identifies a novel CENP-E-interacting protein, CENP-V, essential for mitotic checkpoint

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CENP-E is a kinesin-related microtubule motor protein that is essential for chromosome congression during mitosis. Our previous studies show that microtubule motor

CENP-E represents a link between attachment of spindle microtubules and the mitotic checkpoint signaling cascade. However, the molecular mechanism underlying CENP-E-mediated spindle checkpoint signaling remains elusive. To identify the proteins that participate in the CENP-E-mediated spindle checkpoint signaling, we carried out proteomic search for kinetochore proteins associated with CENP-E in mitotic cells. To this end, mitotic extracts from HeLa cells stably expressing FLAG-CENP-E were purified using a FLAG antibody-affinity chromatography. The bound proteins were eluted with the FLAG peptide and fractionated on SDS-PAGE. For identification of proteins selectively bound to CENP-E, SDS-PAGE separated bands were removed from the acrylamide gel, digested in-gel by trypsin, and the resulting peptide fragments were extracted and analyzed by MALDI-TOF mass spectrometry. BubR1, a well-known component of CENP-E complex, was retained on the affinity matrix, consistent with our previous study. In addition, a 59 kDa protein band was identified with unknown function. Our immunofluorescence microscopic analysis shows that this 59 kDa protein co-distributes with CENP-E to the kinetochore of HeLa cells during early mitosis and departs from kinetochore upon the metaphase alignment. We therefore designated it as CENP-V. To characterize the ultrastructural localization of CENP-V, we carried out immunoelectron microscopic analyses. CENP-V targets to the outermost region of the developing kinetochores of monoorient chromosome upon the nuclear envelope breakdown. After stable attachment, throughout chromosome congression, CENP-V is a constituent of the corona fibers, extending up to 90 nm away from the kinetochore outer plate. Concomitant with metaphase alignment, CENP-V departs from the kinetochore and migrates toward the centrosomes. To characterize the interaction between CENP-V and CENP-E, we performed yeast two hybrid assay that the C-terminal tail of CENP-E binds to the N-terminal 200 amino acids of CENP-V. Pull-down experiment using bacterial recombinant CENP-E and CENP-V proteins confirmed the yeast assay. To elucidate the functional relevance of CENP-V, we suppress the synthesis of CENP-V using siRNA. Depletion of CENP-V abrogates the localization of CENP-E to the kinetochore and results in chromosome mis-segregation. In addition, elimination of CENP-V reduces the tension across the sister kinetochore, suggesting that CENP-V links spindle microtubules to kinetochore. Real-time analyses show that elimination of CENP-V caused a substantial mitotic arrest. Taken together, our studies strongly support a model in which CENP-V functions in spindle checkpoint signaling cascades at kinetochore.

Keywords**:** mitosis; mitotic checkpoint; kinetochore; CENP-E; CENP-V

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Molecular elucidation of Cdc14A runction in mitosis

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Chromosome segregation in mitosis is orchestrated by protein kinase and phosphatase signaling cascades. Previous studies showed that overexpression of human phosphatase hCdc14A, an antagonist of CDK1, affects several aspects of cell division. However, the molecular mechanism underlying this regulation has remained elusive. To elucidate the molecular mechanism underlying Cdc14A functions in mitosis, we expressed various deletion mutants of Cdc14A in HeLa cells and found that over-expression of N-terminal catalytic domain of hCdc14A prevents cells enter into mitosis with a prolonged arrest in G2 phase, consistent with a role of Cdc14 in inhibiting Cdk1 activity. Our biochemical characterization indicates that Cdc14A forms an intra-molecular association which inhibits its phosphatase activity. To identify the factor(s) that regulate Cdc14A activity, we performed a yeast 2-hybrid assay and identified a novel Cdc14A-interacting protein kinase. Interestingly, this kinase interacts with and phosphorylates Cdc14A, which releases Cdc14A intra-molecular association and activates its phosphatase activity. To examine the functional relevance of such phosphor-regulation of Cdc14A, phospho-mimicking mutant of Cdc14A was expressed in HeLa cells. Importantly, over-expression of the phospho-mimicking mutant caused aberrant chromosome alignment with a pro-metaphase delay, suggesting the temporal order of Cdc14A phosphorylation is critical for orchestrating mitotic events. Currently, we employ biosensor to monitor the spatio-temporal profile of Cdc14A activation and illustrate how the kinase-phosphatase interplay orchestrates mitotic exit.

Keywords**:** mitosis; kinase; phosphatase; Cdc14A; Cdk1

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Chromosome alignment and segregation regulated by ubiquitination of survivin

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Mitosis is a remarkably dynamic cellular process, requiring a continuously assembly and disassembly of many protein complexes. We are interested in the chromosome passenger complex containing Survivin and the kinase Aurora B regulates the attachment of sister kinetochores to microtubules from opposite spindle poles to form bi-oriented chromosomes on the metaphase spindle. By identifying the Survivin-interacting proteins, we found that a de-ubiquitinating enzyme, hFAM, regulates chromosome alignment and segregation by controlling both the dynamic association of Survivin with centromeres and the proper targeting of Survivin and Aurora B to centromeres. Survivin is ubiquitinated in mitosis through both Lys48 and Lys63 ubiquitin linkages. Lys63 de-ubiquitination mediated by hFAM is required for the dissociation of Survivin from centromeres, whereas Lys63 ubiquitination mediated by the ubiquitin binding protein Ufd1 is required for the association of Survivin with centromeres. Thus, ubiquitination regulates dynamic protein-protein interactions and chromosome segregation independently of protein degradation.

***Keywords*:** kinetochore; ubiquitination; survivin; FAM; p97/Ufd1/Npl4

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Mitotic phosphorylation of Mis13 is required for kinetochore assembly and proper chromosome segregation

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Chromosome segregation in mitosis is orchestrated by dynamic interaction between spindle microtubules and the kinetochore, a multi-protein complex assembled onto centromeric DNA of the chromosome. Our previous studies show that kinetochore is composed of several interactive protein subcomplexes such as ZW10, Hec1 and CENP-E. However, the mitotic regulation of these protein-protein interaction networks has remained elusive. To identify the proteins that regulate Mis13 in mitosis, we carried out proteomic search for Mis13-interacting protein in mitotic cells. To this end, mitotic extracts from HeLa cells stably expressing FLAG-Mis13 were purified using a FLAG antibody-affinity chromatography. Among various known Mis13-binding proteins identified, Aurora B was found to be retained on Mis13 affinity beads. The Aurora B-Mis13 interaction was then confirmed with recombinant Mis13 and Aurora B proteins in test tubes. Significantly, Aurora B co-localizes with and phosphorylates Mis13 *in vivo*. This Aurora B-mediated phosphorylation of Mis13 was confirmed in vitro using recombinant proteins. Interestingly, suppression of Aurora B kinase protein by siRNA or kinase activity by inhibitors reduced the level of Mis13 associated with kinetochore. Expression of non-phosphorylatable Mis13 resulted in a delay in prometaphase-metaphase transition. Immunofluorescence analyses revealed that localization of the outer plate proteins such as CENP-E, CENP-F and Hec1 was severely reduced in the kinetochore expressing non-phosphorylatable Mis13. These results indicate that Aurora B-mediated phosphorylation of Mis13 plays an essential role in chromosome segregation and contributes to mitotic kinetochore assembly.

***Keywords*:** mitosis; kinetochore assembly; chromosome congression; Aurora B; Mis13

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Protein phosphatase 4 is a new member of centromere passenger

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Protein phosphatase 4, an important phosphatase of PP2A family，has been shown to associate with centrosome. Use of an antibody different from the one previously used made us accidentally observed the novel subcellular pool of PP4. In this pool, PP4 existed in the nucleus in interphase and translocated to centromere in prometaphase and maintained in centromere in metaphase. Then it disassociated from centromeres and translocated to the spindle midzone during anaphase and, finally, to the midbody in telophase. Also we used GFP as a tag combined with PP4RL (a phosphatase-dead mutant of PP4) to further confirmed the novel localization. To understand the multiple functions of the certain protein in cell cycle regulation, it is necessary to get an overall understanding of the cellular distribution of the protein. What we had found provided a necessary complementarity to get an overall understanding of the cellular distribution and function of the protein.

***Keywords*:** PP4; chromosome passenger protein; cytokinesis

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Primary discussion about the relationship between the change of the cell-cycle pathway and genesia of deficiency-cold syndrome

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This paper reports the experimental results and findings from the cDNA microarray test carried out for a family suffering from the Deficiency-Cold Syndrome (DCS).In order to investigate the molecular mechanism of the DCS and provide an objective molecular index for diagnoses and therapy in clinics, four part works were carried out: Firstly, a family including five normal members and seven patients suffering from DCS is selected. Secondly their accumulated clinical scores obtained from the 40-items clinical scoring was statisticed and their peripheral blood samples were tested for cDNA microarray with 18816 clones. Thirdly,Through comparing their gene expression profiles of mRNA, 179 differential expression genes were found, 38 genes are correlated to cell-cycle in them. Fourthly, biological pathways for these differential expression genes were explored by using the Pathway Comparison of BRB-Arrays, the result manifested that there are two pathways correlated to cell-cycle in the first 8 pathways which are significant at the 0.05 level of the LS permutation test or KS permutation test in the BioCarta Map. One pathway is Cell Cycle: G1/S Check Point, which includes 25 genes. The other is Regulation of p27 Phosphorylation during Cell Cycle Progression which has 12 genes. In the first pathway there are 6 differentially expressed genes, including 5 up-regulated genes (CDK2, CDK4, DHFR, CDKN1B, and TFDP1), and one down-regulated gene (TGFB1). There are another 2 up-regulated genes (EIB-AP5 and MGC1366) taking part in the regulation of the pathway. There are 4 genes differentially expressed in the secord pathway. CDK2, TFDP1 and CDKN1B are up-regulated, and TCEB1L is down-regulated. There are some reports supporting our results.for example ,the level of phosphorylation and electric transport is usually decreased in DCS, Perhaps the low phosphorylation effected the p27 phosphorylation, The results indicated that DCS is possibly associated with up--regulated genes within the cell-cycle: G1/S Check Point pathways and down-regulated genes in Regulation of p27 Phosphorylation passway. Besides, In direct comparing and indirect comparing of our test, there are some genes involved in PKC (PTPN13, PIP5K1A, DUSP3, DUSP8, PRKCA, TPTE, ITPR2) and MAPK (CASP6, DUSP3, DDIT3, FGFR3, PRKCA, DUSP8, NRAS, FOS, MAP2K2) signal pathway, which plays a key role in the regulation of cell cycle. The cell cycle transition from G1 to S phase is a key regulatory point in the cell cycle. This transition is regulated by the checkpoint kinase cdk2 that activates the G1 to S transition when it is associated with cyclin E. In addition, it is also regulated by other molecular or signal pathway, such as thyroid hormones, PKC, MAPK etc. Actually, thyroid hormones regulate the cell cycle via PKC and MAPK. In this test, above differentially expressed genes are all involved in cell cycle and the person with DCS is of low metabolism, as is important evidence that there are some disorders of cell cycle in DCS, Of course the specific regulating mechanism is unknown, it's our future work.

***Keywords*:** deficiency-cold syndrome; cell cycle; cDNA microarray; pathway comparision

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Molecular analysis of the subcellular localization of Thg1L

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Thg1L ($tRNA^{His}$ guanylyltransferase-1 like), ever named ICF45, is a cytoplasm protein which is involved in cell cycle regulation. Thg1L is only expressed in interphase, and has a special subcellular localization demonstrating 1-2 dots near the nuclear membrane and sometimes colocalize with centrosomes, or adjacent to each other. We reported here that Thg1L molecules can bind to each other shown by GST Pull-Down assay, and the regions between amino acid residues 30 and 122, 245 and 299, are most likely responsible for the self-association, whereas the region between amino acid residues 1 and 63 limited the interaction level of Thg1L. Interestingly, it was found that overexpression of Thy1L or the expression of some truncated forms can strongly diminish even eliminate the recruit of some known centrosomes proteins such as α tubulin and some autoimmune antigens to the centrosome judged by immunofluoresent staining. These observations strongly suggested a possible interaction between Thy1L and centrosome proteins.

***Keywords*:** Thg1L; centrosome; cell cycle

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Dynamic proteomic analysis of G0-G1 transition based on hepatectomy model and serum starved cell line model

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The mechanism by which quiescent cells reenter to the cell cycle is not well elucidated so far. Dynamic proteomic analysis was carried out on G0-G1 transition using two models: rat liver hepatectomy model and serum starved HEK293 cell line. In the hepatectomy model, the protein expression profiles at 0.5 h, 2 h, 3.5 h, 5 h and 6.5h post-hepatectomy were determined by 2DE followed by ESI-MS/MS. A total list of 89 dynamically changed proteins were selected and identified. Based on the dynamic properties of these identified proteins, five changing patterns were categorized to reveal their temporal order and possible roles during the transition of hepatocytes from quiescent to proliferate states. In HEK293 cell line model, analysis of the proteome profiles at 0 h, 1 h, 2.5 h, 4 h, 6 h resulted a large set of differentially expressed proteins, among which 45 proteins were identified. These proteins are major involved in cellular metabolism, DNA and protein synthesis, signal transduction, cell cycle control and cell skeleton regulation. It revealed multiple pathways may participate in cell cycle reentry and therefore provided more information for understanding the mechanism of G0→G1 transition. Further, one of the dynamic differential proteins during the transition from G0 to G1 of hepatocytes was selected and studied. This new mRNA transcript was cloned and the full length is 3135 nt, the open reading frame is from 1 nt to 1278 nt, and coding a protein with 425 amino acid. Semi-quantitative RT-PCR analysis revealed that this mRNA is abundant in testis, subsequently in spleen, kidney and liver. The GFP-fused protein expression experiment indicates it is a cytoplasmic protein. The studies on its function is under going.

***Keywords*:** dynamic proteomic; G0-G1 transition

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Study on the apoptosis effect induced by isothiocyanates in broccoli on human gastric adenoma cells (SGC-7901) and human hepatocarcinoma cells (HepG-2)

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To investigate the pro-apoptosis effect of isothiocyanates (ITCS) on human gastric adenoma cells SGC-7901, human hepatocarcinoma cells HepG-2 and its mechanism. METHODS: SGC-7901 and HepG-2 were treated with different concentrations of ITCS. MTT assay was used to evaluate the influence of ITCS on cell proliferation. Flow cytometry was used to test ROS levels, intracellular mitochondrial transmembrane potential($\Delta\psi$m), and hypodiploid apoptosis peak in both SGC-7901 and HepG-2. RESULTS: ITCS obviously inhibited proliferation of SGC-7901 cells and HepG-2 cells, and their IC50 were 22.406 μg/mL and 31.429 μg/mL, respevtively. When treated with 0, 15, 30, 60, 120, 240 μg /mL of ITCS for 24 h, intracellular ROS levels of SGC-7901 were (1.6±0.5)%, (2.0±0.3)%, (5.5±0.4)%, (25.8±1.4)%, (83.7±1.2)% and (97.4±4.2)%, respectively; and $\Delta\psi$m were (98.6±4.3)%, (98.4±4.8)%, (95.7±5.4)%, (92.6±4.0)%, (74.0±5.6)% and (63.7±4.0)%, respectively; meanwhile the ROS levels of HepG-2 were (2.1±1.4)%, (23.1±1.8)%, (53.3±3.3)%, (57.9±2.0)%, (79.9±0.4)% and (93.4±1.6)%, respectively; and $\Delta\psi$m were (96.3±5.3)%, (94.8±5.5)%, (91.8±5.4)%, (66.0±5.6)%, (65.5±6.6)% and (44.3±2.7)%, respectively; when treated with 0, 60, 120, 240 μg /mL of ITCS for 48 h, cell apoptotic rates of SGC-7901 were (4.3±1.6)%, (9.1±3.8)%, (20.1±4.2)% and (55.4±4.9)%, respectively; and the rates of HepG-2 were (5.4±3.6)%, (16.6±2.8)%, (21.9±4.4)% and (70.2±5.3)%, respectively. CONCLUSIONS: ITCS generates ROS in both SGC-7901 and HepG-2, which causes mitochondrial membrane permeabilization and $\Delta\psi$m decrease, therefore, leads to apoptosis.

***Keywords*:** broccoli; isothiocyanates; apoptosis

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Saponins of asparagus inducing apoptosis of HepG2 and SGC-7901 and affecting cell cycle

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To study the inhibitory effect on cell proliferation and explore anti-tumor mechanism of Saponins of asparagus. Saponins of asparagus with different concentration was treated with HepG2 and SGC-7901 at different time, MTT assay was used to detect inhibitory rate, fluorescence staining was used to observe apoptosis morphology, flow cytometry was used to detect apoptosis rate and cell cycle. The results showed Saponins of asparagus inhibited cell

proliferation, the IC50 on HepG2 was 172.3 mg·L^{-1} and on SGC-7901 was 177.5 mg·L^{-1}. Apoptosis morphology was observed by fluorescence microscope, after treatment 48 h, the cell number appreciably decreased, and the growth current in pieces also weakened, the clearance between cells expanded. Some cells changed into rotundity from anomalous form, and the profile of cell became clear, the nucleus became small, chromatin aggregated, the ratio of nucleus and chromatin decreased, the fluorescence intensity enhanced. The effect of Saponins of asparagus on cell cycle of HepG2 and SGC-7901 was similar, cell cycle was arrested at S phase, G2/M phase percent decreased. After 72 h the treated group appeared apoptosis peak, and apoptosis rate in high dose group was 30.9%, 20.1%. So we concluded that Saponins of asparagus could inhibit cell proliferation of HepG2 and SGC-7901, effect cell cycle, induce cell apoptosis.

Keywords**:** saponins of asparagus; HepG2; SGC-7901; apoptosis; cell cycle

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Inhibition effects of diallyl disulfide in human colon cancer SW480 cells *in vitro*

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To investigate the inhibition effects of diallyl disulfide (DADS) in SW480 cells in vitro and its related mechanism. Methods: The growth inhibition of SW480 cells were measured by growth curve analysis, vitality detection and MTT assay. Morphology was observed by optics microscope and electron microscope. Phase distribution of cell cycle was analyzed by flow cytometry. Immunocytochemical staining and morphometric quantitative analysis detected the expression of PCNA, p53, p21WAF1, Bcl-2 and Bax. Results: MTT assay showed that DADS from 30 to 70 μg/ml significantly inhibited SW480 cells and exhibited a dose-dependent modal. After exposure to DADS, cell viability of SW480 decreased from controls 97.37% to experimental 80.83% (P<0.05), average doubling time retarded from 34.50 hours in normal cultured SW480 cells to DADS experimented SW480 cells 94.74 hours (P<0.05). As exposed to optics microscope and electron microscope, SW480 cells took on malignance declining as cellular heteromorphism diminished, nucleocytoplasmic proportion was reliable to reasonableness, cellular apparatus were abundant in plasm, nuclear and partial cell organs manifested retrograde alters and partial cells represented apoptosis. All these showed above suggested that SW480 cells malignancy and proliferation capacity is declined. Flow cytometry analysis revealed that the cell content of G1-phase declined whereas G2-phase increased after exposure to DADS, which indicated that colon cancer cells arrested in G2-phase (P<0.05); Hhyodiplod peak is higher, which means cells apoptosis induced by DADS. Immunocytochemical stain and morphometric quantitative analysis indicated that expression of p53, PCNA, Bcl-2 reduced and that expression of p21WAF1 and Bax enhanced (P<0.05). Conlusion: To human colon cancer SW480 cell line, DADS has significance growth inhibition, and this effect is a dose-dependent and time-dependent model. DADS can induce SW480 cells block in G2/M and apoptosis. Its mechanism probably relate to downregulated expressions of p53, PCNA, Bcl-2 and upregulated expression of p21WAF1 and Bax.

Keywords**:** diallyl disulfide; colon cancer; growth inhibition; cell cycle; gene expression

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Cytoplasmic localization of cyclin B1 and its possible role in Diallyl disulfide-induced G2 checkpoint in HL-60 cells

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Diallyl disulfide(DADS), an oil-soluble allyl sulfur compound found in processed garlic, was found to inhibit the growth of various tumors by decreasing the cell growth rate, inducing apoptosis and cell cycle arrest. The previous studies in our laboratory showed that DADS induced the growth arrest of HL-60 cells in the G2/M phase，but the detail mechanism by which DADS induced the cell cycle arrest remains to be elucidated. We show here that treatment HL-60 cell using DADS could induce the expression and cytoplasmic localization of cyclin B1 during interphase. Treatment of HL-60 cells with leptomycin B (LMB), a specific inhibitor of the nuclear export signal (NES)-dependent transport, resulted in nuclear accumulation of cyclin B1 in G2 phase. The treatment of LMB combined with DADS markedly inhibit the nuclear export of cyclin B1 indued by DADS. Moreover, we show that LMB treatment of the cells is able to override the DADS induced G2/Mcheckpoint when combined with caffeine treatment. These results suggest a role of nuclear exclusion of cyclin B1 in the DADS-induced G2 checkpoint.

***Keywords*:** diallyl disulfide; cyclinB1; nuclear export; G2/M checkpoint; HL-60 cell

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The isolation of differentially expressed genes in the G2/M arrest induced by Diallyl disulfide in HL-60 cells using suppression subtractive hybridization (SSH)

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Diallyl disulfide (DADS), an oil-soluble allyl sulfur compound found in processed garlic, was found to inhibit the growth of various tumors by decreasing the cell growth rate, inducing apoptosis and cell cycle arrest. The previous studies in our laboratory showed that DADS induced the growth arrest of HL-60 cells in the G2/M phase， but the detail mechanism by which DADS induced the cell cycle arrest remains to be elucidated. In this report, an SSH cDNA library of HL-60 cells was constructed using DADS-treated HL-60 cell mRNA as tester and untreated-HL-60 cell mRNA as driver, respectively. The patterns of the differentially expressing-gene fragments in DADS-treated- or untreated-HL-60 were analyzed by PCR. Sequences of resulting cDNA fragments were compared with that of genes in GenBank by BLAST. The resulting expressed sequence tags (EST) were confirmed by RT-PCR based on mRNA from HL-60 cells with or without DADS treatment. Our results showed that 120 direct clones and 100 inverse clones, are obtained from the constructed SSH cDNA library. Of random analysis of 57 clones using PCR, 51 clones contained inserted fragments, and were sequenced and analyzed by BLAST. 7 clones are shown to be novel ESTs. One EST, of 7 novel ESTs, only express in DADS treated-HL-60 cells but not in untreated-HL-60 cells. At same time we obtained that one novel gene, which might be involved in HL-60 cells arrested in G2/M phase induced by DADS, by rapid amplification of cDNA ends (RACE).

***Keywords*:** diallyl disulfide; suppression subtractive hybridization; G2/M chengpoint; HL-60 cell

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Tastin is required for spindle assembly during mitosis

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Timely assembly of the mitotic spindle and chromosome segregation are essential for cell division. Tastin was previously characterized as a microtubule-associated protein participating in early embryo implantation by forming a complex with two partners, trophinin and bystin. We report here that tastin is also required for the assembly and organization of the mitotic spindle. We show that tastin is selectively expressed in proliferating cells. Tastin mRNA and protein levels peak at G2/M phase and abruptly decline after cell division. Microscopic analysis of both fixed and live mammalian cells shows that tastin is primarily localized on microtubules and the centrosome in interphase and to the mitotic spindle during mitosis. Two putative centrosome localization domains in the tastin N-terminus are required for centrosomal targeting. Tastin overexpression disrupts spindle organization and preferentially induces mono-/multipolar mitotic spindle poles. Suppression of tastin by RNA interference results in aberrant mitotic spindles and defective chromosome congression, leading to mitotic block and cell death. These results suggest a crucial role for tastin in maintenance of spindle integrity and mitosis progression during mitosis.

***Keywords*:** mitotic spindle apparatus; microtubules; mitosis

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Effects of alpha-synuclein expression on manganese induced neurotoxicity in PC12 vells

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On the basis of former experiments, we have employed a model in vitro that used the PC12 cell line to explored the characteristics of α-synuclein (α-SYN) and the relations between ERK and JNK pathways under the same conditions aiming to reveal the molecular mechanisms of dysfunctions in basal ganglia concentration caused by manganese exposure. PC12 cells were exposed to MnCl2 within a range of 100-900 μmol/L. The inhibition of the cells during 7 d was determined by MTT assay and plate clone formation test. α-SYN, phosphorylation of ERK1/2 and JNK1/2 were determined by Western-blot. Then, pcDNA3.1(+)-sense-α-SYN plasmids and pcDNA3.1(-)-antisense -α-SYN plasmids were transfected into PC12 cells to test the cell inhibition

by MTT and phosphorylation of ERK1/2 and JNK1/2 by Western-blot. In addition we also used inhibiters (PD98059 and SP600125) to block ERK and JNK cascades, and then observed the expression of α-SYN. Results showed that $MnCl_2$ at different concentrations (100, 300, 500, 700 and 900 μmol/L) could inhibit the growth and proliferation of PC12 cells in a dose- and time-dependent manner. Western blot showed that $MnCl_2$ decreased p-ERK1/2 and increased α-SYN, p-JNK expression (P<0.05). After we transfected pcDNA3.1(+)-sense-α-SYN plasmids and pcDNA3.1(-)-antisense-α-SYN plasmids to PC12 cells, we found that, with the change of the expression of α-SYN of MnCl2 treated PC12 cells, the expression of phosphorylation of ERK1/2 and JNK1/2 changed accordingly. The results indicated that MnCl2 might significantly inhibit the growth and proliferation of PC12 cells, possibly by inducing the decrease of the phosphorylation of ERK 1/2 and the increase of the phosphorylation of JNK1/2 and the changes of α-SYN expression might play important role in this process.

Keywords**:** manganese; α-synuclein; PC12 cells; signal transduction; proliferation arrest

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Study on cell culture in *Macrobrachium nipponense*

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Cell culture is a significant tool for studying physiology such as endocrinology and diseases of shrimp. It has also gained recent attention as a potent model for the development of diagnostic reagents and probes for use in the shrimp, crayfish and lobster industries. Yet, for reasons that remain obscure, all endeavors to develop cell lines have been ineffective so far. Macrobrachium nipponense as material, we attempted to cultivate some kinds of cells from some tissues,based with medium 199, supplemented with 15 % fetal bovine serum (FBS) , 100 IU/mL Penicillin , 100μg/mL streptomycin and 1μg/mL amphotericin B. It is requisite work for studying cell line's establishment. (1) For M. nipponense hepatopancreatic cells, under the condition of pH 7. 0~7. 2, osmotic pressure 600 mOsm, the cells exhibites the best. It is also found that linoleic acid added to the defined medium could accelerate growth of the cells obviously, and the optimun concentration of it in the medium is 1.6×10^{-4} mol/L. Cells have been subcultured for twice and survive for 45 d. (2) For the neurosecretory cells in the eyestalk of *M. nipponense* cultured *in vitro*, on the condition of pH 7. 0~7. 2 , osmotic pressure 900 mOsm or so, the cells adhere well and axon can grow in first 3~5 d after inoculation，but these axon start to atrophy after two weeks. The kind of cells can survive for 20 d. Its higher osmotic pressure than cells from other tissues origin may suggest these cells are of a regulation function for osmosis. Besides, it is also found that absence of serum have no effect on cell growth. (3) For testis cells in *M. nipponense* cultured in vitro, it is found that Spermatogonium and spermatocyte are of active division capability, but they are not able to divide consecutively in basal medium and survive less than 1 week. Through the datas about cell adherence, the range of the most sutable pH and osmotic pressure for testis in vitro is determined, respectively, pH 7.2~7.4 and 400~500 mOsm.

Keywords**:** *Macrobrachium nipponense*; cell culture; pH; osmotic pressure

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Culture of endothelial progenitor cells from human peripheral blood and real-time identification by flow cytometry

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To establish the method of isolation of endothelial progenitor cells（EPCs） from human peripheral blood and identify the markers of EPCs by FACS to provide basis for angiogenesis therapy. Methods: Total mononuclear cells (MNC) were isolated from peripheral blood by Ficoll density gradient centrifugation, and then the cells were plated on human fibronectin coated culture dishes and cultured in EGM-2 MV, 4 days later, the adherent cells were stained with Dil-AC-LDL and FITC-UEA-1 and observed by fluorescence and confocal laser microscope. The cell surface markers (CD31, CD34 and KDR) were detected by FACS analysis after differentiation for 4-7 d in cell culture. Results: Adherent cells double-positive for Dil-AC-LDL and FITC-UEA-1 by direct fluorescent staining could be observed under fluorescent and confocal laser microscope 4 days after culture. Flow cytometric analysis revealed that EPCs cells were positive for CD34 (2.50±1.47)%, CD31 (5.72±1.66)% and KDR (36.24±3.24)% at day 4 of culture. At days 5, 6 and 7, CD34+ cells were (8.45±3.97)%,

(14.13±2.79)% and (21.14±2.91)% and CD31+ cells were (22.52±3.86)%, (42.76±3.67)% and (54.67±3.44)%, respectively. Conclusion: EPCs exist in the peripheral blood and can be differentiated into mature endothelial cells (ECs). FACS can be used to efficiently identify EPCs for angiogenesis study.

***Keywords*:** endothelial progenitor cells; cell culture; surface markers

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Effects of tetrandrine on chondrocytes cultured *in vitro*

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The effects of tetrandrine on cultured chondrocytes were investigated *in vitro*. Methods: A whole cell enzyme-linked immunosorbent assay (Cell ELISA) that detects the BrdU incorporation during DNA synthesis and collagen type II secretion was applied to evaluate the proliferation and functional expression of chondrocytes. Cell viability was estimated by the MTT assay and cell function were assessed by measuring glycosaminoglycan (GAG) secreted by chondrocytes. Results: The data suggested that tetrandrine at low concentration could improve the chondrocyte proliferation, viability and function. On the contrary, tetrandrine at high concentration inhibited the proliferation and functional expression of chondrocytes. Conclusion: Tetrandrine at low concentration could enhance the cartilage reconstruction *in vitro*.

***Keywords*:** tetrandrine; chondrocyte; cell culture; cell proliferation; cell ELISA

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Regulation of cell cycle progression, chromatin replication, and DNA damage response by the cullin-containing ubiquitin E3 ligases

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Alteration of the cell cycle is a hallmark of human cancer. A critical mechanism for the cell cycle regulation is the specific and timely removal of key cell cycle regulators through ubiquitin-dependent proteolysis. In multicellular organisms, our studies revealed that two multi-protein ubiquitin E3 ligase complexes, the SCF (-SKP1, CUL1, and F-box proteins) and the CUL4/DDB1 E3 ligases, play pivotal roles in regulating the cell cycle progression from G1 to S phases. The SCFSKP2 E3 ligase complex uses the F-box protein SKP2 as the specific substrate binding subunit to target the CDK inhibitor p27Kip1 for proteolysis at late G1 phase, thereby promoting S phase entry. Our previous data indicate that the CUL4/DDB1 ubiquitin E3 ligase represents a new class of E3 ligase that regulates chromatin replication and DNA damage response. We recently found that the CUL4/DDB1 E3 ligase also regulates the G1/S transition by targeting both p27Kip1 and p53 tumor suppressor protein for degradation. The p53 stability is regulated by MDM2 and our data indicate that CUL4-mediated p53 polyubiquitination and proteolysis are dependent on MDM2, PCNA, and components of CUL4 complexes such as DDB1 and a novel WD40 repeat protein L2DTL/CDT2. In addition, the CUL4/DDB1 E3 ligase also regulates the proteolysis of replication licensing protein CDT1 in response to DNA damage and replication through L2DTL/CDT2 and PCNA. Unscheduled activation of CDT1 leads to re-replication of the genome and partial chromatin polyploidy. The activity and assembly of SCF and CUL4/DDB1 E3 ligase complexes are further regulated by the covalent modification of CUL1 and CUL4 by an ubiquitin-like protein, Nedd8, and the binding of an inhibitory protein, CAND1. We will discuss the identification of additional proteins or factors of cullin E3 ligases and new pathways that are regulated by these E3 ligases. Our studies thus provide new insight into the mechanism of cell cycle regulation by cullin E3 ligases and suggest how the alteration of these proteolytic pathways may contribute to proliferation-related human diseases such as cancer.

***Keywords*:** cell cycle; CDK inhibitor p27Kip1; SCF-SKP2; p53; CUL4/DDB1

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Comparison of hollow-fiber membrane and microcarrier for expanding ability in a rotating bioreactor

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Large-scale expansion of SD (Sprague–Dawley) rat's osteoblasts was studied in rotating wall hollow-fiber membrane bioreactor (RWHMB) by using hollow-fiber membrane as the carrier. For comparative studies, cells were also expanded in T-flask by using hollow-fiber membrane as carrier and rotating wall vessel bioreactor (RWVB) by using microcarrier as carrier. During the culture period, the cells were sampled every 12 hr. After 5 days, the cells were harvested and evaluated with scanning electron microscope (SEM), hematoxylin-eosin (HE) staining and alkaline phosphatase (ALP) staining. Moreover, von-Kossa staining and Alizarin Red S staining were carried out for mineralized nodules formation. The results show that in RWHMB, the cells present better morphology and vitality and secrete much more extracellular matrix. It is concluded that the RWHMB combines the advantages of rotating wall vessel and hollow-fiber membrane bioreactors. The hydrodynamic stimulation within it accelerates the metabolism of osteoblast and mass transfer, which is propitious to the cell differentiation and proliferation.

***Keywords*:** bioreactor; expansion; hollow-fiber membrane; microcarrier; osteoblast

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Regulating chromosome-bound MCAK—a new role for Aurora B in spindle assembly

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Chromosome congression and segregation requires the proper attachment of microtubules to the two sister kinetochores. At centromeres, Aurora B and MCAK (Mitotic Centromere-Associated Kinesin) are key regulators of kinetochore-microtubule attachments and dynamics. Previous studies showed that disruption of either Aurora B or centromeric MCAK causes increased chromosome misalignment and segregation due to improper kinetochore-microtubule attachments. Indeed MCAK localization and activity are regulated by Aurora B, but how Aurora B phosphorylation of MCAK contributes to spindle assembly is not clear. Here we show that MCAK also binds to chromosome arms in an Aurora B dependent manner. Aurora B regulates the amount of MCAK binding to chromosome arms through a two-site regulatory mechanism. Aurora B phosphorylation of T95 on MCAK promotes MCAK association with the chromosome arms while phosphorylation of S196 on MCAK promotes dissociation from arms. Aurora B also regulates MCAK targeting to centromeres through a distinct two-site regulatory mechanism. Phosphorylation of S110 on MCAK is essential to target MCAK to centromeres while dephosphorylation of T95 on MCAK increases the affinity of MCAK for centromeres. Our studies reveal a new role for Aurora B, which is to prevent too much MCAK binding to chromatin to facilitate chromatin-nucleated spindle formation. Our studies also unravel a complicated and sophisticated regulation mechanism of spindle formation through multi-site phosphorylation of a key Aurora B substrate.

***Keywords*:** Aurora B; MCAK; chromosome; kinetochore; phosphorylation

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Lmx1b is essential for Fgf8 expression in the isthmic organizer during tectum and cerebellum development in mice

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The LIM homeobox gene *Lmx1b* is essential for neuronal differentiation in many brain regions, but its function in the brain regionalization remains largely unclear. In this study, we found that *Lmx1b* is required for the induction activity of the isthmic organizer in the mid/hindbrain boundary (MHB). Expression of *Lmx1b* was observed in the anterior embryo as early as E7.5 in normal mice and became restricted to the isthmus at E9.0. In *Lmx1b*$^{-/-}$ mice, the development of the tectum and cerebellum was markedly impeded. Analysis of gene expression in the MHB of the mutant embryos showed that many genes were lost by E9.5. Importantly, the expression of Fgf8, which normally occurs at 4-somite stage, was completely absent throughout the development. Among those genes that are

normally initiated prior to 4-somite stage, Wnt1, En1 and Pax2 were down-regulated before 4-somite stage, whereas Gbx2 down-regulation occurred at 4-somite stage. On the other hand, Otx2 and Pax6 expression was normal in *Lmx1b*$^{-/-}$ embryos. Finally, the requirement of specific *Lmx1b* expression in the MHB was further confirmed by Wnt1-Cre-mediated region-specific conditional knockout of *Lmx1b*. Thus, the homeobox gene *Lmx1b* plays an essential role in the tectum and cerebellum development by regulating expression of Fgf8 and other isthmic organizer-dependent genes in the MHB.

***Keywords*:** Lmx1b; isthmic organizer; Fgf8; tectum; cerebellum

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TRPC channels promote cerebellar granule neuron survival

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Channels formed by the TRP family of proteins serve for a variety of physiological functions. Here we report that two members of the TRPC subfamily, TRPC3 or TRPC6, promotes survival of cerebellar granule neurons (CGNs) in the rat brain and protects cultured CGNs against serum-deprivation (SD)-induced cell death. Knock-down of TRPC3/6 with RNAi induced CGN apoptosis in neonatal rat cerebellum and this effect was rescued by over-expressing either TRPC3 or TRPC6. In CGN cultures, blocking TRPC channels or down-regulating TRPC3/6 expression suppressed BDNF-dependent neuronal protection, BDNF-triggered ERK and CREB phosphorylation. Notably, over-expressing TRPC3/6 prevented SD-induced cell death and this effect could be blocked by dominant negative form of CREB. Furthermore, inhibition or down-regulation of PLC/IP3 receptor suppressed BDNF-dependent protection on CGNs. Thus, our findings provide in vivo and in vitro evidence for a critical role of TRPC channels in promoting neuronal survival.

***Keywords*:** TRPC; cerebellar granule neuron; survival

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Protective effect of sandalwood oil and vitamin D against high glucose stress in HaCaT cells

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In order to abbreviate the symptom of diabetic foot ulcer, we have screened several plant extracts to reduce stress induced by high glucose on human keratinocyte cell line, HaCaT. We have found that sandal wood oil, the essential oil from Santalum Album Lignum, which is known to act as a tonic in the immune system and to be good for dry skin and urinary tract problems, has the protective effect against high glucose stress in HaCaT cells. Sandalwood oil decreased the level of ROS and restored the suppressed cellular proliferation. Cell cycle analysis has showed that sandalwood oil relieved the induced G1 arrest. Similarly, calcitriol, the hormonal form of Vitamin D, which is known to protect keratinocytes from UV- and chemotherapy-induced damage, has been shown to increase the cell proliferation of high glucose stressed keratinocytes and to relieve the induced G1 arrest. The activation of the stress-activated protein kinases is an early cellular response to stress signal and an important determinant of cell fate. The mechanism of increasing keratinocyte proliferation of sandalwood oil and Vitamin D seems to be related with ROS reduction and MAPK activation.

***Keywords*:** sandalwood oil; high glucose stress; calcitriol; ROS level; cell proliferation

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Enhancement of EPO production of r-CHO-S cells by trichostatin under hyperosmotic pressure

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In this study, the enhancement of recombinant protein production was investigated by medium additives in the culture of CHO cells transfected with the human erythropoietin (*rhEPO*) gene. Elevated osmolarity is one of methods that can increase specific productivity in recombinant Chinese Hamster Ovary (rCHO) cell culture. Trichostatin A (TSA) is known to be a toxic chemical of which function is to inhibit histone deacetylase (HDACs) by binding directly to the catalytic site. The expression of recombinant proteins following transduction of CHO cells with recombinant baculoviruses containing a mammalian expression cassette with the CMV-promoter has been known to be enhanced by the addition of TSA. To maximize the specific protein productivity, TSA (1, 0.5, 0.1 μM) was added in hyperosmolar medium at the exponential cell growth phase and

then the synergy effect was investigated. TSA increased recombinant protein production, but did not induce apoptosis. Also, TSA treatment resulted in a G0-G1 phase cell cycle arrest. The G0-G1 phase accumulation after TSA addition seems to be related with the enhancement of EPO production. Interestingly, decrease of sub G1 population was observed after TSA addition. The relationship for the optimization of recombinant protein production with G0-G1 phase cell cycle arrest needs to be elucidated. Taken together, our results demonstrate that the addition of TSA has positive effects on improving the recombinant protein production.

Keywords**:** recombinant protein production; CHO; EPO; cell cycle; Trichostatin A

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Mob2p interacts with the protein kinase Cbk1p to function in cell polarity growth and cytokinesis in *Aspergillus nidulans*

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The molecular mechanisms that temporally and spatially coordinate cell morphogenesis with the cell cycle remain poorly understood. In the yeast *S.pombe*, mob2p-orb6p is a widely expressed serine-threonine kinase complex, homolog of human Mob2-NDR that has been implicated in cell proliferation and tumor progression. Due to the significance of *Aspergillus* fungi as human pathogens, it will assist in understanding the biology, host interactions, and pathogenicity of these organisms, as well as aid in vaccine and drug development. Here we report the characterization of mob2p, a novel protein required for the regulation of cell growth and cell cycle control in *A. nidulans* by the highly efficient gene homologous replacement and GFP-targeting approach. Depletion of Mob2p by repression of the alcA promoter induced a severe growth defect accompany with loss of polarity and random direction of second germ tube formation, This phenotype is similar to that of alcA(P)-GFP:: Cbk1p (CotA) mutant when the expression of Cbk1 protein which is homolog of yeast orb6p in *A. nidulans* was turned off in depression medium. Futhermore, both alcA(P)-GFP::Cbk1p and alcA(P)-YFP:: Mob2p strains in the induce medium of alcA promoter showed the normal hyphal extension and conidiation. In addition, over-expression of Mob2p led to the delay of cytokinesis and the formation of large fluffy colonies than that of wild type. Thus, we concluded that the fusion proteins of GFP-Mob2p and YFP-Cbk1p that we constructed are the functional forms in the two target strains. Mob2p and Cbk1p are essential in *A. nidulans* polarity growth and regulated the coordination between mitosis and cytokinesis. The further details of regulation mechanism are ongoing. This work was supported by the NSFC.

Keywords**:** Mob2p; Cbk1p(CotA); polarity; mitosis; *Aspergillus nidulans*

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Getting into and out of mitosis

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We are trying to understand how cells ensure that their two daughter cells receive an identical copy the genome in mitosis. This is clearly essential to genomic stability and defects in the checkpoints controlling chromosome segregation may contribute to tumorigenesis. Key to the control of chromosome segregation is the regulation of the Anaphase Promoting Complex/Cyclosome (APC/C) by the spindle assembly checkpoint. We have developed a live cell assay to monitor APC/C activity and its inhibition by the spindle checkpoint. This has revealed that the checkpoint is intrinsic to the timing of mitosis. We have found that the APC/C is recruited to unattached kinetochores by the checkpoint proteins that may explain the tight temporal control on the APC/C by the checkpoint. We are currently testing the idea that this temporal control requires that the checkpoint and ubiquitination machineries interact at the spindle. Using time-lapse fluorescence microscopy as a real time assay for the proteolysis of mitotic regulators in living cells we have found that the events of cytokinesis and spindle disassembly may, in part, be coordinated by ubiquitin-mediated proteolysis in mammalian cells. Moreover, the interplay between the APC/C and its regulators are essential to coordinate mitosis with DNA replication.

Keywords: cell cycle; cyclin-dependent kinases; ubiquitin; proteolysis; spindle-checkpoint; APC/C

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The cyclin-dependent kinase Cdc28 regulates the septin cytoskeleton for polarized morphogenesis

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Cell polarization is a universal phenomenon that constitutes an integral part of diverse biological processes including cell morphogenesis, migration, differentiation and development in all organisms. Cytoskeletons such as actin filaments, microtubules and septin fibers play key roles in cell polarity establishment and maintenance. Although a number of central regulators of cytoskeleton structures and functions have been found and extensively investigated, the molecular details of the interactions between the regulators and their effectors remain elusive in most cases. One example is the cyclin-dependent kinase Cdc28 of budding yeast. Cdc28 in association with G1 cyclins Cln1 and Cln2 is well known to activate actin and septin polarization to the presumptive budding site for bud growth, but the molecular link of Cdc28/Cln kinases to the cytoskeleton proteins is largely unknown. Here, we have used the polymorphic fungus *Candida albicans* as a model to study the mechanisms that control the growth switch from yeast to hyphae. First we have found a G1-cyclin-related protein Hgc1 that, by complexing with Cdc28, plays a specific and essential role for hyphal morphogenesis. To identify substrates of the Cdc28/Hgc1 kinase, we used a 2D-Western approach to examine the phosphorylation patterns during yeast and hyphal growth of a large number of proteins that had been known or implicated in cell polarity control, aiming at finding proteins that undergo growth mode- or Hgc1-dependent phosphorylation. We found that the septin Cdc11 undergoes phosphorylation immediately after hyphal induction. Interestingly, Hgc1 is not required for causing but is essential for maintaining the hypha-specific Cdc11 phosphorylation. I will provide evidence that Cdc11 phosphorylation is essential for normal hyphal development.

Keywords: cyclin-dependent kinase; septin; cell polarity; morphogenesis and *Candida albicans*

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Effects of several chemicals on the cell cycle of mouse fetal fibroblast cells

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In research of animal cloning, the cell cycle of the donor cells is one of the important factors which can effect the development of reconstructed embryos following nuclear transfer (NT). Many investigations found that synchronizing donor cells in phase G0 or G1 can improve the viability of embryos in NT, but others considered that donor cells in phase M that coordinate with MII oocytes in cell cycle were benefit to the development of reconstructed embryos. It is known that some chemicals can control the cell cycle of donor cells. To obtain the given stage cells, passage 4-6 mouse fetal fibroblast cells at about 70 % confluent were treated as follow in this experiment: I. 20 μM Roscovitine cultured for 24 h; II. 2 μg/ml Aphidicolin cultured for 24 h; III. 2 μM Nocodozole cultured for 24 h; VI. 0.05 μg/ml Demecolcine cultured for 16 h; V. Control. Fibroblast cells in each group were trypsinized and fixed with 70 % ethanol, then treated with 100 μg/ml RNAase under 37 ºC for 30 min and stained with 10 μg/ml propidium iodide for 10 min. Finally, prepared samples were examined by using flow cytometer. The results of analysis showed that the percentages of G0/G1 cells treated with Roscovitine, Aphidicolin, Nocodazole, Demecolcine and control cells were 78.6±0.3 %, 80.9±6.9 %, 34.6±0.4 %, 54.1±2.5 % and 74.4±1.4 %, and the percentages of S cells of them were 11.2±0.6 %, 13.8±5.7 %, 19±7 %, 29.8±5.6 % and 11.3±5.7 %, while the percentages of G2/M cells of them were 10.3±0.9 %, 5.3±1.3 %, 46.4±7.3 %, 16.0±4.1% and 14.3±4.3 % respectively. The results indicated that the percentage of G0/G1 cells in Aphidicolin was higher than that of cells in Nocodazole, Demecolcine and control significantly (P<0.01), though it had no significant difference with that in Roscovitine. The percentage of S cells in Demecolcine was significantly higher than that in other chemicals or control (P<0.05). The percentage of G2/M phase cells in Nocodazole was the highest among the chemical treatments and control. In conclusion, Aphidicolin can increase the percentage of G0/G1 mouse fetal fibroblast cells and Demecolcine can increase the percentage of S cells, while Nocodazole may enhance the percentage of G2/M cells.

***Keywords*:** cell cycle; chemical treatmen; mouse; fetal fibroblast cells

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C7 is a Plk1-interacting and Cdk1-phosphorylated protein involved in cell cycle process

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Polo-like kinase 1 (Plk1) is a serine/threonine kinase critically involved in cell cycle control. We identified a novel protein, C7, as a potential interacting partner of Plk1 in yeast two-hybrid screens. C7 is phosphorylated by Cdk1 in

M phase and this is required for its interaction with Plk1. C7 is phosphorylated at Ser104 and Thr120. The phosphorylation of C7 at Thr120 is required for its interaction with Plk1. Overexpression of C7 or its knockdown by siRNA causes de-regulation of cell cycle. These findings suggest that C7 plays a role in cell cycle regulation through its interaction with Plk1.

Keywords: Plk1; Cdk1; phosphorylation; cell cyle

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Measuring cytotoxicity of amyloid-β 1-42 and hydrogen peroxide on mammalian astrocyte cultures from senescence-accelerated mice R1 and P8 with the MTT assay

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In our previous study, the difference after the H_2O_2 treatment on astrocytic cultures from a rapidly aging strain of mouse (SAMP8) and its sister control R1 was compared. There was a mild but significant difference observed between SAMR1 and SAMP8 cells after H_2O_2 treatment. This indicated that the two cell lines maybe had different antioxidative and physiological condition during aging. In this article, MTT assay was used to measure cytotoxicity of amyloid-β 1-42 and hydrogen peroxide in the two astrocytic cultures. Our results showed that after treatment by amyloid β 1-42 and hydrogen peroxide, polygonal astrocytes could differentiate into process-bearing stellate cells and expressed high level of GFAP, which was typical of reactive astrocytes. Compared with control SAMR1 cell, 1 μM and 5 μM amyloid-β 1-42 could significantly induce morphological changes in SAMP8 cells 24 h after addition of amyloid-β 1-42. Amyloid-β 1-42 at micromolar concentration could induce the morphological changes, but lower nanomolar concentration was enough to inhibit the cellular redox activity without affecting cell survival. However, hydrogen peroxide at low dose could induce MTT reduction due to influencing cell death, could not accelerate the exocytosis of intracellular MTT formazan granulars and rapidly induce needle-like crystals on astrocyte surface as Aβ did. So amyloid β 1-42 had a different mechanism in inducing cell morphological changes and altering the cell membrane integrity, which contributed to neurodegeneration in AD.

Keywords**:** cytotoxicity; amyloid-β 1-42; hydrogen peroxide; senescence-accelerated mice; MTT assay

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Proliferation of colon cancer cells inhibited by SFPS associated with activation of caspase-3 mediated by caspase-8 and caspase-9 or G0/G1 arrest

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The effects of SFPS (*Sargassum fusiforme* polysaccharides), from the edible seaweed Sargassum fusiforme, on viability of colon cancer cells and induction of apoptosis were investigated. SFPS remarkably reduced the viability of human colon cancer cell lines, i.e. lovo and RKO cells. Furthermore, SFPS treatment induced classical signs of apoptosis, including cell detachment, membrane shrinkage, and nuclear fragmentation in the experiment of lovo and RKO cells. The result from flow cytometry confirmed this idea of G0/G1 arrest in SFPS-treated RKO cells and more cells undergoing apoptosis in lovo than in RKO cells. The occurrence of apoptosis was accompanied by the proteolytic processing of procaspase-3 in lovo and RKO cells treated with SFPS. The present study demonstrates, by using western blot analysis and caspase activity assay, that SFPS induced differential activation of caspases in lovo and RKO cells, of which caspase-3 and -9 were mainly activated in lovo cells, while caspase-3, -8 and -9 were activated significantly in RKO cells. Together, SFPS may induce the apoptosis of lovo cells in vitro resulting in the inhibition of proliferation mediated by the cleavage of caspase-9 and subsequent activation of caspase-3, while the inhibitive viability in RKO cells result from G0/G1 arrest and the apoptosis associated with the activation of caspase-3, -8.

Keywords**:** *Sargassum fusiforme* polysaccharides; human colon cancer; apoptosis; caspase

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