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Effect of yttrium ion on the proliferation, differentiation and mineralization function of primary mouse osteoblasts *in vitro*

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Abstract: A series of experimental methods including MTT test, alkaline phosphatase (ALP) activity measurement, oil red O stain and measurement and mineralized function were employed to assess the effects of Y^{3+} on the proliferation, differentiation, adipogenic transdifferentiation and mineralization function of primary mouse osteoblasts (OBs) *in vitro*. The results indicated that Y^{3+} (1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L) promoted the proliferation of OBs on day 1, 2 and 3. Y^{3+} had no effect on the differentiation of OBs at concentrations of 1×10^{-9} and 1×10^{-8} mol/L, promoted the differentiation of OBs at concentration of 1×10^{-7} mol/L, but turned to inhibit the differentiation of OBs at other tested concentrations on day 1. On day 2, Y^{3+} inhibited the differentiation of OBs at all tested concentrations. On day 3, Y^{3+} promoted the differentiation of OBs at lower concentrations of 1×10^{-9} and 1×10^{-8} mol/L, but turned to inhibit the differentiation of OBs at other concentrations. Y^{3+} promoted the adipocytic transdifferentiation of OBs at most tested concentrations on day 9 and 12. On day 15, Y^{3+} promoted the adipocytic transdifferentiation of OBs at lower concentrations of 1×10^{-9} and 1×10^{-8} mol/L, turned to inhibit at other tested concentrations. Y^{3+} inhibited the formation of mineralized matrix nodules of OBs at concentrations of 1×10^{-9}, 1×10^{-8} and 1×10^{-6} mol/L, but turned to promote the formation of mineralized matrix nodules of OBs at other tested concentrations. These findings suggested that the effects of Y^{3+} on the proliferation, differentiation, adipocytic transdifferentiation and mineralization function of primary OBs depended on the concentration and culture time, moreover, concentration and culture time were pivotal factors for switching the biological effects of Y^{3+} from toxicity to activity, from damage to protection, or from down-regulation to up-regulation.

Keywords: yttrium ion; osteoblasts; proliferation; differentiation; mineralization; rare earths

In the whole life, bone remodeling occurs continuously to maintain the normal bone mass. The loss of bone mass in osteoporosis is due to an imbalance between the formation and resorption of bone, which, in turn, depends on the interactions between osteoblasts (OBs) and osteoclasts (OCs)[1]. OBs stem from bone marrow stromal cells and are bone-forming cells. The formation of bone involves a series of complex events that include the proliferation and differentiation of osteoprogenitor cells and result eventually in the formation of a mineralized extracellular matrix[2]. OCs are multinucleated cells that are responsible for bone resorption[3]. So OBs and OCs play important roles in the pathogenesis of osteoporosis.

The biological properties of the lanthanides, based on their similarity to calcium, have stimulated research into therapeutic application[4]. Moreover, it was also found that they could be enriched in bone and difficult to be ejected. Owing to the similarities between rare earth ions and Ca^{2+} in some physical and chemical properties, it is likely that rare earth elements affect bone cell function and intervene in bone-remodeling process. Jha et al.[5] found that Pr_6O_{11} and Nd_2O_3 exhibited a promoting bone-resorbing property in mice. Quarles et al.[6] found that Gd^{3+} stimulated DNA synthesis in MC3T3-E1 OBs *in vitro* in a dose-dependent fashion. This was related to Gd^{3+}-activated G-proteins in OB membranes. Hartle et al.[7] found that Gd^{3+} inhibited prostaglandin E. (PGE)-stimulated cyclic adenosine monophosphate (cAMP) accumulation, but potentiated parathyroid hormone (PTH)-stimulated cAMP production. Zhang et al.[8] reported that La^{3+} and Ga^{3+} inhibited the proliferation of primary mouse OBs at concentrations of 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, and 1×10^{-5} mol/L La^{3+} at concentration of 1×10^{-5} mol/L significantly increased the alkaline phosphate (ALP) activity of primary mouse OBs up to 3-folds, however, the effects reversed to inhibit at other concentrations. Ga^{3+} played a negative effect on ALP activity. Our experimental results also demonstrated that the effects of Ln^{3+}(La^{3+}, Sm^{3+}, Er^{3+}, Nd^{3+}, Gd^{3+} and Dy^{3+}) on OCs and UMR-106 cell line depended their concentrations and species[9–13]. Wang et al.[14] reported that lanthanum ion enhanced OB differentiation via pertussis toxin-sensitive gi protein and ERK signaling pathway *in vitro*. Huang et al.[15] reported that lanthanum ion

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had effects on composition, crystal size, and lattice structure of femur bone mineral of Wistar rats. Yttrium element has been widely used in medical field[16]. Some of the UltraCap and nanocrystalline HA powders were doped with yttrium because previous studies demonstrated that Y-doped HA in bulk improved osteoblast function over undoped HA[17]. No cytotoxicity has also been observed through Y_2O_3 stabilized zirconia used in animal studies[18]. So far people did not know how and what yttrium element affected the proliferation, differentiation, and mineralization function of primary mouse OBs. In order to elucidate how Y^{3+} affect bone metabolism at cell level, the effects of Y^{3+} on the proliferation, differentiation and mineralization function of primary mouse OBs were studied in this paper.

1 Experimental

1.1 Materials and reagents

Kunming (KM) mice were obtained from the Animal Center of Hebei University. Dulbecco's modified Eagle's medium (DMEM), trypsin and fetal bovine serum (FBS) were purchased from Gibco. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT), benzylpenicillin, streptomycin, β-glycerphosphate, dexamethasone, ascorbic acid, collagen II, insulin, oil red O, alizarin red S (ARS), and cetylpyridium chloride were from sigma. ALP activity kit was obtained from Nanjing Jiancheng Biological Engineering Institute (Nanjing, China), and micro-protein assay kit was purchased from Beyotime Biotechnology (Haimen, China). The YCl_3 was purchased from Beijing Institute of Rare Earth Sci. & Tech. Co., Ltd..

1.2 Methods

1.2.1 Isolation and culture of primary OBs The mouse OBs were isolated mechanically from newborn mouse skull using a modification of the method previously reported[19]. Briefly, skull was dissected from KM mice, endosteum and periosteum were stripped off, and then the bone was cut into approximately 1–2 mm^2 pieces and digested with trypsin (2.5 g/L) for 30 min. The bone was further digested with collagenase II (1.0 g/L) twice with 1 h for each, and the cells were collected. After incubating overnight at 37 ºC, in a 5% CO_2 humidified incubator, the DMEM was removed. Then the medium was changed every 3 d in all experiments.

1.2.2 OB proliferation assay The protocol described by Mosmann was followed by some modifications[20]. Briefly, OBs (2×10^4 cells per well) were plated in 96-well culture plates and cultured overnight at 37 ºC, in a 5% CO_2 humidified incubator. Y^{3+} was then added to the wells at final concentrations of 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L. Control wells were prepared by addition of DMEM. Wells containing DMEM without cells were used as blanks. Wells containing NaF (1×10^{-6} mol/L) without Y^{3+} were used as positive control. Upon completion of the incubation, MTT dye solution (20 μl, 5 mg/ml) was added. After 4 h incubation, the supernatant was removed and DMSO (100 μl) was added. The optical density (OD) was measured on a microplate spectrophotometer (BioRad Model 3550, USA) at a wavelength of 570 nm. The proliferation rate (%) was calculated according to the formula: $[(OD_{treated}-OD_{blank})/(OD_{control}-OD_{blank})-1)]\times100\%$.

1.2.3 ALP activity assay OBs (2×10^4 cells per well) were plated in 48-well culture plates, and treated with Y^{3+} at final concentrations of 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L for 1, 2 or 3 d, respectively. The plates were washed thrice with ice-cold PBS and lysed by two cycles of freezing and thawing. Aliquots of supernatants were subjected to ALP activity and protein measurement using an ALP kit and a micro-protein assay kit, respectively. All results were normalized by protein content.

1.2.4 Oil red O stain and measurement The OBs (2×10^4 cells per well) were plated in 48-well culture plates, after being induced by adipogenic supplement (10 μg/ml insulin, 10^{-7} mol/L dexthamethone) and treated with Y^{3+} at final concentrations of 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L, and fat droplets within differentiated adipocytes from OBs were stained using the oil red O described by Ichiro et al. with some modification[21]. Briefly, cell monolayers were washed by PBS then stained with a 0.6% (*W/V*) oil red O solution (60% isopropanol, 40% water) for 15 min at room temperature. For quantification of oil red O content, the cells were washed with dH_2O three times to remove background staining, and recorded by inverted phase contrast microscopy (Olympus IX 51), then 1 ml of isopropyl alcohol was added to the culture plates. After 5 min, the absorbance of the extract was measured by a spectrophotometer at 510 nm. The adipocytic trans-differentiation promoting rate (%) was calculated according to the formula: $(OD_{treated}/OD_{control}-1)\times100\%$.

1.2.5 Mineralized matrix formation assay The OBs were seeded into 24-well culture plate at 2×10^4 cells/ml and cultured overnight at 37 °C, in a 5% CO_2 humidified incubator. Then the medium was changed to differentiation medium containing 10 mmol/L β-glycerophosphate and 50 μg/ml ascorbic acid in the presence or absence of Y^{3+} of the concentrations of 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L for 21 d. The formation of mineralized matrix nodules was determined by ARS stain. Briefly, Cell monolayers were fixed with ethanol and stained with 0.1% alizarin red S for 30 min at room temperature. Following rinsing with PBS, quantitation of ARS staining was performed by elution with 10% (*W/V*) cetylpyridium chloride for 10 min and the absorbance was measured at 570 nm. Results were expressed as mol of ARS permilligram of total cellular protein[22].

1.2.6 Statistical analysis Data were collected from at least four separate experiments. The results were expressed as means±standard deviation (SD). The statistical differences were analyzed using SPSS' t-test. *P* values less than 0.05 were considered to indicate statistical differences.

2 Results

2.1 Effects of Y^{3+} on the proliferation of OBs

As shown in Fig. 1, Y^{3+} (1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L) promoted the proliferation of OBs with maximum promotion effect on day 1, 2 and 3 at concentrations of 1×10^{-4}, 1×10^{-9} and 1×10^{-5} mol/L, respectively.

2.2 Effects of Y^{3+} on the differentiation of OBs

As shown in Fig. 2, Y^{3+} had no effect on the differentiation of OBs at concentrations of 1×10^{-9} and 1×10^{-8} mol/L, promoted the differentiation of OBs at concentration of 1×10^{-7} mol/L, but turned to inhibit the differentiation of OBs at other tested concentrations on day 1. On day 2, Y^{3+} inhibited the differentiation of OBs at all tested concentrations. On day 3, Y^{3+} promoted the differentiation of OBs at lower concentrations of 1×10^{-9} and 1×10^{-8} mol/L, but turned to inhibit the differentiation of OBs at other concentrations.

2.3 Effects of Y^{3+} on the adipocytic transdifferentiation of OBs

As shown in Fig. 3, Y^{3+} had no effect on the adipocytic transdifferentiation of OBs at a higher concentration of 1×10^{-4} mol/L, but promoted the adipocytic transdifferentiation of OBs at other tested concentrations on day 9. On day 12, Y^{3+} had no effect on the adipocytic transdifferentiation of OBs at concentrations of 1×10^{-7} and 1×10^{-4} mol/L, but promoted the adipocytic transdifferentiation of OBs at other tested concentrations. On day 15, Y^{3+} promoted the adipocytic transdifferentiation of OBs at lower concentrations of 1×10^{-9} and 1×10^{-8} mol/L, and the effects were reversed to inhibit at other tested concentrations. The morphologic observation was in accordance with the above results (Fig. 4).

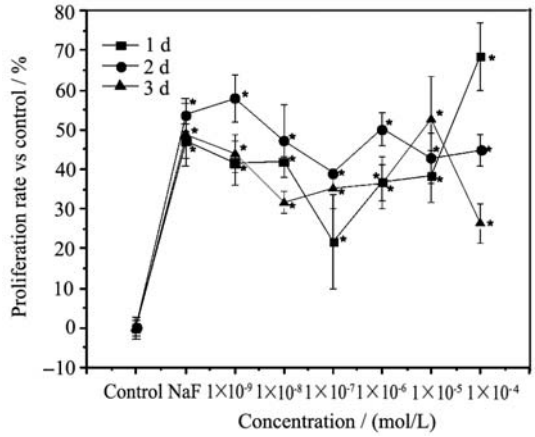

Fig. 1 Effect of Y^{3+} on the proliferation of OBs ($^{*}P$<0.001 vs control, n=4)

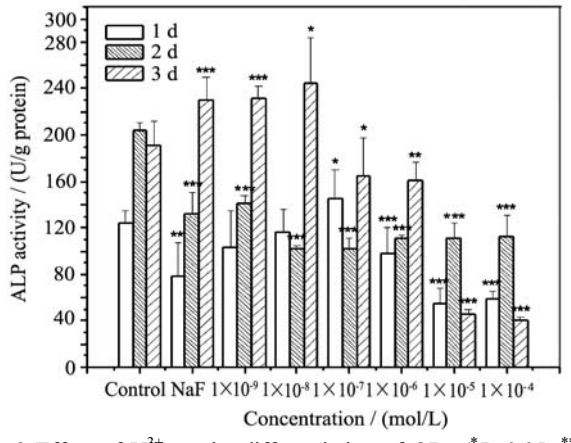

Fig. 2 Effect of Y^{3+} on the differentiation of OBs ($^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 vs control, n=4)

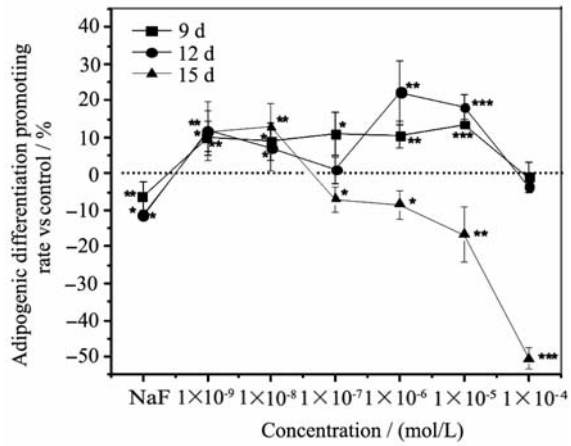

Fig. 3 Effect of Y^{3+} on the adipocytic transdifferentiation of OBs ($^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 vs control, n=5)

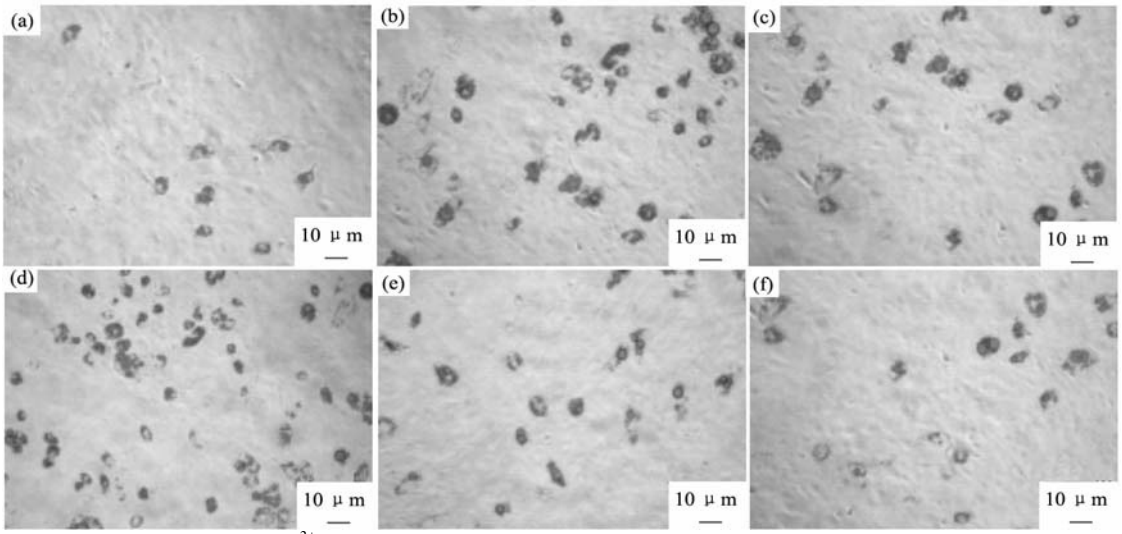

Fig. 4 Effect of Y^{3+} on the adipocytic trans-differentiation of OBs by Oil Red O stain (15 d)
(a) Control without adipogenic supplement; (b) Adipogenic supplement; (c) Adipogenic supplement+1×10^{-6} mol/L NaF; (d) Adipogenic supplement+1×10^{-8} mol/L Y^{3+}; (e) Adipogenic supplement+1×10^{-5} mol/L Y^{3+}; (f) Adipogenic supplement+1×10^{-4} mol/L Y^{3+}

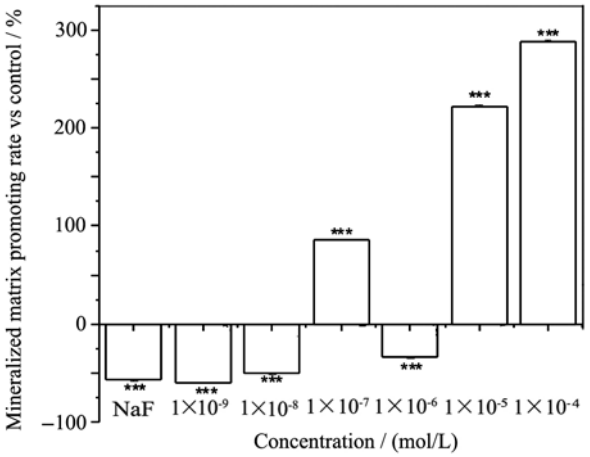

Fig. 5 Effect of Y^{3+} on the mineralized nodule formation of OBs ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs control, $n=4$)

2.4 Effects of Y^{3+} on the formation of mineralized matrix nodules

As shown in Fig. 5, Y^{3+} inhibited the formation of mineralized matrix nodules of OBs at concentrations of 1×10^{-9}, 1×10^{-8}, and 1×10^{-6} mol/L, promoted the formation of mineralized matrix nodules of OBs at other tested concentrations with maximum promotion effect at a higher concentration of 1×10^{-4} mol/L. The morphologic observation was in accordance with the results (Fig. 6).

3 Conclusions

In the present study, the effects of Y^{3+} on the proliferation, differentiation, adipocytic transdifferentiation and mineralization function of primary OBs *in vitro* were studied. Measurement of cell viability and proliferation forms the basis for numerous *in vitro* assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. In our experiments, Y^{3+} promoted the proliferation of OBs at tested concentrations. An increase in bone specific ALP activity *in vitro* reflects the maturation from an earlier to a more mature stage of osteoblast differentiation. ALP hydrolyzes organophosphates thereby releasing inorganic phosphate, which appears to be the actual initiator of mineralization[23]. As osteoblasts continue to differentiate and enter the mineralization stage, levels of proteins associated with maturation such as ALP decline, while expression of osteocalcin and formation of hydroxyapatite become evident[24]. In our experiments, the effects of Y^{3+} on the differentiation and formation of mineralized matrix nodules of primary OBs depended on concentrations and culture time. For example, Y^{3+} inhibited the differentiation of OBs at tested concentrations on day 2. On day 3, Y^{3+} promoted the differentiation of OBs at lower concentrations 1×10^{-9}, 1×10^{-8}, and 1×10^{-7} mol/L, but turned to inhibit the differentiation of OBs at other concentrations. Y^{3+} inhibited the formation of mineralized matrix nodules of OBs at concentrations of 1×10^{-9}, 1×10^{-8}, and 1×10^{-6} mol/L, but turned to promote the formation of mineralized matrix nodules of OBs at other tested concentrations. Adipocytic and osteogenic cells are believed to derive from multipotential stromal cells in the marrow. *In vitro* studies have shown an inverse relationship between the differentiation of adipocytic and osteogenic cells[25]. So a reversal of adipogenesis will provide an important therapeutic approach to prevent aged-related and steroids-induced osteoporosis[26–29]. Our results indicated that Y^{3+} promoted the adipocytic transdifferentiation of OBs at most tested concentrations on day 9 and 12. On day 15, Y^{3+} promoted the adipocytic transdifferentiation of OBs at lower concentrations of 1×10^{-9} and 1×10^{-8} mol/L, and the effects reversed to in-

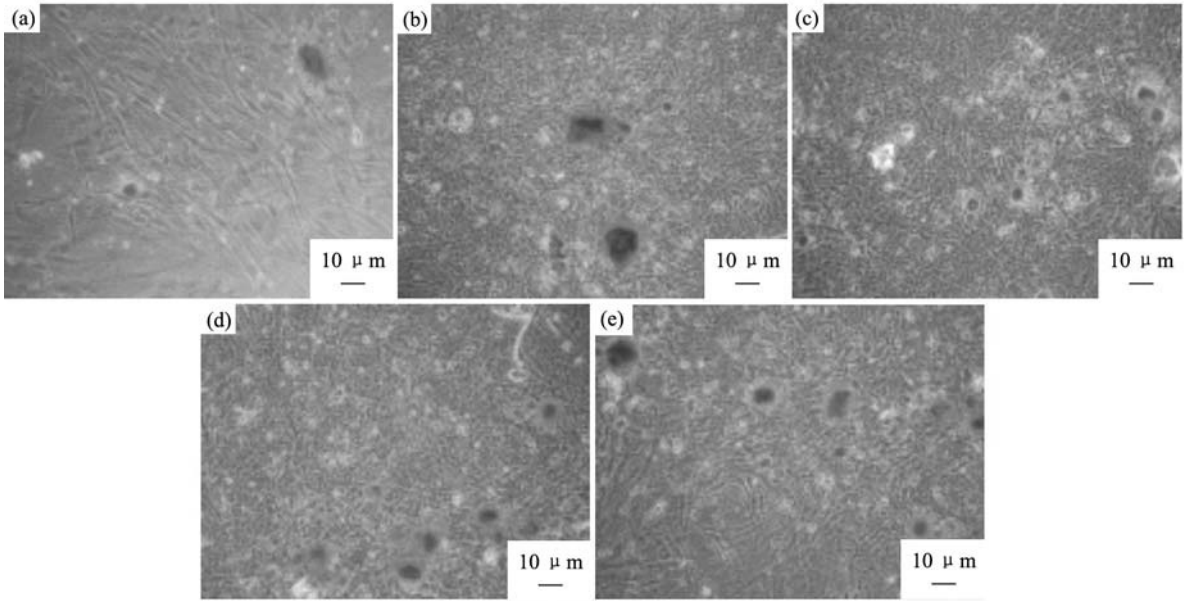

Fig. 6 Effect of Y^{3+} on the formation of mineralized matrix nodules of OBs stained by alizarin red S

(a) Control without differentiation medium; (b) Differentiation medium; (c) Differentiation medium+1×10^{-6} mol/L NaF; (d) Differentiation medium+1×10^{-7} mol/L Y^{3+}; (e) Differentiation medium+ 1×10^{-5} mol/L Y^{3+}

hibit at other tested concentrations. In fact, the different species of rare earth ions may behave differently, which has been recognized in a series of biological effects of lanthanides. These differences may relate to the physico-chemical characteristics of the respective cations depending upon features, such as their ionic radii or charge densities[30].

In summary, the concentration and culture time are key factors for switching the biological effects of Y^{3+} from toxicity to activity, or from down-regulation to up-regulation. This property has been recognized in a series of biological effects of lanthanides[9–13]. The mechanism of the effects of Y^{3+} on the proliferation, differentiation, adipocytic transdifferentiation and mineralization function of primary OBs remains to be further studied.

References:

[1] Riggs B L. Pathogenesis of osteoporosis. *Am. J. Obstet. Gynecol.*, 1987, **156**(5): 1342.

[2] Yuhara S, Kasagi S, Inoue A, Otsuka E, Hirose S, Hagiwara H. Effects of nicotine on cultured cells suggest that it can influence the formation and resorption of bone. *Eur. J. Pharmacol.*, 1999, **383**(3): 387.

[3] Suda T, Nakamura I, Jimi E, Takahashi N. Regulation of osteoclast function. *J. Bone Miner. Res.*, 1997, **12**(6): 869.

[4] Mu K G, Zhang W J, Cui J Y, Zhang F S, Hu L. Review of studies on rare earth against plant disease. *J. Rare Earth*, 2004, **22**(3): 315.

[5] Jha A M, Singh A C. Clastogenicity of lanthanides-induction of micronuclei in root tips of vicia faba. *Mutation Res.*, 1994, **322**(3): 169.

[6] Quarles L D, Hartle J E, Middleton J P, Zhang J, Arthur J M. Aluminum-induced DNA synthesis in osteoblasts: mediation by a G-protein coupled cation sensing mechanism. *J. Cell Biochem.*, 1994, **56**(1): 106.

[7] Hartle J E, Prpic V, Siddhanti S R, Spurney R F, Ouarles L D. Differential regulation of receptor-stimulated cyclic adenosine monophosphate production by polyvalent cations in MC3T3-E1 osteoblasts. *J. Bone Miner. Res.*, 1996, **11**(6): 789.

[8] Zhang D W, Zhang J C, Chen Y, Yang M S, Yao X S. Effects of lanthanum and gadolinium on proliferation and differentiation of primary osteoblasts. *Prog. Nat. Sci.*, 2007, **17**(5): 618.

[9] Zhang J C, Huang J, Xu S J, Wang K, Yu S F, Zhang T L, Yang M S. Effects of lanthanum(III) on bone resorbing activity of rabbit mature osteoclasts co-cultured with osteoblasts. *J. Rare Earths*, 2005, **23**(4): 496.

[10] Zhang J C, Zhang T L, Xu S J, Wang K, Yu S F, Yang M S. Effect of lanthanum(III) on cytosolic free calcium in isolated rabbit mature osteoclasts. *J. Rare Earths*, 2005, **5**(23): 580.

[11] Zhang J C, Li X X, Xu S J, Wang K, Yu S F, Lin Q. Effects of rare earth ions on proliferation, differentiation and function expression of cultured osteoblasts *in vitro*. *Prog. Nat. Sci.*, 2004, **14**(4): 404.

[12] Zhang J C, Zhang T L, Xu S J, Wang K, Yu S F, Yang M S. Effects of lanthanum on formation and bone-resorbing activity of osteoclast-like cells. *J. Rare Earths*, 2004, **22**(6): 891.

[13] Zhang J C, Xu S J, Wang K, Yu S F. Effects of the rare earth ions on bone resorbing function of rabbit mature osteoclasts *in vitro*. *Chinese Sci. Bull.*, 2003, **48**(20): 2170.

[14] Wang X, Yuan L, Huang J, Zhang T L, Wang K. Lanthanum enhances *in vitro* osteoblast differentiation via pertussis toxin-sensitive gi protein and ERK signaling pathway. *J. Cellular Biochem*., 2008, **105**(5):1307.

[15] Huang J, Zhang T L, Xu S J, Li R C, Wang K, Zhang J, Xie Y N. Effects of lanthanum on composition, crystal size, and lattice structure of femur bone mineral of Wistar rats. *Calcified Tissue International*, 2006, **78**(4): 241.

[16] Salem R, Hunter R D. Yttrium-90 microspheres for the treatment of hepatocellular carcinoma: A review. *Int. J. Radiat. Oncal. Biol. Phys.*, 2006, **66**(2): S83.

[17] Sato M, Sambito M A, Aslani A, Kalkhoran N M, Slamovich E B, Webster T J. Increased osteoblast functions on undoped and yttrium-doped nanocrystalline hydroxyapatite coatings on titanium. *Biomaterials*, 2006, **27**(11): 2358.

[18] Piconi C, Maccauro G. Zirconia as a ceramic biomaterial. *Biomaterials*, 1999, **20**(1): 1.

[19] Nishimori S, Tanaka Y, Chiba T, Fujii M, Imamura T, Miyazono K, Ogasawara T, Kawaguchi H, Igarashi T, Fujita T, Tanaka K, Toyoshima H. Smad-mediated transcription is required for transforming growth factor-β1- induced $p57^{Kip2}$ proteolysis in osteoblastic cells. *J. Biol. Chem*., 2001, **276**(14): 10700.

[20] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 1983, **65**(1-2): 55.

[21] Sekiya, Larson B, Smith J, Pochampally R, Cui J G, Prockop D J. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize yields of early progenitors and evaluate their quality. *Stem Cells*, 2002, **20**(6): 530.

[22] Gori F, Thomas T, Hicok K C, Spelsberg T C, Riggs B L. Differentiation of human marrow stromal precursor cells: bone morphogenetic protein-2 increase OSF2/CBFA1, enhances osteoblast commitment, and inhibits late adipocyte maturation. *J. Bone Miner. Res*., 1999, **14**: 1522.

[23] Beck G R. Inorganic phosphate as a signaling molecule in osteoblast differentiation. *J. Cell Biochem.,* 2003, **90**: 234.

[24] Stein G S, Lian J B. Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. *Endocr. Rev.,* 1993, **14**: 424.

[25] Nuttall M E, Gimble J M. Is there a therapeutic opportunity to either prevent or treat osteopenic disorders by inhibiting marrow adipogenesis? *Bone*, 2000, **27**: 177.

[26] Ailhaud G, Grimaldi P, Negrel R. Cellular and molecular aspects of adipose tissue development. *Annu. Rev. Nutr*., 1992, **12**: 207.

[27] Benayahu D, Zipori D, Wientroub S. Marrow adipocytes regulate growth and differentiation of osteoblasts. *Biochem. Biophys. Res. Commun*., 1993, **197**: 1245.

[28] Sakaguchi K, Morita I, Murota S. Relationship between the ability to support differentiation of osteoclast-like cells and adipogenesis in murine stromal cells derived from bone marrow. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 2000, **62**: 319.

[29] Benayahu D, Peled A, Zipori D. Myeloblastic cell line expresses osteoclastic properties following coculture with marrow stromal adipocytes. *J. Cell Biochem*., 1994, **56**: 374.

[30] Wang Kui. The analogy in chemical and biological behavior between non-essential ions compared with essential ions. *South Afr. J. Chem.*, 1997, **50**(64): 232.