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ROLE OF MECHANICAL STRAIN AND ESTROGEN IN MODULATING OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS (MSCS) FROM NORMAL AND OVARIECTOMIZED RATS

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Abstract

Bone's adaptability to loading depends upon the process of bone remodeling. This adaptive mechanism is restricted in post-menopausal osteoporosis. Differentiation of mesenchymal stem cells (MSCs) is crucial to bone remodeling and regeneration. It is well accepted that mechanical loading influences the fate of MSC differentiation. The aim of this study was to explore the possible restricted mechanism in osteoporotic condition, through investigating response of MSCs from both sham-operated and ovariectomized rats. MSCs were exposed to estrogen and mechanical strain (2%, 1Hz, 6h/day) for 3 days. Osteogenic differentiation and β-catenin protein in MSCs were examined. Exposure to estrogen and mechanical strain alone enhanced expression of Runx2 (Cbfα1), type I collagen (ColI) and activated β-catenin protein in MSCs from both sham-operated and OVX rats. MSCs from both sham-operated and OVX rats stimulated with both mechanical strain and estrogen had higher expression of osteogenic genes and activated β-catenin protein than these cells exposed to estrogen and mechanical strain alone. Osteoporotic MSCs had lower expression of osteogenic genes and protein in the absence and presence of stimulation than did MSCs from sham-operated rats. Cumulatively, our results indicate that mechanical strain and estrogen in vitro enhance osteogenic potential and activation of β-catenin in MSCs from both sham-operated and OVX rats. Estrogen augments strain-induced osteogenic potential and activity of β-catenin in MSCs.

Key words: Mechanical strain, estrogen, postmenopausal osteoporosis, mesenchymal stem cells (MSCs), β-catenin.

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INTRODUCTION

Mesenchymal stem cells (MSCs) are capable of differentiating into specialized cell lineages, such as osteoblasts, adipocytes and chondrocytes (19). Normally, the process of bone remodeling depends upon communication between osteoblasts and osteoclasts, including bone formation and bone resorption (17). Osteoblasts, which are derived from mesenchymal stem cells in bone marrow, contribute to build and repair bone (1). Therefore, osteogenic differentiation of MSCs influences balance of bone formation and bone resorption. Postmenopausal osteoporosis is characterized by increase in bone loss and risk of fracture due to imbalance between bone formation and resorption. Our previous studies and other reports demonstrated that bone marrow adipocity increase, and osteogenic potential of osteoporotic MSCs decrease in osteoporotic condition (12, 13, 22). In vitro, estrogen augments expression of osteogenic genes for Runx2 (Cbfα1), ALP, type I collagen (ColI) and osteocalcin (OCN) in MSCs (4, 7, 29). So, the fate of MSCs is critical to recover from postmenopausal osteoporosis.

Mechanical signals play an important role in bone growth, remodeling and healing (18, 26). There is increasing attention that mechanical strain controls differentiation of MSCs, which influences bone regenerative potential. For example, mechanical strain influences osteoblastogenesis of MSCs by up-regulation of transcription factor Runx2, osteoblast genes and growth factors in MSCs (8, 20). These suggest that mechanical strain favors increasing osteoblastogenesis of MSCs. It may be that osteogenic potential of MSCs links with restricted mechanism of adaptation to loading in osteoporotic condition. However, few studies have been focused attention on MSCs response to mechanical strain associated with hormonal status. The mechanism of MSC differentiation response to mechanical strain in etiology of condition remained to be fully defined.

Runx2, a member of Runt family of transcription factor, is essential for MSC differentiation towards osteoblasts. Type I collagen and osteocalcin not only are important bone matrix protein genes, also osteoblast special genes that distinguish from other cell types in differentiated process (10, 16). β-catenin, a key node of classical Wnt signaling pathway, plays an essential role in regulating osteoblastogenesis of MSCs in vivo. β-catenin translocates to nucleus, where it interacts with transcription factors and regulates downstream genes (15). In the present study, differentiation and activity of β-catenin were examined in MSCs from both sham-operated and OVX rats exposed to estrogen and mechanical strain. This study investigated that the effect of estrogen on strain-induction of osteogenic potential of MSCs in rats.

MATERIALS AND METHODS

Animal Models and Cell Culture

Three-month-old female Sprague-Dawley rats underwent bilateral ovariectomy, and sham-operated animals (SHAM) underwent the same surgical procedures without excision of ovaries. After three months, ovariectomized rats (OVX) were induced osteoporotic condition. MSCs were isolated from sham-operated and OVX animals as described previously (25). Briefly, bone marrow was flushed from femur and tibia with Dulbecco's Modified Eagle's Medium-low glucose (phenol red-free DMEM-LG; Gibco) and centrifuged at 720g for 10 minutes. Cells

Table 1. Sequences of primers for real-time PCR.

mRNA	Forward primer	Rever primer	product size (bp)
GAPDH	CAACTCCCTCAAGATTGTCAGCAA	GGCATGGACTGTGGTCATGA	118
Runx2	GCCGGGAATGATGAGAACTA	GGACCGTCCACTGTCACTTT	200
ColI	TGCTGCCTTTTCTGTTCCTT	AAGGTGCTGGGTAGGGAAGT	179
OCN	AGCTCAACCCCAATTGTGAC	AGCTGTGCCGTCCATACTTT	190

were resuspended in DMEM-LG supplemented with 15% charcoal stripped fetal bovine serum (FBS; Biological Industries), 2mM L-glutamine (Gibco), 100U/ml penicillin and 100 μg/ml streptomycin, then cultured at 37°C in a humidified 5%CO_2 incubator. Non-adherent cells were removed by changing culture medium. Adherent cells reached 80% confluence and were harvested with 0.25% trypsin containing 0.02% EDTA, then subcultured into flask. The cells (P3-P4) were detected by flow cytometry, the results found that the cells were positive for CD90, CD44, CD29, and negative for CD31, CD45, CD11b. MSCs were exposed to 100 nM 17β-estradiol (Sigma-Aldrich) and mechanical strain.

Application of Mechanical Strain

The cells were detached by treatment with trypsin, and seeded onto culture chambers at a density of 4×10^5/well. These culture chambers with flexible silicon membranes had been pre-coated with type I collagen. The cells were incubated with DMEM-LG containing 2% charcoal stripped fetal bovine serum for 24h, then MSCs were subjected to 2% biaxial cyclic strain at rate of 1Hz for 3 days (6h/day). Unstrained cells (control) were maintained in chambers without strain.

Assessment of Runx2, Type I Collagen, and Osteocalcin Expression by Quantitative Real Time PCR

The mRNA expression of osteogenic genes for Runx2, type I collagen and osteocalcin was evaluated by Quantitative real time PCR. Total cellular RNA was extracted from MSCs using Trizol ragent according to manufacturer's instructions. Complementary (c) DNA was synthesized from RNA using reverse transcription kit (Bio-Rad, USA). The forward and reverse primers in this study are listed in Table1. The PCR was carried out in a total of 20μl reaction mixture contained 2μl of cDNA, 10μl SsoFast EvaGreen Supermix (Bio-Rad, USA), 1μl of each primer and 6μl of DEPC-treated water. PCR program was initiated by 95°C for 3min followed by 40 cycles of 15s at 95°C, 10s at primer-specific annealing, 72°C for 15s, and a melt curve analysis at 65°C-96°C. Samples were run in triplicate. The expression of GAPDH mRNA was used for normalization, and standardized on a dilution curve from cDNA samples.

Western Blotting

The cells were washed with ice cold PBS, and lysed in lysis buffer (150mM NaCl, 50mM Tris HCl, 0.5% sodium deoxycholate, 1% NP-40, 0.1% SDS and complete protease inhibitor tablets) for 30 min on ice. Samples were

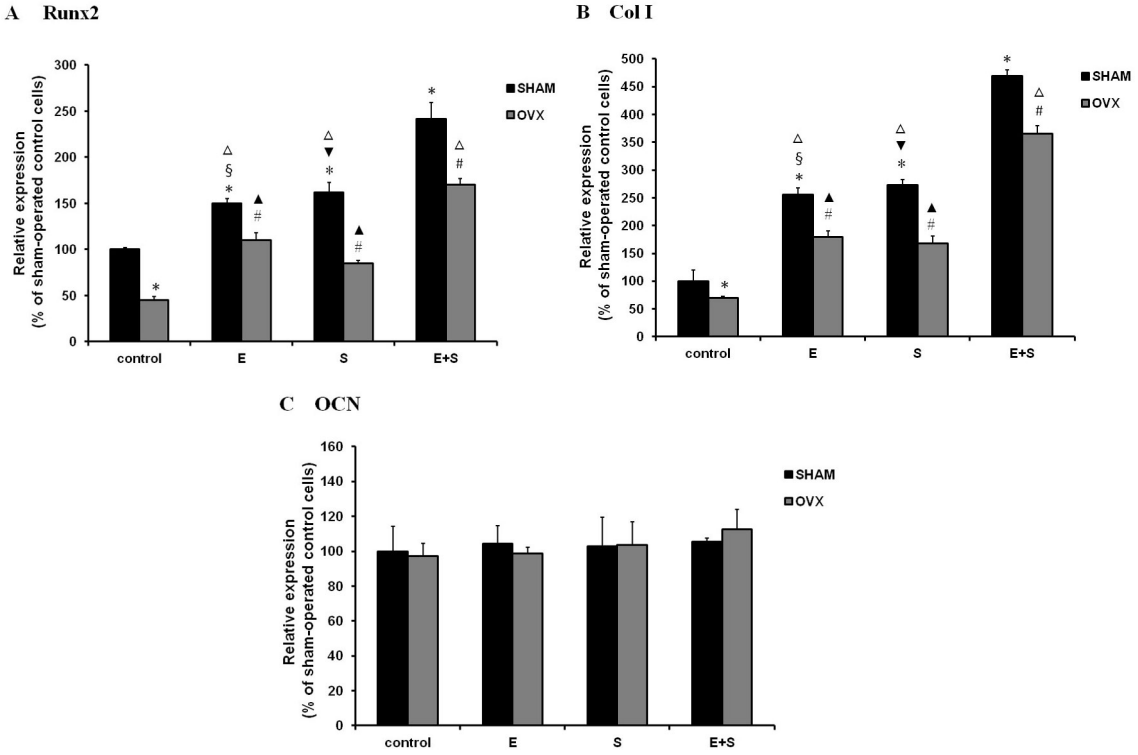

Figure 1. Effect of Estrogen and mechanical strain on expression of osteogenic gene in MSCs. MSCs were stimulated with estrogen (E) and mechanical strain (S) for 3 days. The mRNA expression of Runx2 (A), ColI (B) and OCN (C) were detected by quantitative real-time PCR. Results were normalized to mRNA expression of GAPDH and analyzed by ANOVA (*, $p< 0.01$ vs.sham-operated control cells. #, $p< 0.01$ vs.OVX control cells. §, $p< 0.01$ vs.OVX+E. ▼, $p< 0.01$ vs.OVX+S. △, $p< 0.01$ vs.SHAM+E+S. ▲, $p< 0.01$ vs.OVX+E+S).

centrifugated at 12000g for 5min at 4°C. Protein (15μg) was electrophoresed and transferred to PVDF membrane. These membranes were blocked with freshly prepared TBS/T containing 5% nonfat dry milk. Then membranes were incubated with primary antibodies overnight at 4°C. The primary antibodies were including anti-active-β-catenin (Millipore Corporation, Billerica, MA, USA), total β-catenin (Santa Cruz, USA) and β-actin (Santa Cruz, USA). The antibody used for active β-catenin was specific for active form β-catenin, dephosphorylated on Ser37 or Thr41. Membranes were washed with TBS/T, and incuba ted with secondary antibody conjugated with horseradish peroxidase for 1h at room temperature. Immunoreactive bands were visualized using ECL kit (Thermo Scientific, USA).

Statistical Analysis

All experiments were performed at a minimum of three times. Results are expressed as mean ± SEM. Statistical comparisons were performed using one-way ANOVA. A value of $p < 0.05$ was statistically considered significant.

RESULTS

MSCs from OVX rats had lower expression of osteogenic genes for Runx2, ColI than did MSCs from sham-operated rats with or without estrogen stimulation (Fig. 1). When MSCs from sham-operated rats were exposed to estrogen for three days, expression of Runx2 and ColI mRNA increased (50±5%, 155±13%, p<0.01). Estrogen also significantly increased expression of Runx2 and ColI mRNA in MSCs from OVX rats (144±19%, 159±15%, p<0.01) compared with OVX control. No effects on OCN mRNA level were observed with estrogen treatment for three days (Fig. 1C).

To examine the role of mechanical strain in MSC differentiation, MSCs from both sham-operated and OVX rats were subject to mechanical strain for three days (Fig. 1). The levels of Runx2 and ColI mRNA in MSCs from sham-operated rats were induced up-regulation by mechanical strain in comparison to corresponding control cells (62±11%, 173±9%, p<0.01). The level of Runx2 and ColI mRNA also increased in MSCs from OVX rats exposure to mechanical strain (89±6%, 142±18%, p<0.01), which

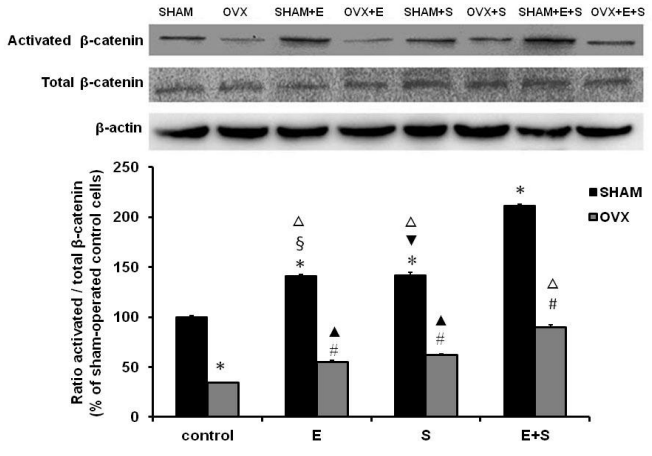

Figure 2. Effect of Estrogen and mechanical strain on activity of β-catenin in MSCs. β-catenin was examined by western blot. (*, p< 0.01 vs.sham-operated control cells. #, p< 0.01 vs.OVX control cells. §, p< 0.01 vs.OVX+E. ▼, p< 0.01 vs.OVX+S. △, p< 0.01 vs.SHAM+E+S. ▲, p< 0.01 vs.OVX+E+S).

were still significantly lower than did MSCs from sham-operated rats. There was a lack of significant effect of mechanical strain on OCN level in MSCs from both sham-operated and OVX rats.

To further know the effect of estrogen on response of MSCs to mechanical strain, MSCs from sham-operated and OVX rats were exposed to estrogen and mechanical strain in combination for 3 days. Shown in Figure 1, expression of Runx2 and ColI mRNA were higher in these cells, as compared with those which were exposed to estrogen and mechanical strain alone.

After OVX, expression of activated β-catenin in osteoporotic MSCs was significantly declined (Fig. 2), as compared with MSCs from sham-operated control rats. After the end of exposure to estrogen, expression of activated β-catenin protein in MSCs from both sham-operated and OVX rats significant increased compared with corresponding control (41±2%, 60±4%, p<0.01). Similarly, these results indicated that activated β-catenin protein significantly increase following mechanical strain in MSCs from both sham-operated and OVX rats (42±3%, 79±3%, p<0.01). Combined induction of activated β-catenin protein was higher than that of MSCs exposure to estrogen and mechanical strain alone. However, expression of activated β-catenin protein in osteoporotic MSCs was still lower than that of MSCs from sham-operated rats in the presence of stimulation.

DISCUSSION

After OVX, levels of Runx2 and ColI mRNA in MSCs were significantly decreased in this study. Estrogen stimulation resulted in increasing levels of osteogenic genes in MSCs obtained from both sham-operated and OVX rats. Runx2 is a critical transcription factor in osteoblastogenesis. Deletion of Runx2 gene in MSCs inhibits differentiation into osteoblasts, and maintains potential to adipogenesis and chondrogenesis (9). Runx2 influences expression or promoters activity of major bone matrix protein genes including type I collagen and osteocalcin (10, 11). OCN, which is secreted in the late stage of osteoblast differentiation, remained constant in this study. The increase in expression of Runx2 and type I collagen supported the evidence for differentiation towards osteoblast phenotype. In agreement with previous reports, estrogen deficiency decrease osteogenic potential of MSCs, and estrogen influence osteogenic potential of MSCs in vitro and vivo (4, 29).

Expression of activated β-catenin protein in MSCs from OVX rats was lower than that of MSCs from sham-operated rats, which was similar to osteogenic genes expression. β-catenin play a critical role in osteoblast lineage differentiation. β-catenin invalidation in mesenchymal progenitor cells completely inhibits differentiation towards osteoblasts, maintains potential to differentiation into chondrocytes (6). The activated of β-catenin inhibits adipogenesis by reducing expression of adipogenic transcription factors (23). In addition to bind to LEF/TCF transcription factors to regulate expression and promoter activity of Runx2 (5), β-catenin interacts with BMP2 to promote osteogenic differentiation (14). Expression of activated β-catenin protein increased in MSCs obtained from both sham-operated and OVX rats after estrogen stimulation, whereas osteoporotic MSCs had a lower level of activated β-catenin pro-

tein compared with MSCs from sham-operated rats. Our results further indicate that estrogen promotes MSCs in commitment to osteogenic lineage. The effect of estrogen on β-catenin coincided with that of Runx2, suggesting that effect of estrogen on β-catenin may play an important role in osteogenic differentiation of MSCs. Shuanhu Zhou (29) found that TGF-β1 and BMP-2 expression was reduced in OVX animal. Most growth factors, including TGF-β1 and BMP-2, take part in controlling osteoblastogenesis by modulating osteoblastic transcription factors expression or activity (11). These suggest that estrogen depreviation decreased the potential of MSCs to differentiation into osteoblast at the level of the regulating element.

To investigate the effect of mechanical strain on MSCs, we quantified expression of osteogenic genes in MSCs from sham-operated and OVX rats. Our results showed that mechanical strain augmented expression of Runx2 and ColI mRNA in MSCs. Previous studies also show that mechanical strain modulates osteogenic differentiation of MSCs (8, 18). Osteoporotic MSCs stimulated with mechanical strain had a lower osteogenic gene expression than did MSCs from sham-operated rats. This result suggests that osteogenic potential of osteoporotic MSCs in response to mechanical strain is lower than that of normal MSCs. However, the precise mechanism of mechanical response of MSC differentiation is not yet well understood. Recently, there were evidences to suggest that β-catenin involves in response to mechanical strain in osteoblasts, and β-catenin levels influence mechanical response in osteoblasts (2, 3, 21). Buer Sen and Case N (3, 24) found that mechanical strain inhibits adipogenisis of MSCs in adipogenic medium, and increases expression of target gene via activated β-catenin in osteogenic condition. These studies suggest that β-catenin play a role in bone cell's response to mechanical strain. Our results demonstrated that mechanical strain increased expression of activated β-catenin protein in MSCs. Expression of activated β-catenin protein in MSCs from OVX rats was lower than that of MSCs from sham-operated rats in the absence and presence of mechanical stimulation. These results suggest that osteogenic potential of osteoporotic MSCs in response to mechanical strain may be associated with level of activated β-catenin protein. The cascaded mechanism of β-catenin response to mechanical strain in MSCs remains to be fully defined.

Further studies showed that MSCs were subjected to both estrogen and mechanical strain. Our present study showed that estrogen augmented strain-inductions of osteogenic gene and activated β-catenin protein in MSCs. Chiuan-Ren Yeh and his team (27) showed that estrogen augments mechanical signals and gene expression induced by mechanical stimulation via increasing integrin expression in osteoblast-like cells. Other study found that estrogen receptor involves in response of osteocytes to both mechanical strain and estrogen (28). It is possible that estrogen pathway is cross-talk with mechanical signaling pathway in some signaling pathways, and estrogen influences mechanical response of bone cells by regulating element.

In summary, our results demonstrated that mechanical strain and estrogen could promote expression of Runx2 and ColI mRNA and activated β-catenin protein in MSCs from sham-operated and OVX rats, and estrogen augments strain-inductions of osteogenic gene and activated β-catenin protein in MSCs. Some information in our study may be helpful in understanding the mechanism of bone cells response to mechanical strain in estrogen depletion.

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REFERENCES

1. Caetano-Lopes, J., Canhão, H. and Fonseca, J.E., Osteoblasts and bone formation. *Acta Reumatol Port.* 2007, **32(2)**:103-110.

2. Case, N. and Rubin, J., Beta-catenin--a supporting role in the skeleton. *J Cell Biochem.* 2010, **110(3)**:545-553.

3. Case, N., Xie, Z., Sen, B., Styner, M., Zou, M., O'Conor, C., Horowitz, M. and Rubin, J., Mechanical activation of β-catenin regulates phenotype in adult murine marrow-derived mesenchymal stem cells. *J Orthop Res.* 2010, **28(11)**:1531-1538.

4. Chen, F.P., Hu, C.H. and Wang, K.C., Estrogen modulates osteogenic activity and estrogen receptor mRNA in mesenchymal stem cells of women. *Climacteric.* 2013, **16(1)**:154-160.

5. Gaur, T., Lengner, C.J., Hovhannisyan, H., Bhat, R.A., Bodine, P.V., Komm, B.S., Javed, A., van Wijnen, A.J., Stein, J.L., Stein, G.S. and Lian, J.B., Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. *J Biol Chem.* 2005, **280(39)**:33132-33140.

6. Hill, T.P., Spater, D., Taketo, M.M., Birchmeier, W. and Hartmann, C., Canonical Wnt/b-catenin signaling prevents osteoblasts from differentiating into chondrocytes. *Dev Cell.* 2005, **8**:727-738.

7. Jin, W.J., Jiang, S.D., Jiang, L.S. and Dai, L.Y., Differential responsiveness to 17β-estradiol of mesenchymal stem cells from postmenopausal women between osteoporosis and osteoarthritis. *Osteoporos Int.* 2012, **23(10)**:2469-2478.

8. Kearney, E.M., Farrell, E., Prendergast, P.J. and Campbell, V.A., Tensile strain as a regulator of mesenchymal stem cell osteogenesis. *Ann Biomed Eng.* 2010, **38(5)**:1767-1779.

9. Kobayashi, H., Gao, Y.h., Ueta, C., Yamaguchi, A. and Komori, T., Multilineage differentiation of Cbfa1- deficient calvarial cells in vitro. *Biochem Biophys Res Commun.* 2000, **273(2)**:630-636.

10. Komori T., Regulation of bone development and extracellular matrix protein genes by RUNX2. *Cell Tissue Res.* 2010, **339(1)**:189-195.

11. Komori, T., Regulation of Osteoblast Differentiation by Transcription Factors. *J Cell Biochem.* 2006, **99**:1233-1239.

12. Li, L., Li, D., Wu, J., Wu, W.C., Chen, H.Q. and Mao, Y.Q., A Potential Role for the Bone Marrow Mesenehymal Stem Cell in the Pathogenesis of Osteoporosis by Ovarietomy in Rat. *J Biomed Eng.* 2006, **23(1)**:129-135.

13. Li, X., Kuo, D., Schafer, A.L., Porzig, A., Link, T.M., Black, D. and Schwartz, A.V., Quantification of vertebral bone marrow fat content using 3 Tesla MR spectroscopy: reproducibility, vertebral variation, and applications in osteoporosis. *J Magn Reson Imaging.* 2011, **33(4)**:974-979.

14. Lin, G.L. and Hankenson, K.D., Integration of BMP, Wnt, and Notch signaling pathways in osteoblast differentiation. *J Cell Biochem.* 2011, **112(12)**: 3491-3501.

15. Ling L., Nurcombe, V., and Cool, S.M., Wnt signaling controls the fate of mesenchymal stem cells. *Gene.* 2009, **433**:1-7.

16. Marie, P. J., Transcription factors controlling osteoblastogenesis. *Arch Biochem Biophys.* 2008, **473**: 98-105.

17. Matsuo, K. and Irie, N., Osteoclast–osteoblast communication. *Arch Biochem Biophys.* 2008, **473(2)**: 201-209.

18. Ozcivici, E., Luu, Y.K., Adler, B., Qin, Y.X., Rubin, J., Judex, S. and Rubin, C.T., Mechanical signals as anabolic agents in bone. *Nat Rev Rheumatol.* 2010, **6(1)**:50-59.

19. Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Doug-

las, R., Mosca, J. D., Moorman, M. A., Simonetti, D. W., Craig, S. and Marshak, D. R., Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999, **284(5411)**:143-147.

20. Qi, M.C., Zou, S.J., Han, L.C., Zhou, H.X. and Hu, J., Expression of bone-related genes in bone marrow MSCs after cyclic mechanical strain: implications for distraction osteogenesis. *Int J Oral Sci.* 2009, **1(3)**:143-150.

21. Robinson, J.A., Moitreyee, C.K., Yaworsky, P.J., Cullen, D.M., Zhao, W.G., Li, C., Kharode, Y., Sauter, L., Babij, P., Brown, E.L., Hill, A.A., Akhter, M.P., Johnson, M.L., Recker, R.R., Komm, B.S. and Bex, F.J., Wnt/β-Catenin Signaling Is a Normal Physiological Response to Mechanical Loading in Bone. *J Biol Chem.* 2006, **281(42)**:31720-31728.

22. Rosen, C.J. and Bouxsein, M.L., Mechanisms of disease: is osteoporosis the obesity of bone? *Nat Clin Pract Rheumatol.* 2006, **2(1)**:35-43.

23. Ross, S.E., Hemati, N., Longo, K.A., Bennett, C.N., Lucas, P.C., Erickson, R.L. and MacDougald, O.A., Inhibition of adipogenesis by Wnt signaling. *Science.* 2000, **289(5481)**:950-953.

24. Sen, B., Xie, Zh., Case, N., Ma, M., Rubin, C. and Rubin, J., Mechanical Strain Inhibits Adipogenesis in Mesenchymal Stem Cells by Stimulating a Durable β-Catenin Signal. *Endocrinology* 2008, **149(12)**:6065-6075.

25. Soleimani, M. and Nadri, S., A protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. *Nat Protoc.* 2009, **4(1)**:102-106.

26. Thompson, W.R., Rubin, C.T. and Rubin, J. , Mechanical regulation of signaling pathways in bone. *Gene.* 2012, **503(2)**:179-193.

27. Yeh, C.R., Chiu, JJ., Lee, C.I., Lee, P.L., Shih, Y.T., Sun, J.S., Chien, S. and Cheng, C.K., Estrogen augments shear stress-induced signaling and gene expression in osteoblast-like cells via estrogen receptor-mediated expression of beta1-integrin. *J Bone Miner Res.* 2010, **25(3)**:627-639.

28. Zaman, G., Jessop, H.L., Muzylak, M., De Souza, R.L., Pitsillides, A.A., Price, J.S. and Lanyon, L.L., Osteocytes use estrogen receptor alpha to respond to strain but their ER alpha content is regulated by estrogen. *J Bone Miner Res.* 2006, **21(8)**:1297-1306.

29. Zhou, S.H., Zilberman, Y., Wassermann, K., Bain, S.D., Sadovsky, Y. and Gazit, D., Estrogen Modulates Estrogen Receptorα and β Expression, Osteogenic Activity, and Apoptosis in Mesenchymal Stem Cells (MSCs) of Osteoporotic Mice. *J Cell Biochem Supplement.* 2001, **36**:144-155.