

Mesenchymal Stem Cells: *In Vitro* Differentiation among Bone and Cartilage Cell Lineages

Mohamadreza Baghaban Eslaminejad, Ph.D.¹, Poopak Eftekhari Yazdi, Ph.D.

Stem Cells Department, Royan Institute

¹ Corresponding Address: P.O. Box: 19395-4644, Stem Cell Department, Royan Institute, Tehran, Iran

Email: bagesla@yahoo.com

Abstract

Received: 1/May/2007, Accepted: 5/Jul/2007

The capacity of mesenchymal stem cells (MSCs) to differentiate among skeletal cell lineages and to undergo extensive proliferation *in vitro* renders them an appropriate source for cell-replacement therapy to heal defects of bone and cartilage tissue. It is argued that in bone and cartilage defects, MSCs would better be transplanted as fully-differentiated cells otherwise they may produce non-specific cells in defect sites. This notion may emphasize the importance of the studies considering *in vitro* bone and cartilage differentiation of MSCs. Indeed, the capacity of producing osteoblastic and chondrocytic cell lineage is among the earliest differentiation potentials of MSCs, being reported at first isolation of the cells. Recent studies, however indicate that MSCs could differentiate into more cell lineages than expected. The present study provides evidence for, *in vitro* potential of MSCs to differentiate into bone and cartilage cell lineages. As an introduction to the differentiation, the characteristics of MSCs have been described and MSCs differentiation reviewed. The culture condition for bone and cartilage differentiation, molecular regulation of the differentiation, signaling pathway involved during the differentiation, and the genes up-regulated upon bone and cartilage differentiation have also been described.

Keywords: Mesenchymal Stem Cells, Bone and Cartilage Differentiation, Signaling Pathway, Culture Condition

Yakhteh Medical Journal, Vol 9, No 3, Autumn 2007, Pages: 158-169

Introduction

Stem cell criteria

Stem cells are defined as undifferentiated somatic cells having the ability to produce varieties of fully-differentiated progenies in response to the appropriate environment either *in vitro* or *in vivo* (1). Nowadays, at least four criteria are considered for cells to be known as stem cells. Stem cells should have the ability to undergo extensive proliferation for a long time. This property is called as self-renewal potential. Stem cells should also have the capacity to undergo multilineage differentiation in the presence of appropriate culture condition, for example, mesenchymal stem cells represent the potential to generate fibroblast, osteoblast, chondrocyte and adipocyte. In addition, stem cells need to have the potential to regenerate a tissue defects upon transplantation. The last but not the least, stem cells should have the potential to produce fully-differentiated

progenies even in the absence of any tissue damages *in vivo* (2).

MSCs characteristics

MSCs, having first been isolated and described from bone marrow aspirates, are a multipotent cell population resided in many tissues of adult body. These cells were originally recognized by their ability to produce three distinct phenotypes of osteoblastic, chondroblastic and adipocytic cell lineages *in vitro* and *in vivo* (3, 4).

The first definitive discovery of mesenchymal stem cells was reported by Friedenstein (1974) who isolated the cells by their plastic adherent property and described them as a non-hematopoietic, clonogenic and fibroblastic cells having the capacity of producing osteoblastic, chondrocytic and adipocytic cell lineages (3, 4). The following studies indicate that MSCs occur in low quantity in bone marrow

aspirate, they constitute approximately 0.01%-0.001 of the whole bone marrow cells (5). In spite of their limited number, MSCs can easily be isolated owing to their plastic adherent property. The expansion of the cells is strongly dependent on bovine serum content of culture media. The cells assume spindly-shape morphology upon cultivation.

Mesenchymal stem cells (MSCs) have been successfully isolated from human, cat, dog, rabbit, rat, chicken, sheep, goat and pig bone marrows, thanks to their plastic adherence property (6-13). The isolation of murine mesenchymal stem cells is far more difficult than that of other species due to the unwanted growth of non-mesenchymal cells in both primary and passaged cultures (14, 15). To date, several protocols have been developed to purify the fibroblastic cells of murine bone marrow. The purified isolation of these cells would be important because they are suitable models for human disease (16-20). Recently, we have shown that the purified murine mesenchymal stem cells could be isolated by low-density primary culture system.

In the past, bone marrow derived-fibroblastic mesenchymal stem cells have been referred to by different terminology as cloney forming unit fibroblasts (CFU-F), marrow stromal fibroblasts (MSF), mesenchymal stem cells (MSC) and mesenchymal progenitor cells (MPC) (2, 3, 21-23). Nowadays, most denominations have been abandoned in favor of MSCs that have mostly been cited in various articles.

One of the defining characteristics of mesenchymal stem cells is their self-renewal potential, the ability to generate identical copies of themselves through mitotic division over extended periods. The absolute self-renewal potential of MSCs, however, remains an open question, due in large part to the different methods employed to derive populations of MSCs and the varying approaches used to evaluate their self-renewal capacity as a population (24). In contrast to embryonic stem cells that undergo indefinite proliferation, mesenchymal stem cells reach to senescence after a several cell doubling

in vitro. It has been shown that *ex vivo* expansion leads to progressive decrease of MSCs proliferation and loss of their multilineage differentiation potential. In this regard, Bruder et al reported that MSCs isolated from fresh bone marrow aspirates extensively expand *in vitro*, undergo senescence and a change in shape and morphology after about 38 population doublings. During differentiation, the adipogenic potential is the first to be lost while osteogenic potential is maintained even up to 38 population doublings (24-26). MSCs, as most somatic cells, do not express telomerase and as a result, their successive subcultures are characterized by progressive decrease of the telomere length and consequent cell aging.

Several studies provide evidence that the addition of FGF2 to culture of MSCs can delay the culture induced senescence in MSCs population by selecting a subpopulation of earlier progenitor among the total bone marrow stromal cells (27, 28). For this very reason, it is assumed that a stem cell compartment does reside in bone marrow within the MSCs population, but culture conditions or the environment so far have not been permissive to support its isolation and expansion.

The issue regarding MSCs for further investigation is the genetic signature. The phenotypes of MSCs are different among various laboratory studies and at present, there is a lack of unifying definition as well as information on specific markers that defines the cell types characterized as MSCs (29). In all studies concerning MSCs isolation, the only way to show the mesenchymal nature of the cells in question is examining the differentiation potential of the cells among the mesenchymal lineages by providing appropriate environment in culture. Recently, we indicated that Thy 1.2 surface antigen increases significantly during murine mesenchymal stem cells culture period and thus, could be used as enriching antigen for these cells (30).

MSCs differentiation potential

Heterogeneity of MSCs cultures

One of the specific characteristics of

MSCs is their colonogenic ability, even in primary culture. Individual colonies derived from single MSCs precursor have been reported to be heterogeneous in terms of their multilineage differentiation potentials. Reports indicated that only one-third of initial adherent bone marrow-derived MSC colonies is pluripotent and capable of differentiating into osteoblastic, chondrocytic, and adipocytic cell lineages, while the remainder display a bi-lineage (osteo/chondro) or uni-lineage (osteo) potential (31, 32). In this regard, the interesting point is that all colonies represent the bone differentiation potential. Baksh et al. proposes a model that may explain the heterogeneity of MSCs *in vitro*. According to their model, MSCs in bone marrow constitute the cells with different multilineage potential e.g. tri-, bi-, and uni-potential MSCs (33).

Multilineage differentiation potential of MSCs

MSCs differentiation is a complex process with a rather unknown molecular mechanism. It is believed that various parameters may involve in commitment of MSCs to differentiate into a particular cell lineages. These factors include the molecular composition of the serum, various treatments, the type of the plastic used in culture plate and the cell interactions. Providing the appropriate conditions, MSCs are able to generate variety of cells primarily including bone, cartilage and fat (34-39). Moreover, MSCs have been reported to enjoy more differentiation potential than that was originally expected, in that they can give rise to various cells such as neurons, keratinocytes, lungs, and intestines (40, 41). An investigation by Herrera et al (2004) has indicated that MSCs engrafted in the damaged kidney, even are able to differentiate into tubular epithelial cells and consequently promote the recovery of morphological and functional alterations (42). Such property is referred to as MSCs' plasticity or transdifferentiation.

Plasticity of MSCs

This property is defined as an ability of the cell to give rise to differentiated progeny other than the tissue cells of their origin.

All the studies documenting plasticity have used models of tissue injury to induce homing and differentiation of MSCs. To explain this property, being referred by others as transdifferentiation, three hypotheses have so far been proposed. One hypothesis states that, in contrast to previous notion considering the adult stem cells as multipotent, it seems that these cells are indeed pluripotent. According to another hypothesis, however, stem cell plasticity is a consequence of their dedifferentiation into more immature state and then differentiation into a tissue cells other than those they belong to. Finally, the third hypothesis indicates that plasticity occurs because stem cells simply fuse to fully-matured cells, called differentiated progeny of the stem cells (43-45).

Recently, a subclass of immature MSCs has been isolated from bone marrow aspirates and has been reported to possess much more *in vitro* differentiation potential than the ordinary MSCs described elsewhere. The cells, referred to as multipotent adult progenitor cells, (MAPCs) have indicated to be able to undergo indefinite proliferation owing to high telomerase activity and possess the potential of differentiation into cells of three germ layers. It has been shown that these cells are not positive to hematopoietic surface antigens such as Sca1 and c-kit and CD45 and express Oct-4 transcription factor similar to embryonic stem cells (46). The interesting point is that the methodology suggested for the isolation of MAPC cells was not applied by many other labs attempting to replicate the procedure.

Osteogenic differentiation of MSCs

The main function of bone in skeletal system is to provide structural support for the body as well as its vital organs. The bone is a main source of minerals and plays a main role during the muscle contraction. Considering these functions, the main changes in bone structure following the wound or any disease could influence the body homeostasis and compromise the individual life (47).

Most defects in bone tissue heal

spontaneously with minimal treatment but in certain cases it requires further treatment for compromised healing due to interposition of soft tissue, improper fracture fixation, loss of bone, metabolic disturbances, impairment of blood supply and infection. In such instances, bone grafts and or metallic implants are recommended (48). Obtaining of the bone is accompanied by probable risk of contamination (49-51) and the main concern regarding metal implant is that they would release harmful ions increasing the risk of cancer (52). The ability of MSCs in differentiating into osteoblastic lineages is appealing promise for cell-based treatment of bone defects especially those with large tissue loss. It is also believed that in bone defects, MSCs should be transplanted as fully differentiated cells, otherwise it may produce non-osteoblastic cells in defect sites. This notion may emphasize the importance of the studies considering *in vitro* bone differentiation of MSCs which has already gained rather considerable attention.

In vitro conditions required for MSCs osteogenesis

For bone differentiation of MSCs, the same procedure is followed in different labs. Usually, after isolation and purifying of MSCs, passageed-2-3 cells are grown into confluency using proliferation medium containing 10-15% fetal bovine serum. Then, the proliferation medium replaces with an osteogenic medium, containing 50µg/ml ascorbic acid 2-phosphate, 10nM dexamethasone and 10mM β-glycerol phosphate. The cultures subsequently were placed in an incubator at 37°C and 5% CO₂ for 21 days, with media changes of 3 times a week. To evaluate the occurrence of bone differentiation, at the end of the cultivation period, the cells fix with 10% formalin for 10 minutes and stain with alizarin red for 15 minutes at room temperature in order to examine the mineralized matrix. For further confirmation of differentiation, RNA extraction and RT-PCR analysis of osteocytic gene expression have also been proposed.

During osteogenesis in cultures, a few cells of monolayer culture usually become detached and float in the medium. Meanwhile, in

some areas of the culture dish, nodule-like structures form. Alizarin red stains red the mineralizing areas (the nodules) of the cultures. In these nodules, bone marker including collagen I, osteocalcin, parathyroid hormone (PTH) receptor, osteopontin, and bone sialoprotein can be detectable using RT-PCR analysis. Our recent morphologic investigation has shown that, in osteogenic nodules, collagen I fibers arrange in perpendicular bundles around the cells (unpublished data).

Molecular regulation of MSCs in vitro osteogenesis

The induction of osteogenesis is a highly programmed process, best illustrated *in vitro*. Treatment with the synthetic glucocorticoid dexamethasone stimulates MSC proliferation and supports osteogenic lineage differentiation (43-54). Organic phosphate, such as β-glycerophosphate, also supports osteogenesis by playing a role in the mineralization and modulation of osteoblast activities (55, 56). Free phosphates can induce expression of osteogenic markers such as osteopontin. The phosphates also exert specific effects on the production and nuclear export of a key osteogenesis regulatory gene, Cbfa1 (core binding factor alpha 1) (57-59). Other supplements, such as ascorbic acid phosphate and 1,25-dihydroxyvitamin D₃, are commonly used for osteogenic induction, with the latter involved in increasing alkaline phosphatase activity in osteogenic cultures and promoting the production of osteocalcin (60).

In addition to established supplements, members of the bone morphogenetic protein (BMP) family of growth factors are also routinely used for osteoinduction. BMP-2 alone appears to increase bone nodule formation and the calcium content of osteogenic cultures *in vitro*, while concomitant application of BMP-2 and basic fibroblast growth factor increases MSC osteogenesis both *in vivo* and *in vitro* (61, 62).

BMPs have been postulated to play a role in the selective differentiation of

mesenchymal precursors into either the osteoblastic or adipogenic lineages (63). Selective blocking of the BMP receptor type 1B (BMPR-1B) results in differentiation into adipocytic lineage rather than osteoblastic differentiation, suggesting that expression of BMPR-1B is required for mesenchymal stem cell commitment to the osteoblastic lineage. Conversely, over expression of BMPR-1A blocks adipogenic differentiation and promotes osteoblastic differentiation, suggesting that the temporal expression or loss of BMP receptor may play a key role in determining the lineage commitment of the mesenchymal precursors into osteoblasts or adipocytes (64).

There is a reciprocal relationship between adipocyte and osteoblast differentiation. Some investigations indicate that peroxisome proliferator-activated receptor γ (PPAR γ), a key transcription factor involved in adipocyte differentiation (65), negatively regulate osteoblast differentiation by repressing the osteoblast specific transcription factor Runx2 (66-75). PPAR γ exists in two isoforms PPAR γ 1 and PPAR γ 2 as a result of alternative splicing. PPAR γ 2 is expressed at high levels in fat tissue and is essential for adipogenesis *in vitro* and *in vivo*.

Signaling pathway involved in MSCs bone differentiation

The analysis of MSCs differentiation potential has been aided by the discovery of specific *in vitro* conditions for differentiation along different mesenchymal lineage. Osteogenic factors usually used in cultures are a combination of dexamethasone, β -glycerolphosphate and ascorbic acid phosphate.

Members of Wnt signaling pathway have been shown to participate in MSC osteogenesis (76, 77).

Wnts are a family of secreted cysteine-rich glycoproteins that have been implicated in the regulation of stem cell maintenance, proliferation, and differentiation during embryonic development. In the presence of the Wnt signal, the signaling pathway starts with the binding of the Wnt factor to the membrane bound receptor.

The receptor transfers the signal into cell and several other intracellular proteins activated. The best characterized effect is the inhibition of the GSK3 β .

In the normal cells, this inhibition leads to an accumulation of the protein β -catenin which is normally regulated by the APC protein. Next, β -catenin can form a complex with a transcription factor of TCF family. This complex enters the nucleus and activates transcription and proliferation. In the absence of Wnt signal, the kinase GSK3 β is not inhibited. GSK3 β phosphorylates protein APC; axin and β -catenin complex. The result is the decrease of the intracellular amount of the β -catenin. The transcription and thus the cell proliferation are not activated. The simplified representation of the wnt signaling pathway is shown in Fig 1.

Two members of Wnt family namely wnt3a and Wnt5a are known to involve in MSC proliferation and differentiation respectively. When MSCs are exposed to Wnt3a, a prototypic canonical Wnt signal (i.e. through intracellular β -catenin), under standard growth medium conditions, they show markedly increased cell proliferation and a decrease in apoptosis, consistent with the mitogenic role of Wnts in MSCs (77). Interestingly, exposure of MSCs to Wnt3a conditioned medium or overexpression of ectopic Wnt3a during osteogenic differentiation inhibits osteogenesis *in vitro* through β -catenin mediated down-regulation of TCF activity (77). In this regard, the interesting point is that the expression of several osteoblast specific genes including alkaline phosphatase, bone sialoprotein, and osteocalcin, is dramatically reduced, while the expression of cbfa1/Runx2, an early osteoinductive transcription factor was not altered, implying that Wnt3a-mediated canonical signaling pathway is necessary, but not sufficient, to completely block MSC osteogenesis. On the other hand, Wnt5a, a typical non-canonical Wnt member, have been shown to promote osteogenesis *in vitro* (77).

Chondrogenic differentiation of MSCs

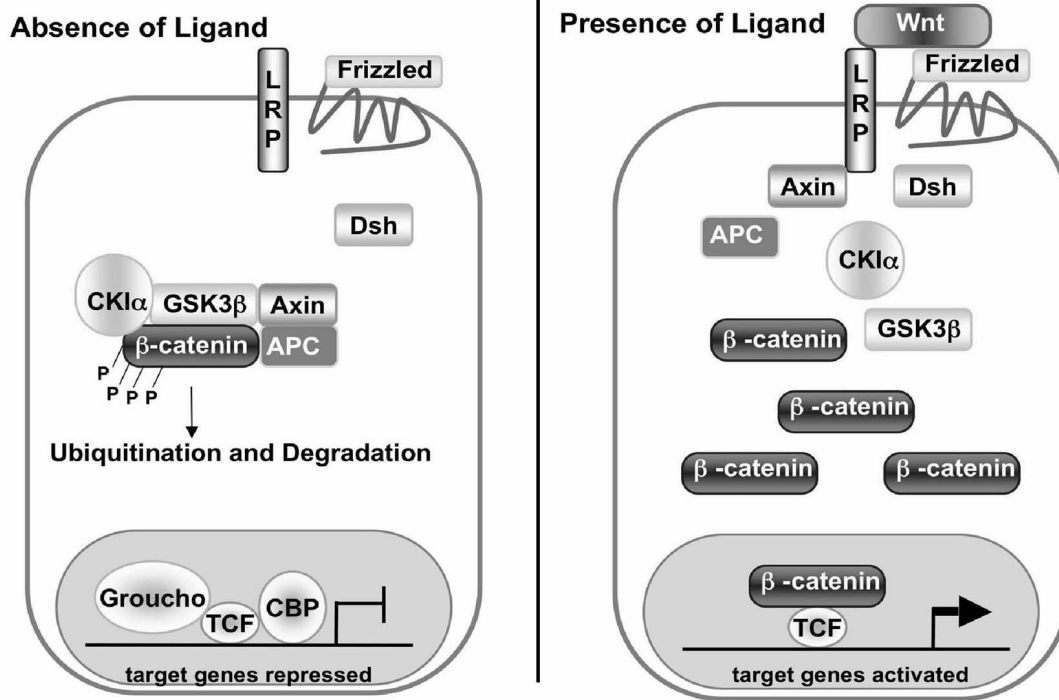


Fig 1: The simplified representation of the wnt signaling pathway

Cartilage tissue damages are a rather worldwide problem of many people and are produced by either trauma or age-dependent degenerative diseases (78, 79). Despite considerable attempts to find a way to improve cartilage regeneration, this issue still remains challenging in the field of regenerative medicine primarily due to the specific nature of cartilage biology (80, 81). The normal mechanism of tissue repair is not working on cartilage regeneration because the tissue is avascular. In natural process of tissue repair of living body, damaged tissue is regenerated by means of humeral and cellular elements brought to the spot from the adjacent areas as well as the intact cells of the tissue after injury. Regeneration of damaged cartilage tissue is impaired due to its inherent low cell density and avascularity (82-85). MSCs could be considered as an appropriate source for cell-based treatment of cartilage defects owing to their capacity in undergoing extensive self-renewal proliferation as well as the potential of giving rise to chondrocytic cell lineage.

In typical cell therapy strategies, the implanted cells are terminally differentiated cells that are intrinsic to the healing site. Therefore, one important step in preparing MSCs cells

for clinical use is providing the condition in which the cells can differentiate into mature cartilage before being implanted. This will guarantee the transplantation of only chondrocytic cells and therefore avoid the unwanted bone formation when undifferentiated cell is implanted.

In vitro conditions required for MSCs cartilage differentiation

Cartilage differentiation of MSCs is usually conducted in a micromass culture system (86-94). Approximately 200,000 cells (passage 2-3) are pelleted by centrifugation at 300 g for 4 minutes, followed by incubation at 37°C and 5% CO₂ in a 0.5 ml chondrogenic medium, composed of 10ng/ml transforming growth factor β 3, 500ng/ml bone morphogenetic protein-6, 100nM dexamethasone, 50 μ g/ml ascorbic 2-phosphate, 50 μ g/ml ITS and 1.25 mg/ml bovine serum albumin. The cultures are maintained for 3 weeks with medium change of 3-day intervals. At the end of this period, the pellets are usually analyzed for differentiation using toluidine blue staining for methachromatic matrix and RT-PCR analysis for cartilage-specific markers including collagen II,

aggrecan, and collagen X.

Molecular regulation of MSCs in vitro chondrogenesis

The induction of chondrogenesis in MSCs depends on the coordinated activities of many factors, including parameters such as cell density, cell adhesion, and growth factor. Culture conditions conducive for chondrogenic induction of MSCs require high-density pelleting and growth in serum free medium containing specific growth factors and supplements. The TGF- β super family of protein and their members, such as the bone morphogenetic proteins (BMPs) are well-established regulatory factors in chondrogenesis. TGF- β 1 was initially used for *in vitro* culture and can induce chondrogenesis under this conditions (95, 96), although TGF- β 3 has recently been shown to induce a more rapid and thorough expression of chondrogenic markers (97, 98). Another TGF- β family member, BMP-6, appears to increase the size and weight of pellet cultures and to increase amount of matrix proteoglycan produced (99). BMP-2 and BMP-9 have also been used in three-dimensional MSC culture systems, such as those seeded in the hydrogel alginate. It is under such conditions that they can induce markers of chondrogenesis (100).

Signaling pathway involved in MSCs cartilage differentiation

Recent studies have demonstrated that TGF- β , BMP-2, and growth differentiation factor-2 (GDF-2) rapidly induce type II collagen expression, suggesting critical roles of signaling by the TGF- β superfamily for chondrocyte-specific gene expression (101-103). In chondrogenic differentiation of MSCs, several TGF- β -dependent signaling pathways are involved. The Smad pathway is one of them that is widely represented in most of the cell types and tissue being studied (104). Investigations have shown that other additional pathways may be activated following treatment with TGF- β in specific contexts. Activation of Ras, extracellular signal- regulated kinase $\frac{1}{2}$ (ERK1/2), and c-Jun N-terminal kinase (JNK) by TGF- β signaling has been reported

in primary intestinal epithelial cells and chondrogenic cell line derived from mouse teratocarcinoma (105, 106). In an attempt to define the contribution of specific TGF- β dependent signaling pathway involved in the regulation of chondrogenesis from human mesenchymal stem cells, the ERK1/2 pathway was inhibited by U0126, a specific inhibitor of mitogen activated protein kinase 1/2 (MEK1/2), which is an upstream molecule that activates ERK1/2. For inhibition studies, U0126 was added with TGF- β 3 treatment at the same time. According to the results MEK inhibition resulted in complete down-regulation of type II collagen. In contrast, aggrecan expression was detected in the same level by treatment of U0126. It seems that type II collagen expression might be critically regulated by downstream signaling molecules of ERK1/2. It seems that ERK1/2 mediated signaling pathway might be one of the key signaling factors and the inactivation resulted in blocking of chondrogenesis of mesenchymal stem cells. Chondrogenesis of mesenchymal stem cell is regulated by complicated protein kinase signaling cascades. Recently, it has been reported that aggrecan gene expression was regulated by cross-talk between Smad, ERK1/2, and p38 mitogen-activated protein kinase (MAPK) pathway but not protein kinase A (107).

Some evidences have indicated that Wnt and Wnt-related family of signaling proteins would also involve in adult cartilage formation. It has been reported that Wnt3a have chondro-stimulatory effects in mouse C3H10T1/2 cells. The other evidence for Wnt involvement in chondrogenesis is the identification of the constitutive expression of Wnt5a in pellet culture *in vitro* (108-111).

Genes involved in bone and cartilage differentiation of MSCs

In a study, Song and Tuan used affymetrix humangenomeU133arrayset and compared the transcriptum profiles associated with three mesenchymal lineages derived from human MSCs, namely, osteoblast, chondroblast and adipocyte, to that of uncommitted MSCs (33). Genes with 1.5 fold or higher level of increased expression during differentiation were selected and

categorized into three subclasses, depending on their upregulation in one, two, or all three lineages. Among 39,000 transcripts analyzed for osteogenesis, adipogenesis, and chondrogenesis, respectively, 914, 947, 52 genes increased their expression in one mesenchymal lineage, while 235, 3 and 10 genes shared upregulation expression between two lineages. Most interestingly, there are eight genes whose expression are increased during all three mesenchymal lineage differentiations, suggesting that they might function in all three lineages, and thus may represent the putative master control genes. These genes are identified as period homolog1 (PER1), nebullette (NEBL), neuronal cell adhesion molecule (NRCAM), FK506 binding protein 5 (FKBP5), interleukin 1 type II receptor (IL R2), zinc finger protein 145 (ZNF145), tissue inhibitor of metalloproteinase 4 (TIMP4), and serum amyloid A2. The function of these genes cover a broad range of cellular processes, including cell adhesion, protein folding, organization of actin microfilament, as well as inflammatory response, implying that initiation and commitment of adult stem cells is a complex process requiring the coordination of multiple molecules and signaling pathways.

Asymmetric division as the first event in MSCs commitment for differentiation

The key question about the MSCs differentiation is how and when MSCs tend to stop proliferation and initiate the differentiation. In response to this fundamental question, a model has been proposed that explains the regulation of adult stem cell differentiation (33). In this model, two continuous yet distinct compartments were considered for MSCs. In the first compartment named stem cell compartment, the cells remain quiescent and growth arrested in G0/G1, until stimulated for example by the supplementation of growth factors. At this time they undergo asymmetric division and, giving rise to daughter cells, one being the exact replica of the mother and maintaining multi-lineage potential and, the other daughter cell becoming precursor cell, with a more restricted developmental

program. Precursor cells continue to divide symmetrically, generating more tri-potent and bi-potent precursors that were morphologically similar to multi-potent cells and differed in their gene transcription repertoire, and as a result, still residing in the stem cell compartment. The exit from stem cell compartment to the committed compartment occurs when precursor cells divide symmetrically and generate uni-potent progenitor capable to differentiate into specific lineage. Indeed, the commitment compartment includes the cells with only one differentiation potential.

References

1. Fillip S, Mokry J, Hruska J. Adult stem cells and their importance in cell therapy. *Folia Biologica* 2003; 49: 9-14
2. Zech NH. Adult stem cell Manipulation and possible clinical perspectives. *Reproduc Med Endocrinol* 2004; 2: 91-99
3. Friedenstein AJ, Deriglasora UF, Kulagina NN, Panasuk AF, Rudakowa, Luria EA, Ruadkow IA. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay methods. *Exp Hematol* 1974; 2: 83-92
4. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panansyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hematopoietic tissues. *Cloning in vitro and retransplantation in vivo*. *Transplantation* 1974; 17: 331-40
5. Koc ON, Peters C, Aubourg P, Raghavan S, Dyhouse S, DeGasperi R, Kolodny EH, Yoseph YB, Gerson SL, Lazarus HM, Caplan AI, Watkins PA, Krivit W. Bone-marrow derived mesenchymal stem cells remained host-derived despite successful hematopoietic engraftment after allogeneic transplantation in patient with lysosomal and peroxisomal storage diseases. *EXP Hematol* 1999; 27:1675-1681
6. Awad HA, Butler DL, Boivine GP, Smith FN, Malaviya P, Huibregtse B, Caplan AI. Autologous mesenchymal stem cells mediated repair of tendon. *Tissue Eng* 1999; 5: 267-277
7. Devine SM, Bartholomew AM, Mahmud N, Nelson M, Patil S, Hardy W, Sturgeon C, Hewett T, Chung T, Stock W, Sher D, Weissman S, Ferrer K, Mosca J, Deans R, Moseley A, Hoffman R. Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. *Exp Hematol* 1999; 29: 244-255
8. Jessop HL, Noble BS, Cryer A. The

differentiation of a potential mesenchymal stem cells population within ovine bone marrow. *Biochem Soc Trans* 1994; 22: 248

9. Kadiyala S, Young RG, Thiede MA, Bruder SP. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. *Cell Transplant* 1997; 6:125-134

10. Martin DR, Cox NR, Hathcock TL, Niemeyer GP, Baker HJ: Isolation and characterization of multipotential mesenchymal stem cells from feline bone marrow. *Exp Hematol* 2002; 3: 879-886

11. Mosca JD, Hendricks JK, Buyaner D, Davis-Sproul J, Chuang LC, Majumdar MK, Chopra R, Barry F, Murphy M, Thiede MA, Junker U, Rigg RJ, Forestell SP, Bohnlein E, Storb R, Sandmaier BM. Mesenchymal stem cells as vehicles for gene delivery. *Clin Orthop* 2000; 379: 71-90

12. Ringe J, Kaps C, Schmitt B, Buscher K, Bartel J, Smolina H, Schultz O, Bumester GR, Haupl T, Sittlinger M. Porcine mesenchymal stem cells. Induction of distinct mesenchymal cell lineages. *Cell Tissue Res* 2002; 307:321-327

13. Schwarz EJ, Alexander GM, Prockop DJ, Azizi SA: Multipotential marrow stromal cells transduced to produce L-DOPA: engraftment in a rat model of Parkinson disease. *Hum. Gene Ther* 1999;10:2539-2549

14. Meirelles LDS, Nardi NB. Murine marrow derived mesenchymal stem cell: Isolation, in vitro expansion, and characterization. *Brit J Hemat* 2003; 123: 702-711

15. Phinney DG, Kopen G, Isaacson RL, Prockop DJ: Plastic adherent stromal cells from the bone marrow of commonly used strains of inbred mice: variations in yield, growth, and differentiation. *J Cell Biochem* 1999; 72: 570-585

16. Badoo M, Hill K, Wilkinson R, Gaupp D, Hughes C, Kopen GC, Phinney DG. Characterization of mesenchymal stem cells isolated from murine bone marrow by negative selection. *J Cellul Biochem* 2003; 89: 1235-1249

17. Eslaminejad MB, Nikmahzar A, Taghiyar L, Nadri S, Massumi M. Murine mesenchymal stem cells isolated by low density primary culture system. *Dev Growth Differ* 2006; 48: 361-370

18. Peister A, Mellad JA, Larsen LL, Hall BM, Gibson LF, Prockop DJ. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. *Blood* 2004; 103: 1662-1668

19. Tropel P, Noel D, Platet N, Legrand P, Benabid AL, Berger F. Isolation and characterization of mesenchymal stem cells from adult mouse bone marrow. *Exp Cell Res* 2004; 295: 395-406

20. Van Vlasselaer P, Falla N, Snoeck H, Mathieu E: Characterization and purification of osteogenic cells from murine bone marrow by two-color cell sorting using anti sca-1 monoclonal antibody and wheat germ agglutinin. *Blood* 1994; 84: 753-763

21. Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *J Cell physiol* 1999; 181: 67-73

22. Piersma AH, Brockbank KG, Ploemacher RE, Van Vliet E, Brakel-van Peer KM, Visser PJ. Characterization of fibroblastic stromal cells from murine bone marrow. *Exp Hematol* 1985; 13: 237-243

23. Prochop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissue. *Science* 1997; 276: 71-74

24. Bruder SP, Krause KH, Haynesworth SE. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem* 1997; 62: 247-294

25. Banfi A, Muraglia A, Dozin B, Mastrogiacomo M, Cancedda R, Quarto R. Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implication for their use in cell therapy. *Exp Hematol* 2000; 28: 707-715

26. Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate in vivo according to hierarchical model. *J Cell Sci* 2000; 113: 1161-1166

27. Bianchi G, Muraglia A, Daga A, Corte G, Cancedda R, Quarto R. Microenvironment and stem properties of bone marrow derived mesenchymal cells. *Wound Repair Regen* 2001; 9: 460-466

28. Martin I, Muraglia A, Campanile G, Cancedda R, Quarto R. Fibroblast growth factor-2 supports ex vivo expansion and maintenance of osteogenic precursors from human bone marrow. *Endocrinology* 1997; 138: 4456-4462

29. Tuan RS, Boland G, Tuli R. Adult stem cells and cell-based tissue engineering. *Arthritis Res Ther* 2003; 5: 32-45

30. Eslaminejad MB, Nadri S, Hajji Hosseini R. Expression of Thy 1.2 surface antigen increases significantly during the murine MSCs cultivation period. *Develop. Growth Differ.* 2007 49: 351-364

31. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Sciences* 1999; 248: 143-147

32. Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human marrow differentiation in vitro according to hierarchical model. *J Cell Sci* 2000; 113: 1161-1166

33. Baksh D, Song L, Tuan RS. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. *J Cell Mol Med* 2004; 3: 301-316

34. Takeuchi Y, Shibuya H, Gotoh Y, Matsumoto K, Kato S. Cytokines suppress adipogenesis and PPAR-gamma function through the TAK1/TAB1/NIK cascade. *Nature cell Biol* 2003; 5: 224-230

35. XU W, Zhaug X, Qian H, Sun W, Sun Y, Hu J, Zhou H, Chen Y. Mesenchymal stem cells from Adult human bone marrow differentiate in to cardiomyocyte phenotype in vitro. *Exp Biol Med* 2004; 229: 623-631
36. Wang PP, Wang JH, Yan P, Hu MY, Lau GK, Fan ST, Luk JM. Expression of hepatocyte-like phenotype in bone marrow stromal cells after HGF induction. *Bioch Biophys Res commun* 2004; 320: 712-716
37. Chen LB, Jiang XB, Yang L. Differentiation of rat marrow stem cells in to pancreatic islet beta-cells. *World J Gastroentrol* 2004; 10: 3016-3020
38. Kotto DN, Ma By, Cardoso W, Sanderson EA, Summer RS, Williams M.C, Fine A. Bone marrow-derived cells as progenitors of lung alveolar epithelium. *Development* 2001; 128: 1581-1588
39. Kicic A, Shen WY, Wilson AS, Constable IJ, Kobertson T, Rakoczy PE. Differentiation of marrow stromal cells into photoreceptors in the Rat eye. *J Neurosci* 2003; 23: 7742-7749
40. Sugaya, K. Potential use of stem cells in neuroreplacement therapies for neurodegenerative diseases. *Int Rev Cytol* 2003; 228: 1-30
41. Chapel A, Bertho JM, Bensidhoum M, Fouillard L, Young RG, Frick J, Demarquay C, Cuvelier F, Mathieu E, Trompier F, Dudoignon N, Germain C, Mazurier C, Aigueperse J, Borneman J, Gorin NC, Gourmelon P, Thierry D. Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J Gene* 2003; 5: 1028-1038
42. Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. *Int J Mol Med* 2004; 14: 1035-1041
43. Grove JE, Bruscia E, Krause DS. Plasticity of bone marrow-derived stem cells. *Stem cell* 2004; 22: 487-500
44. Orlic D, Hill JM, Arai AE. Stem cells for myocardial regeneration. *Circulation Res* 2002; 91: 1092-1102
45. Forbes SJ, Vig P, Poulsom R, Wright NA, Alison MR. Adult stem cell plasticity: new pathways of tissue regeneration become visible. *Clini Sci* 2002; 103: 355-369
46. Schwartz RE, Reyes M, Koodie L, Jiang y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu W, Verfaillie M. Multipotent adult progenitor cells from bone marrow differentiate in to functional hepatocyte-like cells. *J clini Investi* 2002; 109: 1291-1302
47. Salgado AJ, Coutinho OP, Reis RL. Bone tissue engineering: state of the art and future trends: *Macromol Biosci* 2004; 9:743-765
- 48-Logeart-Avramoglou D, Anagnostou F, Bizios R, Petite H. Engineering bone: challenges and obstacles. *J Cell Mol Med* 2005; 9:72-84
49. Rah DK. Art of replacing craniofacial bone defects. *Yonsei* 2000; 41:756-65
50. Hannouche D, Raould A, Nizard RS, Sedel L, Petite H. Embedding of bone samples in methylmethacrylate: A suitable method for tracking LacZ mesenchymal stem cells in skeletal Tissues. *J Histochem Cytochem* 2006; 55: 255-262
51. Shors EC. Coralline bone graft substitutes. *Orthop Clin North Am* 1999; 30: 599-613
- 52-Riminucci M, Banco P. Building bone tissue: matrices and scaffolds in physiology and biotechnology. *Braz J Med Biol Res* 2003; 36: 1027-36
53. Nuttall ME, Gimble JM. Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications. *Curr Opin Pharmacol* 2004; 4: 290-294
54. Friedenstein AJ. Osteogenic stem cells in bone marrow. *J of bone and Min Resear* 1990; 7: 243-272
55. Bennett JH, Joyner CJ, Triffitt JT, Owen ME. Adipocytic cells cultured from marrow have osteogenic potential. *J Cell Sci* 1991; 99:131-139
56. Beresford JN, Bennett JH, Devlin C, Leboy PS, Owen ME. Evidence for an inverse relationship between the differentiation of adipocytic and osteogenic cells in rat marrow stromal cell cultures. *J Cell Sci* 1992; 102:341-351
57. Gimble JM, Robinson CE, Wu X, Kelly KA. The function of adipocytes in the bone marrow stroma. *Bone* 1996; 19:421-428
58. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284: 143-147
59. Oyajobi BO, Lomri A, Hott M, Marie PJ. Isolation and characterization of human clonogenic osteoblast progenitors immunoselected from fetal bone marrow stroma using STRO-1 monoclonal antibody. *J Bone Miner Res* 1999; 14:351-361
60. Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI. A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. *J Bone Miner Res* 1999; 14: 700-709
61. Ahdjoudj S, Lasmoles F, Oyajobi BO, Lomri A, Delannoy P, Marie PJ. Reciprocal control of osteoblast/chondroblast and osteoblast/adipocyte differentiation of multipotential clonal human marrow stromal F/STRO-1(+) cells. *J Cell Biochem* 2001; 81: 23-38
62. Waterman-Storer CM, Karki SB, Kuznetsov SA, Tabb JS, Weiss DG, Langford GM, Holzbaur EL. The interaction between cytoplasmic dynein and dynactin is required for fast axonal transport. *Proc Natl Acad Sci U S A.* 1997; 94: 12180-12185
63. Ochi K, Wakisaka S, Youn SH, Hanada K, Maeda T. Calretinin-like immunoreactivity in the Ruffini endings, slowly adapting mechanoreceptors,

of the periodontal ligament of the rat incisor. *Brain Res* 1997; 769: 183-187

64. Chen D, Ji X, Harris MA, Feng JQ, Karsenty G, Celeste AJ, Rosen V, Mundy GR, Harris SE. Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. *J Cell Biol* 1998; 142: 295-305

65. Tontonoz P, Hu E, Spiegelman BM. Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor gamma. *Curr Opin Genet Dev* 1995; 5: 571-576

66. Gori F, Thomas T, Hicok KC, Spelsberg TC, Riggs BL. Differentiation of human marrow stromal precursor cells: bone morphogenetic protein-2 increases OSF2/CBFA1, enhances osteoblast commitment, and inhibits late adipocyte maturation. *J Bone Miner Res* 1999; 14: 1522-1535

67. Lecka-Czernik B, Gubrij I, Moerman EJ, Kajkenova O, Lipschitz DA, Manolagas SC, Jilka RL. Inhibition of Osf2/Cbfa1 expression and terminal osteoblast differentiation by PPARgamma2. *J Cell Biochem* 1999; 74: 357-371

68. Lecka-Czernik B, Moerman EJ, Grant DF, Lehmann JM, Manolagas SC, Jilka RL. Divergent effects of selective peroxisome proliferator-activated receptor-gamma 2 ligands on adipocyte versus osteoblast differentiation. *Endocrinology* 2002; 143: 2376-84

69. Gimble JM, Robinson CE, Wu X, Kelly KA. The function of adipocytes in the bone marrow stroma: an update. *Bone* 1996; 19: 421-428

70. Rzonca SO, Suva LJ, Gaddy D, Montague DC, Lecka-Czernik B. Bone is a target for the antidiabetic compound rosiglitazone. *Endocrinology* 2004; 145: 401-406

71. Lazarenko OP, Rzonca SO, Suva LJ, Lecka-Czernik B. Netoglitazone is a PPAR-gamma ligand with selective effects on bone and fat. *Bone* 2006; 38: 74-84

72. Ali AA, Weinstein RS, Stewart SA, Parfitt AM, Manolagas SC, Jilka RL. Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation. *Endocrinology* 2005; 146: 1226-1235

73. Sottile V, Seuwen K, Kneissel M. Enhanced marrow adipogenesis and bone resorption in estrogen-deprived rats treated with the PPAR gamma agonist BRL49653 (rosiglitazone). *Calcif Tissue Int* 2004; 75: 329-337

74. Akune T, Ohba S, Kamekura S, Yamaguchi M, Chung UI, Kubota N, Terauchi Y, Harada Y, Azuma Y, Nakamura K, Kadowaki T, Kawaguchi H. PPARgamma insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors. *J Clin Invest* 2004; 113: 846-855

75. Marie PJ, Kaabeche K. PPAR Gamma Activity and Control of Bone Mass in Skeletal Unloading. *PPAR Res* 2006; 3: 64807

76. De Boer J, Wang HJ, Van Blitterswijk C. Effects of Wnt signaling on proliferation and differentiation of human mesenchymal stem cells. *Tissue Eng* 2004; 10: 393-401

77. Boland GM, Perkins G, Hall D, Tuan RS. Wnt 3a promotes proliferation and suppresses differentiation of adult human mesenchymal stem cells. *J Cell Biochem* 2004; 15: 1210-1230

78. Eisenmann, DM. Wnt signaling, WormBook, ed. The C. elegans Research Community, WormBook, 2005; doi/ 10.1895/ wormbook.1.7.1

79. Buckwalter JA. Articular cartilage injuries. *Clin Orthop* 2002; 402: 21-37

80. Martin JA, Buckwalter JA. Aging, articular cartilage chondrocyte senescence and osteoarthritis. *Biogerontology* 2002; 3: 357-264

81. Buckwalter JA, Mankin HJ. Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. *Instr course Lect* 1998; 47: 487-504

82. Frenkel SR, Di Cesare PE. Scaffolds for articular cartilage repair. *Am Biomed Eng* 2004; 32: 26-34

83. Hardingham T, Tew S, Murdoch A. Tissue engineering: chondrocytes and cartilage. *Arthritis Res* 2002; 4: 563-568

84. Mitchell N, Shephard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. *J Bone joint surg Am* 1976; 58: 230-233

85. Mankin H, Mow V, Buchwalter J. Form and function of articular cartilage. In: Simon S, ed. *Orthopaedic Basic science*. Rosemont IL: American Academy of orthopaedic surgeons 1994; 443-470

86. Frenkel SR, Clancy RM, Ricci JL, Di Cesare PE, Rediske JJ, Abramson SB. Effects of nitric oxide on chondrocyte migration, adhesion, and cytoskeletal assembly. *Arthritis Rheum* 1996; 39: 1905-1912

87. Manning WK, Bonner WMJR. Isolation and culture of chondrocytes from human adult articular cartilage. *Arthritis Rheum* 1967; 10: 235-239

88. Johnstone B. Mesenchymal stem cells and chondrogenesis. *Europ Cell Mat* 2002; 27

89. Mackay AM, Beck SC, Murphy JM, Barry FP, Chichester CO, Pittenger MF. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng* 1998; 4: 415-428

90. Carlberg AL, Pucci B, Rallapalli R, Tuan RS, Hall DJ. Efficient chondrogenesis of mesenchymal cells in micromass culture by retroviral gene transfer of BMP-2. *Differentiation* 2001; 67: 128-138

91. Bosnakovski D, Mizuno M, Kim G, Ishiguro T, Okumura M, Iwanaga T, Kadosawa T, Fujinaga T. Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells in pellet culture system. *Exp Hematol* 2004; 32: 502-509

92. Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T. Gene expression profile of bovine bone marrow mesenchymal stem cell during

- spontaneous chondrogenic differentiation in pellet culture system. *JPN J Vet Res* 2006; 53: 127-139
93. Indrawattana N, Chen G, Tadokoro M, Shann LH, Ohgushi H, Tateishi T, Tanaka J, Bunyaratvej A. Growth factor combination for chondrogenic induction from human mesenchymal stem cell. *Biochem Biophys Res Commun* 2004; 320: 914-919
94. Yoo UY, Barthel TS, Nishimura K, Solchaga L, Caplan AI, Goldberg VM, Jhonstone B. The chondrogenic potential of human bone-marrow-derived mesenchymal progenitor cells. *J Bone Joint Surg* 1998; 12: 1745-1757
95. Eslaminejad MB, Nikmahzar A, Piriea A. The structure of human mesenchymal stem cells differentiated into cartilage in micro mass culture system. *Yakhteh* 2006; 8: 162-171
96. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. In vitro chondrogenesis of bone marrow-derived mesenchymal stem cells. *Exp Cell Res* 1998; 238: 265-275
97. Cassiede P, Dennis JE, Ma F, Caplan AI. Osteochondrogenic potential of marrow mesenchymal progenitor cells exposed to TGF- β 3 or PDGF-BB as assessed in vivo and in vitro. *J Bone Miner Res* 1996; 11: 1264-1273
98. Barry F, Boynton RE, Liu B, Murphy JM. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation dependent gene expression of matrix component. *Exp Cell Res* 2001; 268: 189-200
99. Macky AM, Beck SC, Murphy JM, Barry FP, Chester CO, Pittenger MF. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng* 1998; 4: 415-428
100. Sekiya I, Colter DC, Prockop DJ. BMP-6 enhances chondrogenesis in a subpopulation of human marrow stromal cells. *Biochem Biophys Res Commun* 2001; 284: 411-418
101. Majumdar MK, Wang E, Morris EA. BMP-2 and BMP-6 promotes chondrogenic differentiation of human multipotential mesenchymal cells and overcomes inhibitory effect of IL-1. *J Cell Physiol* 2001; 189: 275-284
102. Shea CM, Edgar CM, Einhorn TA, Gerstenfeld LC. BMP treatment of C3H10T1/2 mesenchymal stem cells induces both chondrogenesis and osteogenesis. *J Cell Biochem* 2003; 90: 1112-1127
103. Ma HL, Hung SC, Lin SY, Chen YL, Lo WH. Chondrogenesis of human mesenchymal stem cells encapsulated in alginate beads. *J Biomed Mater Res* 2003; 64: 273-281
104. Fukumoto T, Sperling JW, Sanyal A, Fitzsimmons JS, Reinholz GG, Conover CA, O'Driscoll SW. Combined effects of insulin-like growth factor-1 and transforming growth factor-beta1 on periosteal mesenchymal cells during chondrogenesis in vitro. *Osteoarthritis Cartilage* 2003; 11: 55-64
105. Hatakeyama Y, Nguyen J, Wang X, Nuckolls GH, Shum L. Smad signaling in mesenchymal and chondroprogenitor cells. *J Bone Joint Surg Am* 2003; 85-A Suppl 3:13-18
106. Sanders MA, Basson MD. Collagen IV-dependent ERK activation in human Caco-2 intestinal epithelial cells requires focal adhesion kinase. *J Biol Chem* 2000; 275: 38040-38047
107. Nakamura K, Shirai T, Morishita S, Uchida S, Saeki-Miura K, Makishima F. P38 mitogen-activated protein kinase functionally contributes to chondrogenesis induced by growth/differentiation factor-5 in ATDC5 cells. *Exp Cell Res* 1999; 250: 351-363
108. Watanabe H, de Caestecker MP, Yamada Y. Transcriptional cross-talk between Smad, ERK1/2, and p38 mitogen-activated protein kinase pathways regulates transforming growth factor-beta-induced aggrecan gene expression in chondrogenic ATDC5 cells. *J Biol Chem* 2001; 276: 14466-14473
109. Rudnicki JA, Brown AM. Inhibition of chondrogenesis by Wnt gene expression in vivo and in vitro. *Dev Biol* 1997; 185: 104-118
110. Tufan AC, Tuan RS. Wnt regulation of limb mesenchymal chondrogenesis is accompanied by altered N-cadherin-related functions. *FASEB J* 2001; 15: 1436-1438
111. Fischer L, Boland G, Tuan RS. Wnt 3a enhances bone morphogenetic protein-2-mediated chondrogenesis of murine C3H10T1/2 mesenchymal cells. *J Biol Chem* 2002; 277: 30B70-30B78