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The role of telomeres and telomerase in aging and cancer

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Cellular senescence is a general stress-response program that restrains cellular proliferation. There are pathways leading to an irreversible arrest of cell proliferation that are independent of telomere length (overexpression of oncogenes, reactive oxygen species). However, under optimal growth conditions, the onset of replicative senescence depends on telomere status. When telomeres are sufficiently short (uncapped) there is a DNA damage response-induced growth arrest. The senescence pathway involves the formation of telomere dysfunction induced foci that contain DNA damage response factors. The DNA-damage response in senescent cells is not a transient phenomenon, but consists of a permanent activation of the DNA damage checkpoint machinery. The long-term growth arrest at senescence may be thought of as an initial anti-tumor protection mechanism. In situations where normal cell cycle checkpoints are altered, cells can bypass the normal senescence signaling pathway and continue to grow until they reach a second growth arrest state known as crisis. In crisis, telomeres are terminally short and, in the presence of other genetic and epigenetic changes, can result in telomeric fusions, subsequent breakage-fusion-bridge cycles and in rare cells, up-regulation or reactivation of telomerase, the cellular RNP that is able to add telomeric repeats to the ends of chromosomes and thus prevent their shortening. In both senescence and crisis ectopic expression of the catalytic subunit of telomerase (hTERT) leads directly to an immortal state demonstrating that telomeres are important in both replicative senescence and crisis. In most pre-neoplasias, telomeres are exceptionally short. Almost all human malignant tumors express telomerase to maintain these short telomeres and this activity is probably required for the long-term growth of most advanced malignancies. Telomerase activity is absent or at lower levels in normal tissues, making the inhibition of telomerase an attractive target for cancer therapeutics. Cancer stem cells (a subset of cells within a tumor mass that is capable of regenerating the tumor) are both telomerase expressing and contain short telomeres. Telomerase inhibitors are thus likely to target both the bulk of the more differentiated tumor cells as well as the stem cells that are thought to be the source of chemotherapy and radiotherapy resistance in most advanced cancers. The expression of the catalytic subunit of human telomerase (hTERT) reconstitutes telomerase activity and circumvents the induction of senescence. We have used hTERT to immortalize a variety of human cell types including skin keratinocytes, dermal fibroblasts, muscle satellite (stem) cells, endothelial cells, retinal-pigmented epithelial cells, breast epithelial cells, both corneal fibroblasts (keratocytes) and corneal epithelial cells and lastly human bronchial epithelial and stromal cells. The epithelial and stromal cells can be reconstituted in 3D to produce tissue-like structures. The production of hTERT engineered cells offer the possibility of producing tissues to treat a variety of diseases and aged-related medical conditions that are due to telomere-based replicative senescence.

***Keywords*:** senescence; stem cells; immortalization; regenerative medicine; telomeres;

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Myogenesis potential of porcine adipose-derived mesenchymal stem cells

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Recently, some studies suggested that human adipose tissue contains pluripotent stem cells similar to bone marrow-derived stem cells which have the capability to colonize different tissues, replicate, and differentiate into multilineage cells. Animals such as farm animals provide both a source of fat-derived stem cells and an immunocompetent, autologous host animal in which to investigate the capacity of the fat-derived cells to differentiate and form tissues *in vivo*. Our goal was the isolation and characterization of porcine adipose tissue mesenchymal to study their myogenic potential *in vitro*. Porcine adipose mesenchymal stem cells (pAMSCs) were selected from the dorsal neck, shoulder, and back regions of Large-White pigs after extensive washing with PBS and treatment with collagenase. After 24h in primary culture in growth medium (Dulbecco's modified Eagle's medium +10% fetal bovine serum), pAMSCs adhered to the plastic surface, presenting a small population of single cells. 7 to 10 days after initial plating, the cells looked like long spindle-shaped fibroblastic cells and began to form colonies. When the cells approached densities over 80%, the cell morphology changed to a more spindle-shaped, fibroblastic morphology. After expansion to 20 passages, the fifth passage cells were selected and Flow cytometry analysis of isolated pAMSCs demonstrated the following phenotype, CD44 (pos), CD105 (pos) and CD14 (neg),

CD106 (neg), HLA-DR and S100(neg), similar to that described for human AMSCs. To induce optimal myogenesis, the fifth passage pAMSCs were plated in myogenic medium (Dulbecco's modified Eagle's medium +10% fetal bovine serum+5% horse serum+0.1 mM dexamethasone +50 mM hydrocortisone) for 3 weeks. Myogenic differentiation was assessed by desmin and myoglobin staining, and expression of myogenic specific genes were detected by RT-PCR.At first week, desmin was expressed in the treated cells. Three weeks later, cells were stained with a monoclonal antibody specific to skeletal muscle myoglobin . Low levels of Myf5 and MyoD were observed in induced pAMSCs at first week and Myosin heavy chain was observed when induced for 3 weeks. In control groups, expression of these genes was barely detectable. Thus, pAMSCs derived from adipose tissue were able to differentiate "*ex vivo*" into myoblast-like cells. These elements may be a suitable source for research on skeletal muscle development and studying relationship between muscle and adipose.

***Keywords*:** porcine; adipose tissue; myogenic; cell culture

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Surface antigens of murine mesenchymal stem cells

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The aim of this study is to issue the surface antigen determinants of murine mesenchymal stem cells (mMSCs) from the bone marrow samples. For this purposes, 6-8 weeks old either NMRI or Balb/c mice were sacrificed, their bone marrow were harvested and plated in 75-cm^2 flask at a density of 10^7 cells per ml. Two weeks after culture initiation, the cells were tripsinized, the majority of them sub cultured in a new culture dishes in split ratio of 1:2 and 2×10^5 cells of primary culture were prepared for flow cytometry analysis to detect some surface antigens including CD135, CD44, CD31, Thy1.2, CD11b, CD45, CD34, Vcam1, Sca-1and c-Kit. Cultivation of the cells was continued for 3 passages in each of which the culture was examined for given antigens. Passaged-3 cells from each strain were investigated whether or not they could generate three principle cell lineage including osteoblast, chondroblast and adipocyte as a proof for their mesenchymal indentity. Our results indicated morphologically heterogeneous nature of the primary culture that becomes homogenous in the late passage. Flow cytometric analysis indicated that CD 44 antigen was persistently expressed in more than 90% of the cells in all examined passages. Although Thy1.2 was not expressed in primary culture, it was observed on about half to one fifth of the cells in passage 3. CD31 antigen was never expressed on the cells. The expressional level of CD135, CD45 and CD11b were gradually decreased and c-kit, Sca1 and CD34 expression were slightly increased from primary culture on to passage 3. Two studied mice strains showed some differences in expressing certain markers. Unlike Balb/c cells, some cells of NMRI strain expressed VCAM antigen. Passage-3 cells from both strains were easily differentiated into bone, fat and cartilage when being cultured in appropriate culture condition. In conclusion: First, the culture of mMSCs remains heterogonous in terms of their surface antigens although it gradually becomes morphologically homogenous during cultivation period from the primary culture to passage 3. Second, more than 90% of the cells were CD 44 positive throughout the culture period. Third, the cells with hematopoietic specific antigens were not eliminated even in the passage 3 in which the majority of the cells were typically fibroblastic cells. Fourth, the MSCs from two mouse strains were somewhat different in terms either of morphology in culture or having certain surface antigens.

***Keywords*:** murine mesenchymal stem cells; surface antigens; bone, adipocyte and cartilage differentiation; Balb/c and NMRI strains

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PPARα is involved in cardiomyocyte differentiation of murine embryonic stem cells

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Peroxisome Proliferator-activated Receptors α (PPARα) is a fatty acid-activated nuclear receptor which plays a key role in the transcriptional regulation of genes involved in cellular lipid and energy metabolism. It is abundantly expressed in tissues with high energy demand, including the heart. Although multiple roles for PPARα have been proposed, the significance of PPARα in early cardiac development, especially during the differentiation of cardiomyocytes remains unclear. To address this issue, murine embryonic stem (ES) cells were adopted in this study, taking the advantage that these cells would differentiate in vitro into cardiomyocytes which faithfully recapitulate cardiomyocyte differentiation *in vivo*. Using RT-PCR and Western-blot, we found that PPARα and PGC-1α were

both induced during cardiomyocyte differentiation of ES cells. A strong correlation between PPARα and Troponin-T (cardiac specific) during differentiation was also observed by immunofluorescence. Inhibition of PPARα by its specific inhibitor GW6471 significantly prevented cardiomyocyte differentiation and resulted in reduced expression of cardiac α-Actinin and Troponin-T. However, GATA4, Nkx2.5, and MEF2C were not notably affected at transcription level. Furthermore, application of PPARα agonist WY14643 significantly improved area positive for α-Actinin and Troponin-T. Taken together, these results show that PPARα activity is necessary for cardiomyocyte differentiation of murine ES cells *in vitro* but not due to large changes in the expression of those key transcription factors.

***Keywords*:** PPARα; PGC-1α; embryonic stem cells; cardiomyocytes; embryogenesis

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Morphological observations of cell-clusters in hemolymph of mamestra brassicae

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Hemocytes isolated from the larval hemolymph were classified in to several types; plasmatocyte, granulocyte, spherulocyte, oenocytoid and prohemocyte. In addition, we observed cell-clusters in hemolymph of Mamestra brassicae, which may be dispersed from hematopoietic organs and eventually disappear at the pre-pupal stage. The origin and function of the cell-cluster has not been fully described. Hemocytes, fat body tissues and cell-clusters were cultured in MGM- 450 medium. During long-term culture, cell-clusters change their morphology to granulocyte-like cells and to plasmatocyte-like cells. In short-term culture, the morphology of cell-cluster did not change, although change when co-cultured with fat body tissues or cultured under supplement with fat body extract or larval plasma. However, there was no effect in heat-treated plasma, protease-k treated plasma and co-cultured with hemocyte, suggesting that the fate of the cell-cluster is regulated by some factor(s) in larval plasma and fat body.

***Keywords*:** mamestra brassicae ; hemolymph; cell-cluster; hemocytes; fat body

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Quantitative structure-activity relationships for the effects of different chlorinated organic compounds on apoptosis of fish lymphocytes *in vitro*

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A simple and rapid *in vitro* toxicological assay, utilizing apoptosis EC50 by the flow cytometric PI staining method, has been used to evaluate the apoptosis cytotoxic effects of 23 chlorinated environmental organic chemicals. The apoptosis EC50 assay allows the quantitative evaluation of the apoptosis cytotoxicity of these chemicals. According to the factor analysis and cluster results of physiochemical parameters, all tested chlorinated chemicals were obviously divided into two groups with different levels of toxicity. For chlorinated chemicals with phenyl ring (type II), EC50 values ranged from 0.060 to 0.263 mM, whereas for chlorinated chemicals without phenyl ring (type I) EC50 values ranged from 0.039 to 0.117 mM. On the average, the cytotoxicities of type I chemicals are greater than that of type II ones. Quantitative structure-activity relationships (QSAR) were developed between apoptosis EC50 values and various molecular descriptors. The involvement of the electronic factor ELUMO in the final QSAR models of both types of chemicals suggests that during the apoptosis induction ROS materials may act as a Lewis base to participate in substituted nucleophile reactions with these chemicals functioning as an electron-pair acceptor or an electrophile. Structural features of tested two types of molecules associated with apoptosis cytotoxicity reveal that the substituent location and type are two important factors. For chlorinated disinfection by-products (CDBPs), the apoptosis cytotoxicity of chemical substituted by Br atom is lower than that substituted by Cl atom, which may be related to the stronger electron attracted abilities of Cl atom. Also for DBPs, the EC50 values decrease with more substituent Cl atoms in the chemical structure, which should be the results of the electron attracted effects of these Cl atoms. On the contrary, for both chlorinated benzenes (CBs) and polychlorinated biphenyls (PCBs), the EC50 values increase with more substituent Cl atoms in the molecular conformation, which should affect by the p-π conjugation of C-Cl bond that can reduce the molecular reactive abilities. On the basis of the presenting findings, our method has a potential for the low dose chemical exposure risk research by the apoptosis EC50 evaluation.

***Keywords*:** apoptosis; QSAR; lymphocytes; cytotoxicity

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Spatiotemporal patterning of N-cadherin expression on rat cardiomyocytes

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To investigate the spatiotemporal patterning of N-cadherin expression during development of rat cardiomyocytes and age-related changes of N-cadherin expression, Immunohistochemical methods were used to examinemyocardial N-cadherins distribution in fetal rat and postnatal development (1 postnatal day to old rat), and quantitied by HIPAS-1000 computerized image analytical instrument. The Results showed thatexpression of N-cadherin was located in myocardium of atrial and ventricule and septum interventriculare and mm.papillares. The N-cadherin immunolabeling dispersed distributions in myocyte cell membranes and within cytoplastic in fetal rat heart. From young to old rat heart, the classic N-cadherin was located in transversely orientated intercalated disk. From neonatal to infant rat, the pattern of N-cadherin immunolabeling changed progressively, from being dispersed over the entire cell surface as in the fetal to the transverse terminals of the myocytes, toward the distribution within the intercalated disk. The percentage of N-cadherin immune positive area in rat ventricular myocardium showed a progressively change with age. The present results demonstrated that the N-cadherin expression got progressive age-related changes and the changing pattern of N-cadherin was closely related with development of the intercalated disk in rat myocardium. The mechanical coupling provided by adherens junctions is essential for the stable cell-cell contact.

***Keywords*:** N-cadherin; adherents junction; myocardium; intercalated disk

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Effects of cerium on lifespan and oxidative stress resistance in *Drosophila melanogaster*

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To investigate the effects of rare earth element cerium (Ce) on lifespan and oxidative stress biomarkers in the fruit fly (*Drosophila melanogaster*). Methods Fruit flies were feed on medium with different dose of ceric sulfate (1, 4, 16, 64, 256, 1024 mg/L), the mean lifespan, half lifespan, maximum lifespan and fecundity were calculated by using Descriptive Statistics method respectively, and the activities of superoxide dismutase (SOD) and catalase (CAT), the levels of lipid peroxidation products, namely malondialdehyde (MDA)content were measured. Results With the increased dose of the rare earth element cerium, cerium caused a significant decrease in mean lifespan, half lifespan and maximum lifespan in fruit fly, and the obvious dose-effect relationship was observed between lifespan and $Ce(SO_4)_2$ concentrations, the reproductive output of fruit fly decreased at different concentration, but significantly at the concentration of $Ce(SO_4)_2$ over 16 mg/L comparing with control group. Meanwhile, cerium caused a significant increase in MDA content and decrease in SOD and CAT activity in fruit flies at the concentration of $Ce(SO_4)_2$ over 16 mg/L, and decrease in MDA content and increase in SOD and CAT activity at the concentration of $Ce(SO_4)_2$ under 16 mg/L comparing with control group (basic medium). Conclusion Rare earth element cerium-caused lifespan shortening in *Drosophila melanogaster* is associated with oxidative stress; orally administrated cerium can generate active oxygen species. Therefore, the present study suggests that cerium compound may be oxidant toxic for *Drosophila melanogaster*.

***Keywords*:** cerium (Ce); lifespan; oxidative stress; *Drosophila*

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Engineering skin ectodermal organ *in vitro* and *in vivo*

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The bioengineering of tissues and organs is emerging as an important new discipline of science and technology. Feather is a unique organ with robust regenerative ability in the adult animal (Jiang *et al*, 2004). Discovery of feather stem cells will help to explain how feathers grow, molt, and generate different functional forms. It becomes a model for stem cell biology (Yue *et al*, 2005, 2006). It may lead to new clues and management to human organ regeneration in the future. The objective of this study is to engineer skin ectodermal organ by using chicken embryonic skin *in vitro* to form new feather follicles and to identify the adult feather stem cell *in vivo*. We also study the reconstitution of stem cells to form hair follicles in scid mouse skin. Methods: For *in vitro* study, the reconstitution of feather bud using intact epithelium and dissociated mesenchyme were made from stage 29-35 developing skin. DiI labeling, *in situ* hybridization and immunostaining were used to trace the new feather bud formation and molecular changes of en-

gineered skin. For *in vivo* study, long-term label retention, transplantation and DiI tracing were applied to identify and map stem cell activities in the adult chicken feather follicles under physiological conditions. For hair study, we inject aggregated newborn mouse epidermal and dermal cells into the dermis of scid mouse (Zheng *et al*, 2005). Results: In reconstitution assay, all skin mesenchyme cells are reset to equivalent state and can reform periodic patterns in reconstituted skin. The size of feather primordial remained constant when different numbers of mesenchymal cells were used in the reconstituted skin. In long-term labeling we found that feather follicles containe slow-cycling long-term labeling-retaining cells (LRCs). The LRCs are enriched in a collar bulge niche in the growing follicle and a papillar ectoderm niche near the dermal papilla in molting follicles. On transplantation, LRCs show multipotentiality. For the hair reconstitution study, we analyze the molecular and cellular process of *de novo* hair formation. Conclusion: The dissociated mesenchyme cells have the properties of adult skin cell multipotentiality and can reform feather follicles and inter-follicle skin. The LRCs located in a collar bulge niche have a high efficiency of incorporation into developing skin and can form different components of feather follicles. These studies provide novel understanding for tissue engineering of ectodermal organs.

***Keywords*:** skin ectodermal organ; reconstitution; stem cell

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Phosphatidylcholine-specific phospholipaseC, P53 and ROS are associated with apoptosis and senescence in vascular endothelial cells

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To find the key factors that associate with senescence and apoptosis in human vascular endothelial cells (VEC), we first measured the changes of phosphatidylcholine-specific phospholipase C (PC-PLC) activity during senescence of VECs. Then, we investigated the effects of suppressing PC-PLC on apoptosis of the cells, and examined the alterations of P53 expression and intracellular reactive oxygen species (ROS) levels before and after inhibiting PC-PLC in the young and senescence cells. We found that PC-PLC activity decreased remarkably with the cell aging, and that suppressing PC-PLC could inhibit apoptosis in the young cells, but it could trigger apoptosis in the senescence cells. The results also showed that, in young VECs, when PC-PLC was inhibited, the elevation of P53 expression induced by apoptosis was depressed. Contrarily, in the senescence cells, suppressing PC-PLC could not inhibit the elevation of P53 expression. Inhibiting PC-PLC pulled down intracellular ROS levels both in the young and senescence cells, but the level of ROS in population doubling level (PDL) 36 cells was much more dramatically depressed than that in PDL 16 cells. The data suggested that PC-PLC was a key factor associated with apoptosis and senescence, and its function might be related with the level of P53 in VECs.

***Keywords*:** phosphatidylcholine-specific phospholipase C; senescence; apoptosis; P53; reactive oxygen species

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D609 blocks cell survival and induces apoptosis in neural stem cells

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In order to investigate the effects of tricyclodecane-9-yl-xanthogenate (D609) on the survival of neural stem cells (NSCs), which were isolated from rat forebrain and identified by the monoclonal antibody against Nestin, we treated the NSCs with D609 in the presence of basic fibroblast growth factor (bFGF). Viable cell number was analyzed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium) method. We found that when NSCs were exposed to 18.76-56.29μM D609, the survival signal was blocked and the viability of the cells remarkably declined. Subsequently, we examined the nuclear fragmentation of NSCs by TUNEL method under laser scanning confocal microscopy and measured the levels of LDH. Our results showed that when the cells were incubated with D609, the cell growth could not continue and apoptosis occurred in NSCs. We also examined the intracellular reactive oxygen species (ROS) levels after the cells were treated with D609. The result showed that the ROS level in NSCs was depressed. The data suggested that D609 was a powerful growth inhibitor and apoptosis inducer in NSCs.

***Keywords*:** D609; neural stem cells; cell growth; apoptosis; fibroblast growth factor

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Suppressing phosphatidylcholine-specific phospholipase C and elevating ROS and Rb levels induce neuronal differentiation in mesenchymal stem cells

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To find a simple and effective method of inducing mesenchymal stem cell (MSC) differentiation into neurons, and to understand the underlying mechanism, we first cultured two kinds of MSCs that were human bone marrow derived MSCs and human placenta-derived MSCs. Then, in the absence of growth factors, we treated the MSCs with D609, a specific inhibitor of phosphatidylcholine-specific phospholipase C (PC-PLC). The results showed that, the cells treated with D609 became neurons in morphology. The neuron specific enolase, neurofilament-L and synapsin were increased significantly. Subsequently, we investigated the changes of PC-PLC activity. The results showed that D609 obviously inhibited the activity of PC-PLC in the hMSCs. Then, we examined the expression of integrin β4, retinoblastoma (Rb) and the changes of the levels of intracellular reactive oxygen species (ROS). We found that the levels of integrin β4 and Rb protein were evidently elevated during neuronal differentiation of hMSCs, and that ROS levels dramatically increased during hMSC differentiation towards neurons. Our data suggested that PC-PLC mediated the differentiation signaling by regulating the expressions of integrin β4 and Rb protein, and that ROS may serve as important signaling molecules during neuronal differentiation of hMSCs.

***Keywords*:** mesenchymal stem cells; PC-PLC; cell differentiation; neuron

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Integrated effects of Notch and MAP kinase signaling influence B versus T lymphoid lineage decision

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Notch signals influence lymphocyte lineage decisions by inhibiting B cell development while promoting T cell differentiation. Mice deficient of Notch1 or its downstream transcription factor, RBP-Jk, harbor increased number of B cells in the thymus. However, the molecular mechanism underlying Notch signals remains poorly understood. Previously, we have demonstrated that activation of Notch receptors accelerates the turnover of E2A proteins, which are basic helix-loop-helix transcription factors crucial for B and T cell development. Phosphorylation in a highly conserved region of E2A, referred to as EHD3 domain, by MAP kinases is a prerequisite for Notch-induced degradation, which involves ubiquitination by the SCFskp2 ubiquitin ligase complex. Importantly, N1-IC induces E2A degradation in B but not T cells due to the different basal levels of MAP kinase activities in these cells. We have now generated knockin mice (termed E2AMm) in which the MAP kinase phosphorylation sites in EHD3 domain are mutated so that the encoded E2A proteins are resistant to Notch-induced degradation. Bone marrow progenitors from these mutant mice but not wild type mice differentiated into B cells on OP9 stromal cells expressing Notch ligand, delta-like1. In the thymus of the E2AMm mice, the number of B cells increased by over 2-fold compared to that of wild type mice. These results suggest that Notch-induced E2A degradation contributes to inhibition of B cell development but additional factors must also be involved. Indeed, we recently found that activation of Notch signaling also diminished STAT5 activation by inducing the ubiquitination and degradation of Jak2. Analogous to E2A degradation, Notch-induced degradation of Jak2 was also dependent on MAP kinase activities and only occurs in bone marrow progenitors and B cells but not in thymocytes. Since JAK-STAT signaling is crucial for B cell development, suppression of STAT5 activation in the thymus may also contribute to Notch-mediated B versus T lineage decision. Consistently, transgenic mice expressing a dominant positive form of STAT5 harbor significantly more B cells in their thymuses. Combination of the STAT5 and E2A mutations resulted in further increase in B cell production in the thymus. Taken together, these results provide insight into the mechanisms by which Notch signaling influences the B versus T lineage decision. Furthermore, they also illustrate that the lineage decision is dependent on both Notch signaling and MAP kinase activities.

***Keywords*:** Notch; ubiquitination; lineage decision; E2A; Jak-STAT

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Maximal expansion of stem cells from human dental pulps and evaluation of their multipotentiality for differention

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Stem cells residing in human dental pulps of both permanent teeth (DPSCs) and exfoliated teeth (SHEDs) are able to cumulate calcium *in vitro* and differentiate into odontoblasts and osteocytes *in vivo*, which makes them the optimal candidates for tooth regeneration. In this study, a series of experiments were performed to testify whether these stem cells could display the same characteristics as marrow stroma stem cells (BMSSCs) which expand more rapidly at low density without the loss of multipotentiality for differentiation, such as adipogenesis and chondrongenesis. P0 DPSCs from a 3rd molar and P0 SHEDs were plated at 1.5 and 3000cells/cm2 to investigate effects of plating density on expansion. DPSCs plated at 1.5 cells/cm^2 expanded 50 population doublings (PDs) in 8 weeks. In contrast, when plating at 3000 cells/cm^2 they only gave rise to 25 PDs in the same period of time. Similarly, when plating at 1.5cells/cm^2, the SHEDs underwent 60PDs, whereas when plating at 3000 cells/cm^2 they only reached 25PDs in 8 weeks. Thus, our results showed that Plating at low density benefits rapid expansion of DPSCs and SHEDs *ex vivo*. Further assay indicated that the optimal density for maximal expansion was 1.5-3.0 cells/cm^2. In order to test whether these cells retain their multpotentiality for differentiation after low density expansion, osteogenic medium was used to examine the capability of calcium deposition of expanded DPSCs and SHEDs. We found that, when the cells were plated at the optimal low density of 1.5-3.0 cells/cm^2 and doubled for 40 times, they maintained the capacity for calcium deposit. There were no differences in the density and size of calcium nodules compared with the cells that were plated at high density of 3000 cells/cm^2 and doubled for 25 times. However, cells plated at that high density totally lost their capacity of calcium deposition after 40PDs. In addition, we performed the Colony Forming Efficiency (CFE) assay to identify the mutidifferentiality of the expanded cells. Results showed that CFE of DPSCs decreased from 55% to 35% and that of SHEDs dropped slightly from 65% to 50% after 40PDs at low plating density. In conclusion, DPSCs and SHEDs retained their capacity for calcium deposit and CFE after 40PDs when plated at optimal low density of 1.5-3.0 cells/cm^2 and therefore apparently retain their potential for multiple differentiations.

Keywords**:** stem cell; DPSC; SHED; cell expension

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Integrated roles of SOX9 with BMP-2 and cAMP/PKA signaling pathways in osteoblastic differentiation

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SOX9 is a member of the SRY-type high-mobility-group transcription factor family and is mutated in Campomelic dysplasia, a severe syndrome characterized by congenital bowing of the long bones and other skeletal malformations. Collaborating with various co-factors including BMP-2, SOX9 is widely known to be a molecule key to the chondrocyte differentiation of mesenchymal progenitors during development that also give rise to osteoblasts. Our recent studies have shown that cAMP/PKA signaling significantly contributes to the BMP-2-induced osteogenetic differentiation. Herein we demonstrate that SOX9 can promote the BMP-2-induced osteoblastic differentiation and the SOX9 action is regulated by cAMP/PKA signaling at multilevel. Overexpression of SOX9 was firstly shown to efficiently propel the BMP-2-induced osteoblastic differentiation of murine progenitor cells, C2C12, as indicated with the increased ALP activity and the enhanced expression levels of collagen type I and osteocalcin. The activity of the promoter of the collagen type I gene was particularly increased by SOX9. Addition of the PKA activator 8-bromo-cAMP and the inhibitor H-89, was shown to enhance and suppress the effect of SOX9, respectively. Overexpression of PKIγ, the cognate inhibitor of PKA, also exhibited a strong restraining effect on the SOX9 action. Furthermore, mutation of two PKA phosphorylation sites (S64A, S211A) of SOX9, known to alter the nuclear location of SOX9, deprived its stimulatory effect. Other mutations of SOX9, including W143R, R152P, and truncation of the C- terminus transactivation domains (T327X), identified in Campomelic Dysplasia patients, also severely impaired the facilitation effect on the expression of osteogenic markers. Unlike the wild type SOX9 and SOX9 with only phosphorylation sites removed, these diseases-causing mutations impaired the direct interaction of SOX9 with CREB, the most representative transcription factor downstream of the PKA pathway. Taken its high expression in fully differentiated chongdrocytes into account, our data suggest that by integrating with BMP-2 and cAMP/PKA pathways, SOX9 may also play a role in endochondral bone development, i.e. the

transition of chondrocytes into hypertrophy.

Keywords**:** SOX9; osteogenesis; BMP-2; PKA; CREB

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Establishment of embryonic stem (ES) cell lines derived from new-born or adult mouse ovary grafted underneath the kidney capsule

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There are a number of ovarian tissues with different genetic background which are resected from clinical cancer therapy and may be stored for future transplantation. Transplantation of ovarian tissue not only provide a means of restoring endocrine function and fertility for patients faced with endocrine disruption or infertility, but also is likely to generate embryonic stem cells or parthenogenetic embryonic stem cells from grafts of ovarian tissue for ES cell-based therapies. In this study, we choose a mouse strain to develop a model that combines the transplantation of ovarian tissue and generation of ES cells. Methods: The basic steps involve (1) transplantation of new-born and adult mouse ovarian tissue underneath the kidney capsule, (2) oocytes maturation and fertilization or parthenogenetic activation from grafts of ovarian tissue, (3) characterization of ES and PGES cells isolated from inner cell mass of the blastocyst derived from ovarian tissue grafts. C57B/6 mice were used as ovarian tissue donors and as graft recipients. Nineteen days after grafting, gonadotrophins were given a single i.p. injection of 5 IU pregnant mare serum gonadotrophin (PMSG). Mice receiving PMSG were killed for collection of oocytes from grafts 48h later. Oocytes matured in MEM medium were directly activated or fertilized in vitro. The resulting parthenogenetic blastocysts or fertilized blastocysts were seeded on the MEF feeder layers for ES cells isolation. Results: Here, we report two ES cell lines and two PGES cell lines, which are respectively derived from blastocyst and parthenogenetic blastocyst generated from grafts of ovarian tissue. All these ES cells and PGES cells could be passaged continuously in culture, could produce teratomas with all three embryonic germ layers (skeletal muscle, Rosette neural tube, respiratory epithelium) and have a normal chromosome karyotype (38+2). All the ES cells and PGES cells are immunoreactive for alkaline phosphatase, octamer-binding transcription factor 4 (Oct-4), and stage-specific embryonic antigen 1 (SSEA-1). Conclusion: This study shows that embryonic stem cells can be derived from grafts of ovarian tissue and offers a model system for alternative generation of human pluripotent stem cells.

Keywords**:** ES cells; ovarian tissue; the kidney capsule; transplantation; model

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Isolation and characterization of embryonic stem (ES) cells directly derived from transgenic mice strongly expressing greenfluorescence protein(GFP)

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Following the birth of "dolly" and the establishment of human ES cell lines, the concept of "therapeutic cloning" was developed. Due to so many wonderful hope that "therapeutic cloning" brings us, scientists show more interests in inducing ES cells to differentiate into various types of tissue cell and in cell transplantation. Since differentiated cells were derived from the ES cells, successful isolation of embryonic stem cells is of significance, especially those ES cells which can express green fluorescence protein (GFP). GFP not only can be observed directly by fluorescencre microscope, but also can be detected using GFP antibody in immunohistochemistry. ES cells expressing GFP can be used to trace differentiation process, migration pathway and final lodging site of cells. Here we report the isolation of GFP-expressing embryonic stem cells directly from inner cell mass (ICM) of blastocysts of EGFP-transgenic mouse. Methods: blastocysts from EGFP-transgenic C57B/6 mice were seed on the mitomycin-treated MEF feeder layer. Six days later, the well-formed ICM colonies were picked up and transferred to a medium containing EDTA-Trypsin with glass pipette. The clumps were pipetted with 0.05% trypsin. Aggregates with 2-8 cells were transfered onto the other feeder cells layer and recorded Passage 1. 4-6 days later, the cell aggregates were dispersed into single cells, and transferred onto other feeder cells layer and recorded Passage 2. Afterwards, the ES cells were passaged every other day. Result: The results showed that resulting ES cells expressed high levels of GFP, maintained the developmental potential to form teratoma with all three embryonic germ layers and retain normal chromosome karyotype. These ES cells were immunoreactive for alkaline phosphatase,

Oct-4 and SSEA-1. Conclusion: EGFP-expressing ES cells obtained from EGFP-transgenic mice possessed typical characterization of ES cells, and it may be used to trace differentiation process, migration pathway and final lodging site of cells through EGFP marks, and may possess more functions than generic ES cells.

***Keywords*:** EGFP-transgenic mouse; embryonic stem cells; green fluorescence protein; MEF feeder layer; blastocyst

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The ultracytochemical localization of ATPase in the developing anthers of *Lattuce sativa* L.

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ATPase distributes widely in cells of plants and takes part in many physiological activities of cells. Anther development of higher plants is a complex process including a series of delicate differentiation processes of tissues and cells. The physiological functions of ATPase are not clear during anther development. We investigated the distributional characters and physiological function of ATPase during the anther development of lettuce (*Lattuce sativa* L.). During anther early development, few ATPase precipitates located in small vacuoles and the nucleus of microspore mother cell and tetrad microspores. The precipitates in young microspore just released from the tetrad began to increase and abundant ones specially accumulated in the germ pore. When some small vacuoles appeared in the cytoplasm of microspore, ATPase precipitates increased in the small vacuoles. Meanwhile the microspore began to synthesize its intine, and many precipitates accumulated in intine, suggesting it might play an important function in this process. At the same time, some vacuoles which contained bigger ATPase precipitates fused each other, and finally formed a big central vacuole which contained numerous ATPase precipitates. On the contrary, ATPase precipitates decreased in the cytoplasm and nucleus except at the germ pores where numerous ATPase precipitates were accumulated. As a big vacuole appeared in the microspore, its polarity was created. The mitosis of polar microspore created a 2-cellular pollen grain. ATPase precipitates were more in both nuclei of vegetative and generative cell than in the cytoplasm. When the generative cell detached from the intine of the vegetative cell, abundant ATPase precipitates specially accumulated in the space between generative cell and vegetative cell. When the generative cell completely suspended in the cytoplasm of the vegetative cell, ATPase precipitates on the surface of the generative cell entirely disappeared. The mature pollen of lettuce was 3-cellular, few ATPase precipitates appeared in pollen. The anther wall of lettuce was composed of four layers of cells: epidermis, endothecium, middle layer and tapetum. During anther early development, epidermis, endothecium, middle layer became high vacuolization, and had few ATPase precipitates in their cytoplasm, while most of precipitates mainly located on their plasma membrane, suggesting that the function of the three layers of cells was transportation of nutriment into locule. The quantity of ATPase precipitates of tapetal cells changed obviously: during early tetrad stage the ATPase precipitates in the cytoplasm evidently increased, and the number of the precipitates reached the peak in early microspore stage. Furthermore, the inner surface of tapetum accumulated more ATPase precipitates than the outer surface. The polar distributional character indicated that the primary function of tapetum was to transport the nutrition materiel into locule.

***Keywords*:** *Lattuce sativa* L.; Anther; ATPase

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Chondrogenesis potential of unrestricted somatic stem cells

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Recently, tissue engineering has merged with stem cell technology with interest to develop new sources of transplantable material for injury or disease treatment. Eminently interesting are bone and joint injuries /disorders because of the low self-regenerating capacity of the matrix secreting cells, particularly chondrocyte. The utilization of stem cells derived from cord blood in tissue engineering is a promising solution to the problem of tissue or organ shortage. The cord blood derived mesenchymal stem cells are undifferentiated, multipotential cells which are capable of giving rise to differentiate into multiple connective tissue types, including cartilage. Here a new intrinsically pluripotent, CD45 negative population from human cord blood, termed Unrestricted Somatic Stem Cells (USSCs) is described. This rare population grows adherently and can be expanded to cells without losing pluripotency. This was followed by the appearance of type collagen and aggrecan when cultured for 21 days *in vitro* in a defined medium supplemented with transforming growth factor-beta (TGF-β), dexamethasone , ascorbic acid -2-phosphate,

ITS, bFGF, Linuleic acid and poly L-lysine to induce cell aggregate. RT-PCR, immunohistochemistry and Alcian blue staining reveal a chondrogenic differentiation after 21 days following stimulation. In conclusion, we showed that TGF-beta induced chondrogenesis of USSC derived from cord blood involves the rapid deposition of a cartilage - specific extracellular matrix.

***Keywords*:** unrestricted somatic stem cell; chondrogenesis; TGF-β (transforming grow factor-beta); cartilage tissue engineering

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Calcium distribution during the development of tobacco anther

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Ca^{2+} is a significant element in organism with numerous physiological functions, and the roles calcium plays have been revealed more and more in plant development. Some researchers investigated the Ca^{2+} distribution in the fertile and sterile anthers of photoperiod-sensitive genic male-sterile rice, and found the sterile anthers displayed abnormal calcium distribution. This result put out a question: what are the spatial and temporal features of calcium distribution during anther development, especially in dicotyledon, and what function of calcium plays during anther development? The current study used potassium antimonite and TEM to localize pools of loosely bound calcium in tobacco anther, and to investigate the relation between calcium distribution and anther development. Before the stage of microspore mother cell (MMC), few calcium-induced precipitates appeared in sporogenous cells and the somatic cells of anther wall. During MMCs preparing meiosis, abundant calcium precipitates were accumulated in cytoplasm of MMC and its callus wall. Meanwhile, there were many calcium precipitates accumulated in the vacuoles of tapetum. After meiosis of MMC and microspores released from tetrad, numerous calcium precipitates appeared in its germ pore, and some small vacuoles containing calcium precipitates appeared in its cytoplasm, suggesting calcium entering into microspore from germ pore. When microspore constructs its intine many vacuoles containing calcium precipitates were secreted out its plasmal membrane and accumulated in the place of intine, displaying calcium functioning during intine construction. With the development of microspore, some calcium precipitates appeared in the endoplasmic reticulum (ER) which began to expand to form many small vacuoles which fused to form a big vacuole to press the microspore nucleus to periplasmodial area created a polarity preparing first division of male gamete. Calcium accumulated in ER and induced vacuoles formation was its another function during microspore development. After an asymmetrical mitosis of microspore, a two-cellular pollen grain is formed which consists of a big vegetative cell and a small generative cell. Then the large vacuole of vegetative cell decomposed into some small ones in which calcium precipitates appeared again. Meanwhile, the quantity of calcium precipitates decreased in tapetal cells, suggesting the transportation of calcium from vascular bundle into locule slowing. The calcium precipitates of two-cellular pollen grain became small in size and disappeared following cytoplasm of vegetative cell becoming densely and storage materials accumulate. Before anthesis, some calcium precipitates specially accumulated on the surface of germ pore and a few in its cytoplasm. The feature of Ca^{2+} distribution during anther development of Nicotiana tabacum displays that it plays some biological significance during microspore development.

***Keywords*:** *nicociana tabacum* L.; anther development; calcium

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The role of telomeres in relicative aging in *Saccharomyces cerevisiae*

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Understanding the processes of replicative cellular aging of an organism is one of the important topics for biology. The erosion and protection of the chromosome ends, telomeres, is thought to play a key role in determination of replication potential of cells. As a unicellular organism, the budding yeast *Saccharomyces cerevisiae* divides asymmetrically, making it an optimal system to investigate the correlation between telomere length and cell life span. Mutant yeast haploids with the knockout of telomerase components were subjected to serial passages, and telomere length and life span of different passages of cells were determined. The early passage of mutant cells exhibit similar life span with the wild-type cells, indicating that telomere length-independent pathway dominates to determine yeast life span. In contrast, the mutant cells with shorter telomeres show a significantly reduced life span; the more shortened of telomeres, the more decreased of life span. These findings suggest that the activation of the telomere length dependent pathway of cellular aging requires shortened telomeres, and

support the notion that telomere length serves as a mitotic clock for cell division in eukaryotes.

***Keywords*:** telomere; aging; life span

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Effects of cyclic-tension force on the expression of receptor activator of NF-κB ligand(RANKL) in mouse bone marrow stromal cells

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To investigate the effect of mechanical tension force on the expression of RANKL in mouse bone marrow stromal cells (BMSCs) and the mechanism of cyclic-tension that may be related to RANKL changes in BMSCs, the four-point bending system was used to load the BMSCs ST2, cells were subjected to 1875 με strain. The expression of RANKL was determined by PCR and immunodeposition western blotting. Under the application of 1mm strain, mRANKL and RANKL mRNA are decreasing after 6 hours' loading, suggesting that Cyclic-tension may inhibit the expression of RANKL .

***Keywords*:** bone marrow stromal cells; mechanical strain; receptor activator of NF-κB Ligand

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Induction of chondrocytes differentiation from rat bone marrow stromal cells and gene expression of immune proteins

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To study an effective differentiation condition of chondrocytes from rat bone marrow stromal cells *in vitro* by immunohistochemistry, in situ hybridization and RT-PCR, and identify gene expression pattern of immune proteins in this process via cDNA microarray analysis. The results suggested that Dexamethasone (DEX) had an ability to promote cell proliferation. The group of transforming growth factor-β1 (TGF-β1) could highly express collagen II while its cell proliferation was weaker than that of DEX group. These indicated that TGF-β1 had the ability to make marrow stromal cells differentiated into chondrocytes. The group of combination of TGF-β1 and DEX express collagen II and maintain proliferation. Analysis by cDNA microarray revealed that 7 genes of immune proteins out of the 2242 rat genes had differential expression (>2 fold or <0.5 fold)during this process, including membrane-spanning 4-domains, subfamily A, member 1; T-cell receptor active alpha-chain C-region, partial cds, clone TRA29; MHC class I-related protein (MR1); peptidylprolyl isomerase C-associated protein; complement C8 β (C8b), partial cds; complement component 4 binding protein, α, and complement component 4 binding protein, β. This study showed that combination cultural condition in which bone marrow stromal cells were induced into chondrocytes lineage was effective to repair of articular cartilage. Various immune genes were involved in chondrocytes differentiation. This would provide foundation for repair of articular cartilage defect with chondrocytes.

***Keywords*:** chondrocyte induction; bone marrow stromal cell; DEX; TGF-β1; gene expression

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Type II collagen modulates osteogenesis processes during endochondral bone development

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During endochondral bone development, mesenchymal stem cell undergoes condensation and forms cartilage in the shape of ensuing bone. This cartilage is rich in type II collagen matrix and served as a template for the subsequent bone formation. These observed processes in bone development lead to a hypothesis that "the cartilage formation is essential for and precedes bone formation, which is promoted by the matrix replacement events in the preformed cartilage". To test this hypothesis, type II collagen (CII), which is the major extracellular matrix (ECM) component during the osteochondral stage of developmental bone, was used to study the ECM-guided osteogenic differentiation. STRO-1+/-, SH2+, SH3+, CD34- human mesenchymal progenitor cells (hMPCs) were isolated from bone marrow aspirate. Various cell differentiation assays were applied to examine the mesenchyme differentiation potential of hMSCs.

Real-time PCR data revealed that under monolayer culture condition, hMPCs treated with beta-glyceralphosphate for 2 to 3 weeks underwent osteogenic differentiation which was companied with changes in collagen typing and production. We further investigated whether the cartilaginous type II collagen exerts any differentiation modulating effect on hMPCs. Up regulation in MAPK signaling accompanied with expression of osteocalcin and down regulation of type II collagen mRNA production was observed when hMPCs were cultured on CII coating plates in osteogenic medium. An enhanced calcium deposition was also observed in CII treated hMPCs under micro-mass pellet culture condition. Subsequently, artificial bone tissues fabricated with either type I collagen scaffold or with type II collagen scaffold were compared histologically and immunohistochemically. The data indicate that CII mediated osteogenic modulating effects may through integrin-MAPK-runx2 axis and the remodeling of cartilaginous matrix may be crucial to osteogenesis processes.

Keywords**:** endochondral ossification; osteogenesis; differentiation; bone development; extracellular matrix

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Aging profiles of a dimorphic fungus, *Candida albicans*

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The unicellular yeast *Saccharomyces cerevisiae* represents one of the major model organisms to understand cellular aging in eukaryotes and has been studied extensively nowadays. As the minor difference between young and old cells, complicated procedure and special equipment are required for isolation of old cells. Our recent findings have shown that the dimorphic yeast, Candida albicans, which allows large-scale separation of old and young cells, is much more convenient. The two types of Candida cells, yeast form and hyphal form, have similar replicative life span. Taking the advantage of morphologic changes, we are able to harvest cells with different age in culture. The age of the separated cells are confirmed by life span assay. Deletion of one copy of the CaSIR2 or CaSGS1 causes a decrease of mean life span, which suggests common mechanism and function in *C. albicans*. We found that in old cells oxidative damaged proteins tend to accumulate, and the ability of nascent protein synthesis is decreased. Interestingly, we didn't observe rDNA circle accumulation or changes in rDNA copy number during cellular aging. Chromosome integrity and telomere length of cells with different age were also examined. Furthermore, we also observed glycogen accumulation in aging process, suggesting an energy shift in old cells. The genome-wide analysis of DNA microarray has been used to search for genes and pathways whose expression is modified during aging process. We compared the profiles of gene expression between old and young *C. albicans* cells during replicative aging and found expression changes of several group of genes, which may help to reveal the molecular mechanism of cellular aging. We think this new aging model not only allows to verify the controversial results in *S. cerevisiae* but also facilitates the biochemical characterization and genomics research.

Keywords**:** *Candida albicans*; aging; life span; microarray

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The Est3p and Est1p interaction is required for telomerase activity in *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae*, the telomerase holoenzyme consists of at least Est1p, Est2p (the catalytic subunit), Est3p and Tlc1 (the RNA subunit) four subunits. The specific function of Est3p subunit in telomerase is unclear. Here we show that the telomere lengthening caused by Est1p overexpression was comprised in est3-Arg110His, -Lys68Ala, -Lys71Ala, -Phe103Ala and -Thr115Ala mutants, but the telomere shortening caused by Est2p or Tlc1 overexpression was not affected in these est3 mutants as in EST3 wild-type cells, suggesting that the Lys68, Lys71, Phe103, Arg110 and Thr115 residues in Est3p are important for its genetic or functional interaction with Est1p, and dispensable for its interaction with Est2p or Tlc1. The further characterization of est3-R110 mutants showed that mutation of Arg110 in Est3p to Lys or His resulted in shorter telomeres, while the Arg110 to Ala, Leu, Glu, Asp or Tyr mutation caused both telomere shortening and cellular senescence, indicating that Arg110 of Est3p is critical for its function in telomerase holoenzyme. Taken together, these data indicate that *in vivo* telomerase requires the genetic or functional interaction of Est3p with Est1p to elongate telomeric DNA.

Keywords**:** telomerase; telomere; Est3p; Est1p; senesence

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Cell differentiation of *Hypericum perforatum* L. and accumulation of hypericins

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Hypericum perforatum L. is a kind of herbal medicine that has been used as an anti-depression folk medicine in Europe for centuries. In resent years, its biological active compound, hypericins, also displays potent anticancer and antiviral activities, which promises an extensive usage of *H. perforatum* L. in the future. Tissue and cell culture were adopted in order to obtain the experimental materials. The callus of *Hypericum perforatum* L. was studied to understand the ultrastructure changes in the dedifferentiation of cells and relationship between the development of callus and hypericins accumulation. The result showed that in tissue culture high concentration of 2, 4-D was effective on callus formation and in addition, high percent of plantlet differentiation could be induced directly under low concentration of NAA and BA. The established suspension culture system of H. perforatum L. showed that cell aggregates with the size between 100μm and 1000μm were beneficial and hypericins could be produced and accumulated in the cell aggregates. Several characteristic in structural changes of chloroplast and vacuole as the symbol and status of the cell dedifferentiation were confirmed. The chloroplast in the parenchyma first began to change in shape with the increase of starch grain in number and volume after two-day's culture and then recovered its shape with the exhaustion of starch in the cell dedifferentiation. Finally, the chloroplasts divided and bud into proplastids while the cells began to divide. In addition, the center vacuole in parenchyma became to change into smaller ones, the process of dedifferentiation being completed. After inducing differentiation, parts of callus projected from the surface and its color gradually changed from pink to red and finally to dark brown. These cells before differentiation had its characters of meristem cells with small volume, dense cytoplasm, large nucleus and rich organelles. During the development of callus, the mass of dense substance (hypericins) was first produced in cytoplasm and then got into vacuole through membrane and gradually accumulated more along the inner surface while the dense cytoplasm became less dense. The dense substance (hypericins) finally accumulated in the center of vacuole while the color of projected part changed to dark brown. However, hypericins in callus and suspension cells were accumulated in the projected part (a group of secretory cells) that are similar to the secretory structure (the black nodules) in the leaf of *H. perforatum* L, although under two different development environments, the differentiation of secretory structue, *in vivo* and *in vitro*, showed something different. Our study enriches the cytological knowledge of the process of dedifferentiation and differentiation and gives some useful information regarding mass-production of hypericins by tissue and cell engineering.

***Keywords*:** *Hypericum perforatum* L.; Hypericins; tissue and cell culture; dedifferentiation; ultrastructure

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Establishment and characterization of a human telomerase catalytic subunit-transduced fetal bone marrow-derived osteoblastic cell line

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The fate of human hematopoietic stem cells (HSCs)/progenitor cells (HPCs) is influenced by bone marrow (BM) stromal cells. To investigate the role of stromal cells in the hematopoietic support, we have transduced human fetal BM stromal cells (FBMSCs) with human telomerase catalytic subunit (hTERT). One of the resultant cell lines was identified as osteoblasts, because it contained mineral deposits and constitutively expressed osteogenic genes osteocalcin, osteopontin, collagen type I, osteoblast marker alkaline phosphatase, but not marrow stromal cell marker STRO-1 and CD105. The hTERT-transduced fetal BM-derived osteoblastic cells (FBMOB-hTERT) can actively maintain the capacity of self-renewal and multipotency of HSCs/HPCs at least partly through transcriptional up-regulation of hematopoietic growth factors such as stem cell growth factors (SCFs) and Wnt-5A during interaction with HSCs/HPCs. The enhanced transcription of SCFs and Wnt5A appears to be mediated by CD29 signaling. Moreover, the FBMOB-hTERT cells seems superior to primary FBMSCs in supporting hematopoiesis, because they are more potent than primary FBMSCs in supporting the ex vivo expansion and LTC-IC activity of HSCs. The FBMOB-hTERT cell line has been maintained in vitro more than 125 population doublings (PDs) without tumorigenicity. The results indicate that the FBMOB-hTERT is useful for the study of molecular mechanisms by which osteoblasts support hematopoiesis.

***Keywords*:** hTERT; FBMSCs; osteoblast cell line; hema-

topoietic support; molecular mechanisms

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Polarization of human bone morrow-derived hepatocytes *in vitro*

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The polarity is a characteristic property of the hepatocyte and is essential for hepatocyte function in the liver. Bone marrow cells have the potential to differentiate to mature hepatocytes *in vitro*. Under induced condition, these cells express hepatocyte-specific markers CK8, CK18, ALB, and AFP. They are also capable of secreting ALB to the culture medium. However, less is known concerning their morphological arrangement and cell polarity established in the culture. In this study, we have developed an *in vitro* culture system to induce human bone marrow stem cells into mature hepatocytes. Human bone marrow cells were harvested and cultured in the presence of hepatocyte growth factor (HGF), fibroblast growth factor (FGF) and lymphocyte inhibitory factor (LIF). Hepatocyte-specific markers could be detected immunohistochemically in a 2-week induced culture. The cells started to produce biochemically detectable ALB on culture day 10, and the ALB level reached to a peak on day 12 to day 15. Immunohistological studies using antibodies against domain-specific membrane proteins demonstrated polarity during this time. The cells were capable of up-taking DiI AcLDL in 1 week of the culture. The distribution of DiI AcLDL was in an asymmetry pattern, indicating the direction of intracellular transportation. In conclusion, our study showed that *in vitro* differentiated hepatocytes not only exhibited biochemical function, but also presented polarized pattern of the hepatocyte. Therefore, this culture system may provide a model for studying the development and maintenance of polarity in this cell type. The hepatocytes induced from this system may contribute to a stable source of hepatocytes for clinical hepatocyte transplantation.

***Keywords*:** hepatocyte; polarization; stem cell

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Isolation, establishment and *in vitro* differentiation potential of porcine adipose mesenchymal stem cell

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The potential of adipose mesenchymal stem (AMSC) to replicate undifferentiated and to mature into distinct mesenchymal tissues suggests these cells as an attractive source for tissue engineering. Animals such as farm animals provide both a source of fat-derived stem cells and an immunocompetent, autologous host animal in which to investigate the capacity of the fat-derived cells to differentiate and form tissues *in vivo*. The objective was to establish a protocol for the isolation of porcine adipose mesenchymal stem cells (pAMSCs) from bone marrow and to demonstrate their in vitro differentiation into various mesenchymal tissue cells. pAMSCs were selected from the dorsal neck, shoulder, and back regions of Large-White pigs after extensive washing with PBS and treatment with collagenase. After 24h in primary culture in growth medium (DMEM + 10% FBS), pAMSCs adhered to the plastic surface. During cell cultre espansion, AMSCs mononuclear cells gave rise to adherent layers of fibroblast-like cells. The fifth passage cells were selected and Flow cytometry analysis demonstrated the following phenotype, CD44 (pos), CD105 (pos) and CD14 (neg), CD106 (neg), HLA-DR and S100 (neg), similar to that described for human AMSCs. AMSCs from passage 3 were selected for differentiation analysis. Differentiation along the osteogenic lineage was documented by deposition of calcium, visualization of alkaline phosphatase activity, and by analysis of osteogenic marker genes. adipocytes were identified morphologically, stained with oil red O and by analysis of adipogenic marker genes. Myogenic differentiation was assessed by using structure, histology and RT-PCR. Therefore, pAMSC may be introduced as a valuable model system with which to study the mesenchymal lineages for basic research and tissue engineering.

***Keywords*:** porcine; adipose mesenchymal stem cell; isolation; differentiation; cell culture

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Differentiation of embryonic progenitor cells into insulin-producing cells for diabetes therapy

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Insulin-dependent type 1 diabetes may be cured by transplantation of isolated human pancreatic islets and thus relived from tedious insulin injections associated with the risk of hypoglycemia. However, severe shortage of cadaveric pancreata has restricted application of this promising therapy. Consequently, alternative approaches toward generating functional insulin-secreting surrogate cells have been extensively explored, in particular using stem/progenitor cells that have potential to give rise to various cell lineages. We have differentiated early (E5.5-6.5) mouse embryo-derived progenitor cells into insulin-producing cells by a stochastic culture protocol. These cells expressed high level (~1/4 of that in adult pancreatic islets) insulin with equal molar amounts of C-peptide. They also expressed other typical beta-cell markers such as Pdx-1, Glut2, and glucokinase. Significantly, the cells are glucose-responsive *in vitro*, as reflected by induction of membrane depolarization, increasing cytosolic free Ca^{2+} levels and insulin release upon elevation of glucose concentrations, like mature islet beta-cells. The functionality of the insulin-producing cells was also assessed in streptozotocin-induced diabetic SCID mice (blood glucose levels > 400 mg/dL). Transplantation of the cells into the kidney sub-capsule or subcutaneously under the front limb in diabetic animals was capable of reversing the hyperglycemia. In addition, histological examinations revealed that there was no significant regeneration of native pancreatic islet beta-cells in cell-transplanted diabetic mice while insulin-producing cells were observed in the removed cell grafts. Furthermore, the blood insulin concentration was dramatically reduced in diabetic mice, but elevated to a level higher than pre-diabetic after the insulin-producing cells were transplanted. On the other hand, hyperglycemia recurred when the subcutaneous cell grafts were removed. Correspondingly, removal of the cell grafts rendered the blood insulin levels in these mice hardly detectable, indicating functional hyperglycemia-lowering activity of transplanted insulin-producing cells. These results have demonstrated a novel strategy that the insulin-producing cells can be differentiated from the mouse embryo progenitor cells and that these cells, like normal pancreatic islet beta-cells, are glucose-responsive and capable of correcting hyperglycemia in diabetes. This work points to the viability and may facilitate differentiation of functional insulin-secreting cells eventually from human embryonic stem cells for treatment of type 1 diabetes.

***Keywords*:** insulin-producing cell; stem cell; differentiation; diabetes; transplantation

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The effect of gamma irradiation on plant generation ability of irradiated rice calluses

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Rice is major food crop for more than one third of the world's population. Drought is a main constraint to rice production in rainfed and water-limited environments. Plant tissue and cell culture techniques in combination with induced mutation give the great possibilities for production of various types of mutants including those tolerances to abiotic stresses. The gamma irradiation of the rice calluses produced from Chichua 1 and C71 rice varieties was performed with the aim to produce drought resistant mutants. The calluses were produced from mature embryos of rice seeds on MS medium supplemented with 2 mg/l of 2.4-D. The obtained calluses were irradiated with 2, 3, 4, 5 krad using Co60 rays and then transferred onto regeneration medium containing MS salts + 0.2 mg/l NAA + 1mg/l BAP + 2 mg/l kinetin to generate mutant plants. In this report, the data on the effect of gamma irradiation on plant generation ability of irradiated rice calluses are presented. The obtained results showed that the calluses from two varieties react differently on the irradiation doses. For the C71, the plant generation was considerable decreased when the doses of gamma rays increased from 2 to 5 krad. In the case of Chichua 1 the generation of plants was effected only by high irradiation (5 krad) and the plant generation was not correlated with the used gamma doses. In overall, the C71 gave higher plant generation percentage (3.12 – 8.13 %) comparing to the Chichua 1 (2.38 – 5.79 %). The obtained mutants were screened with SSR and STS marker tightly linked to drought QTL regions. Many of the lines possessed the drought resistant markers. The obtained results indicated that the use of tissue culture, induced mutation and molecular techniques could be useful for production of drought mutants in rice.

***Keywords*:** gamma irradiation; generation ability; calluses; drought mutant; rice

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Committed differentiation of hair follicle bulge cells into sebocytes: an *in vitro* study

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Several studies showed that the hair follicle bugle cells could differentiate into the hair follicle and contribute to the formation of epidermis and the sebaceous gland. Although many lines of evidence suggest that the renewal and maintenance of the sebaceous gland depend upon hair follicle bulge cells, direct evidence supporting *in vitro* conversion of follicle bulge cells to sebaceous gland cells had not been available. To investigate the possibility of the differentiation of hair follicle bulge cells into sebocytes *in vitro*, we isolated and cultured vibrissa follicle bulge cells of rat and transferred them into the conditional medium. Afterwards, the morphological changes of cells were observed, the expression of epithelial membrane antigens was detected immunohistochemically, and the adipogenesis of cells was evaluated. Next we transfected the sebocyte-like cells with green fluorescent protein (GFPC1) plasmid carrying peroxisome proliferator-activated receptor γ2 (PPARγ2) gene and cultured the cells under the same experimental conditions to study the mechanism which regulates the differentiation of hair follicle bulge cells. The results showed that after about three weeks of induction culture, the cellular bodies enlarged gradually and contained abundant cytoplasm, lipid droplets appeared in the cytoplasm of some cells, and the cells resembled the sebocytes of sebaceous gland, exhibiting positive findings upon oil red O and EMA staining. The expression of PPARγ2 mRNA and protein were significantly upregulated in the PPARγ2 transfection group. The detection of oil red O staining and EMA expression demonstrated that the rate of positive cells was significantly higher in the PPARγ2 transfection group than the other two groups. In conclusions, hair follicle bulge cells of rat vibrissa may differentiate into sebocytes *in vitro*, during which period the PPARγ2 gene plays a crucial role.

Keywords**:** vibrissa hair follicle; stem cell; differentiation; sebocyte

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Embryogenesis of microspore derived multicells in *Capsicum annuum* L

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Microspores and derived multicells were isolated and cultured in modified liquid CP medium after a 15d's preculture of anthers on solidified medium. Thirty days later in suspension culture, embryoids with different developmental stages were formed. Fluorescence and light microscope observations revealed that these embryoids derived from microspores. After several symmetrical division of the nuclei of uninucleated microspores, multi-nuclei cells or multi-cells were formed, and developed further into embryoids. There were white hairs on the surface of pepper embryoids, and some embryoids showed low vigor while others showed normal by TTC staining. Plants can formed from torpedo and cotyledonary stage embryoids on solidified medium. Embryoids could be induced by 7 ºC， 32 ºC or 35 ºC treatment on anthers, but 32 ºC, 35 ºC condition were better. Flow cytometric analysis revealed that there were haploidy, doubled haploidy and haploid-diploid chimera in the regenerated plants.

Keywords**:** microspore; embryogenesis; multicells; cytological observation; *Capsicum annuum* L

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Differentiation of mesenchymal stem cells from cord blood into osteoblastic cells

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This study was carried out to isolate mesenchymal stem cells from full-term cord blood and to induce differentiation of the isolated mesenchymal stem cells into osteoblastic cells. Methods: After separation by Ficoll Hypaqe, the cord blood mononuclear cells were cultured. The culture medium was exchanged every week. The adherent cells were then collected and expanded. Differentiation of cord blood mesenchymal stem cells were induced towards osteoblastic cells by dexamethasone, ascorbic acid and beta glycerol phosphate. Results: The expanded cells were similar to fibroblastic cell and had low alkaline phosphatase activity. Differentiation occured after 12 days of induction and showed an enhanced activity of alkaline phosphatase. Conclusion: The results showed that cord blood mesenchymal stem cells could be an alternative source for bone marrow mesenchymal stem cells and be utilized in research and clinical studies.

Keywords**:** differentiation; mesenchymal stem cells; cord blood; osteoblastic cells

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Inducing embryonic stem cells to differentiate into pancreatic beta cells by a novel three-step approach

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Experimental induction of embryonic stem cells (ESCs) to become pancreatic beta cells can potentially provide ample resource for cell transplantation therapy of type I diabetes mellitus. Most of the previously reported induction strategies were long and complicated, and some required genetic manipulation. Moreover, it has been indicated that the insulin staining of ESC progeny was insulin uptake from the culture medium. We establish a three-step experimental approach based on the combination induction by activin A, all-trans retinoic acid, and other mature factors, which is able to induce murine ESCs to differentiate into insulin-producing cells in 2 weeks, and that insulin release of these induced cells is regulated by the glucose concentration. Our insulin-enhanced green fluorescent green protein reporter system excludes the possibility of insulin uptake. Transplantation of these ESC-derived insulin-positive cells can normalize blood glucose levels and rescue the survival of streptozocin-induced diabetic mice. Based on this, we recently further developed an improved method to induce human ES cells into insulin-producing cells. These findings offered a novel in vitro model to study the differentiation mechanism of pancreatic beta cells and a potential source of insulin-producing cells for transplantation therapy of type I diabetes mellitus.

***Keywords*:** ES cell; pancreatic beta cell

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Genes by oligonucleotide microarray during rat liver regeneration after partial hepatectomy

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To study the actions of the genes associated with mitochondrial apoptosis pathway in liver regeneration (LR) at transcriptional level. These genes were obtained by collecting the data of databases and referring to theses. Their expression changes in regenerating liver were checked by Rat Genome 230 2.0 array and identified by the sham operation. Their actions were analyzed by the systems biology approach. It was found that 108 genes were associated with liver regeneration, that they were classified into 5 and 14 groups separately based on similarity and time relevance of their expression, that their frequencies of up- and down-regulation were 372 and 159 respectively, and that the expression patterns of these genes exhibited 23 types. Their synergy values $E_{0.5\text{-}16,\ 30,\ 42,\ 66\text{-}168}$ calculated by the gene synergy model exceeded control. The above results showed the cellular physiological and biochemical activities in liver regeneration were complicated and staggered, and the mitochondrial apoptosis pathway worked mainly at the early stage, initial stage and post stage of liver regeneration.

***Keywords*:** partial hepatectomy (PH); rat genome 230 2.0 array; apoptosis; genes associated to liver regeneration; gene synergy

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Sorting nexin 10 induces giant vacuoles in mammalian cells

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Eukaryotic cells maintain a sophisticated network of intracellular membranous system to ensure the proper distribution and compartmentalization of cellular proteins critical for diverse functions such as cell division or cell-cell communication. Yet, little is known about the mechanism that regulates the homeostasis of this system. While analyzing the impact of sorting nexins on the trafficking of membrane type matrix metalloproteinases, we unexpectedly discovered that the expression of SNX10 induced the formation of giant vacuoles in mammalian cells. This vacuolizing activity is sensitive to mutations at the putative PI3P binding residue Arg53. Domain-swap experiments with SNX3 demonstratehat the PX binding domain of SNX10 alone is insufficient to generate vacuoles and the downstream CD domain is required for vacuolization. BFA, a chemical known to block the ER to Golgi transport, inhibited the vacuolization process. Together, these results suggest that SNX10 activity may be involved in the regulation of endosome homeostasis.

***Keywords*:** protein sorting; sorting nexins; endosomes

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A novel function of Id in neural stem cells

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Vertebrate neurogenesis is a stepwise process, including expansion of neural stem cell populations at the early stage and the following neuronal differentiation. At the early stage, negative bHLH transcription factors, Id and Hes, play similar roles in neural stem cell maintenance. However, little is known about the relationship between them. In this report, we show that at the early stage of neurogenesis, Id1-3 and Hes1 are co-expressed in the dorsal region of chick metencephalon and inhibit premature neuronal differentiation. Ectopic expression of Id2 can up-regulate endogenous Hes1 expression; conversely, inhibition of Id expression by RNA interference (RNAi) decreases Hes1 expression. Moreover, inhibition of Hes1 expression by RNAi attenuates Id's inhibitory effect on neuronal differentiation. Co-immunoprecipitation and electrophoresis mobility-shift assay (EMSA) reveal that Id proteins can directly interact with Hes1 through HLH domain, and inhibit Hes1's DNA-binding activity. The reporter assays in chick brain and P19 cells show Id can suppress self-feedback inhibition of Hes1 on its own promoter. Our results identify the regulatory relationship between Id and Hes1, and reveal a novel mechanism of Id functions at the early stage of neurogenesis.

***Keywords*:** Id; Hes1; negative-feedback loop; chick embryo; metencephalon

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Transdifferentiation of bone marrow-derived cells into hepatocytes *in vitro*

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The differentiation potential of adult stem cells has long been believed to be limited to the tissue or germ layer of their origin. However, recent studies have demonstrated that adult stem cells may encompass a greater potential then once thought. Multiple studies indicated that bone marrow include a large variety of progenitor cells which are capable of differentiating into a number of lineages under proper treatments, including skeletal myoblasts, cardiac myoblasts, endothelium, lung, gut, skin epithelia, and neuron ectodermal cells. In our study, we examined whether mouse bone marrow-derived cells are able to differentiate into functional hepatocyte-like cells *in vitro*. Mouse bone marrow mononuclear cells (MNCs) were isolated from mice femurs and tibias, and cultured in IMDM supplemented with 10% fetal bovine serum. To induce hepatic differentiation, we designed several novel protocols, including a combination of cytokines (an optimization model at 20 ng/ml HGF, 10 ng/ml FGF-4 and 10 ng/ml OSM), culturing cells in a conditional medium from the injured liver, a conditional medium of hepatocytes, or liver-injured mouse sera. Cells at different time points of differentiation were harvested and subjected to examine the hepatocyte characteristics by a variety of evidence at morphological and molecular levels. Morphologically, epithelioid and binucleated cell colonies appeared after several days of induction, and hepatocyte characteristics such as the expression of AFP, HNF-3β, CK19, CK18, ALB, TAT and G-6-Pase were detected during the differentiation at the mRNA and/or protein levels by RT-PCR and immunocytochemistry, respectively. Functionally, the differentiated cells demonstrated typical characteristics of liver cells, including glycogen storage and urea secretion. Moreover, the hepatic differentiation of Mesenchymal stem cells and Hematopoietic stem cells isolated from MNCs were further investigated, and the results suggested that both MSCs and HSCs are able to transdifferentiate into hepatocyte-like cells. Besides, the differentiated cells were transplanted into liver-injured mice, and the liver function was restored 2~7 d after transplantation. The present study revealed that bone marrow origin stem cells can be induced to differentiate into functional hepatic cells under proper treatments. The induction systems we established for derivation of hepatic cells exhibited several advantages: (1) There is no problem of limited donors as bone marrow cells can be obtained easily; (2) Use of bone marrow derived cells overcomes many moral and ethical barriers of ES cell manipulation; (3) Utilizing bone marrow cells from patients themselves could avoid immunological rejection

in transplantation. We hope that these findings may serve as a model system for the study of mechanisms involved in stem cell transdifferentiation, and contribute to the improvement of cell-based therapies for acute and chronic end-stage liver disease.

***Keywords*:** transdifferentiation; bone marrow; stem cells; hepatocyte; *in vitro*