·论著·

巴戟天含药血清对原代破骨细胞 RANK 和 CA II mRNA 表达的影响

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摘要: **目的** 观察巴戟天含药血清对原代破骨细胞 RANK 和 CA II mRNA 表达的影响。**方法** 取 SPF 级大鼠 48 只,随机分成正常组 12 只和去势组 36 只,正常组切去卵巢周围部分脂肪,去势组切除卵巢。3 个月后测定两组雌激素水平并将去势组随机分为骨质疏松组,骨质疏松 + 雌激素组,骨质疏松 + 巴戟天含药血清组。采用机械分离法提取各组的破骨细胞,培养 5 d 后,TRAP 染色及电镜扫描骨片等的方法鉴定破骨细胞。最后用雌激素或巴戟天含药血清干预 3 d,以 RT-PCR 法检测各组 RANK 和 CAIImRNA 表达。采用单因素方差分析或多样本均数两两比较进行统计分析。**结果** 去势组后大鼠雌激素水平低于正常组($P<0.01$)。骨质疏松组破骨细胞 RANK 和 CAII 表达均高于正常组($P<0.05$,$P<0.05$);巴戟天含药血清可降低骨质疏松后大鼠破骨细胞 RANK 和 CA II 的表达($P<0.05$,$P<0.05$)。**结论** 巴戟天和雌激素均可降低骨质疏松大鼠破骨细胞 RANK 和 CAII 的表达,从而达到抑制骨质疏松的作用。

关键词: 巴戟天;雌激素;破骨细胞;含药血清;RANK mRNA;CAII mRNA

Effect of morinda officinalis-containing serum on the mRNA expression of RANK and CAII in primary osteoclasts in ovariectomized rats

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Abstract: **Objective** To observe the effect of morinda officinalis-containing serum on the mRNA expression of RANK and CAII in primary osteoclasts in ovariectomized rats. **Methods** Forty-eight female SPF rats were randomly divided into 2 groups: SHAM group ($n=12$) and OVX group ($n=36$). **Rats** in SHAM group received the operation in which retroperitoneal fat with the same mass as bilateral ovaries was removed. And rats in OVX group were bilaterally ovariectomized. The serum levels of estradiol (E2) of rats in both groups were detected 3 months after the operation. And the rats in OVX group were randomly divided into 3 groups: osteoporosis (OP) group, OP + E2 group, and OP + RMO group. Osteoclasts in each group were collected using mechanical separation method. After 5-day culturing, the osteoclasts were identified using TRAP staining and scanning electron microscope. Finally, the osteoclasts were treated with RMO or E2 for 3 days, then the mRNA expression of RANK and CAII was detected using RT-PCR. One-way ANOVA and the LSD multiple comparison were performed. **Results** The serum levels of E2 in OVX group were lower than those in SHAM group ($P<0.01$). The mRNA expression of RANK and CAII in osteoclasts in OP group was higher than that in SHAM group ($P<0.05$,$P<0.05$, respectively). The mRNA expression of RANK and CAII in osteoclasts treated with RMO containing serum was lower than that in SHAM group ($P<0.05$,$P<0.05$, respectively). **Conclusion** Both morinda officinalisa and E2 can decrease the mRNA expression of RANK and CAII, which can exert the function of inhibiting OP.

Key words: Morinda officinalis; Estradiol; Osteoclast; RMO-containing serum; RANK mRNA; CAII mRNA

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Osteoporosis, characterized by a loss of bone mass and microarchictectural deterioration of bone tissues, is a major health problem, especially for elderly women. Bone under goes renewal and repair termed "bone remodeling" through the intimate interaction between osteoclasts, which resorb bone, and osteoblasts, which form newbone[1].

Osteoclasts, multinucleated cells responsible for bone resorption, are differentiated from monocyte/macrophage lineage cells[2]. Bone-forming osteoblasts tightly regulate the differentiation of the osteoclast precursors into osteoclasts[3]. Understanding of the molecular mechanisms that regulate osteoclast formation and activation has advanced rapidly since the discovery of the RANKL/RANK signaling system. During the process, the receptor activator of macrophage colony-stimulating factor (M-CSF) and nuclear factor kappaB ligand (RANKL) have been identified as crucial for differentiation of osteoclasts. Osteoprotegerin (OPG) and RANK are two types of receptors for RANKL. The RANKL signal in osteoclast precursor cells stimulate the activation of the three major mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase (ERK), c-Jun-N-terminal kinase (JNK), and p38-MAPKs[4]. In addition, several pathways are also affected by RANKL such as the phosphoinositide kinase-3/Akt, activator protein1 (AP-1), nuclear factor of activated T-cells c1 (NFATc1), and nuclear factor kappa B (NF-κB)[5-7]. These signaling pathways ultimately lead to induction and activation of the transcription factors involved in the expression of genes that characterize osteoclasts. Carbonic anhydrase isoenzyme II (CAII) existing in the osteoclastic cytoplasm accompanies variations of osteoclast activity. It has been reported that fluid shear stress has an effect on CAII inpolarized rat osteoclasts[8]. Additionally, CAII appears that high gene expression during the bone absorbing period affects proliferation and activity of osteoclasts.

The Root of Morinda Officinalis How (RMO), named "Ba-ji-tian", is a famous and classical Chinese medicine, which has been used as a treatment for women's disease relative to estrus and as a stimulant of sex drive for its effects and nourshing kidney[9]. Animal experiments found that RMO extract reduced bone loss in ovariectomized rats, probably via the inhibition of bone resorption, but was not involved with bone formation.[10]. Pharmacological studies showed that RMO enhanced the expression of core-binding factor α1 (Cbfα1) in both morrow stromal cells (BMSCs)[11] and osteoblasts[12] increased the proliferation, alkaline phosphatase (ALP) activity and osteocalcin of osteoblast[13]. Additionally, it could promote the proliferation of osteoblasts and increase the expression of TGF-β1[14]. However, although the biological effects of RMO on osteoblasts and osteoclasts have been studied for many years, the manner in which RMO interacts with osteoclasts and influence their behavior is still poorly understood. In this study, we investigate the effects of RMO-containing serum on the expression of RANK and CAIImRNA in osteoclast cell cultures derived from the bone marrow of ovariectomized rats.

METHODS

Animals

Female rats (SPF) at 3 months of age with body weights of 295 ± 35g were purchased from Medical college of Xiamen University Laboratory Animal Center, Xiamen, China. The rats were housed individually in a regulated SPF environment (20 ± 2℃), with a 12-light/dark cycle (lights on:08:00-20:00), and unlimited access to standard rat food and water.

Reagents ang drugs

The387A-1 kit was used for cytological staining (Sigma chemical USA); Minimum essential medium alpha medium (α-MEM), fetal bovine serum (FBS) and penicillin-streptomycin were purchased from Gibco (USA); E2 (premarin or conjugated E2, Favor-clan HectoPalace Pharma ceutical Factory, Soochow; Bao Dai Lu No. 4, Soochow, Jiang Su, China); Morinda officinalis granule (The Nong's Concentrated Chinese Medicine Granules, PuraPharm International (H. K.) Ltd, Ke Yuan DaDao No. 46, High-tech development area of Nan Ning, Guang xi, China).

Ovariectomy

In the first set of the experiments, 48 healthy female SPF rats were randomlyassigned to two groups.

Sham group includded 12 rats in which retroperitoneal fat with the same mass as bilateral ovaries was removed under gengeral anaesthesia with 5% chloral hydrate (0.6ml/100g). The remaining rats were bilatterally Ovariectomized under similar conditions. After 12 weeks, 1.5ml blood were collected from all the rats and serum was separated for measurement of serum estradiol (E2).

Preparation ofRMO-containing serum and E2-containing serum

Twenty healthy female SPF rats were divided into two groups and orally treated with Premarin (0.065g• kg^{-1}) or RMO (0.1g• kg^{-1}) by intubation for total seven doses, twice a day. The animals were fasted for 12h but given water ad libitum before collection of serum. Sisty minutes afer the last treatment, the animals were exposed to etheranaesthesia, their blood were extracted one by one via the chambers of the heart under aseptic condition, and then centrifuged at 2500rpm for 15min. The serum of respective group were mixed together and filtered through a 0.22μm filter membrane, termed as E2-S and RMO-S, respectively, and then stored at −80℃ until use.

Hormone Measurements

Levels of serum E2 were determined by chemiluminescence analysis using a Centaur automatic immunoassay apparatus (BayCo. Germany) and counting with a XH-6010 gamma counter in parallel double-tubetests. The intrabatch and interbatch coefficient of variation (CVs) of E2 were 45.2% and 26.1%, respectively.

Osteoclast Cell Formation in Rat Marrow Cultures

Rat bone marrow cells were harvested 12 weeks after ovariectomized by the method of Chamber and Magnus[15] with minor modifications. For superficial antisepsis, the sacrificed rats were soaked 10 min in 75% alcohol. After rapid removal of soft tissues under aseptic conditions, the femora and tibiae were cleaned twice with phosphate buffered saline (PBS, pH7.4). They were then rinsed two times with α-MEM culture medium. Bone marrow cells were obtained from both femora and tibiae of the SPF rats by flushing them arrow space with α-MEM (α-MEM contained 15% fetal bovine serum [FBS], Cibco) and seeded into 6-well chamberslides Nunc, Naperville, IL, USA) at 2mL cell suspension/well in α-MEM containing FBS. Bone slices and glass slides were added. After 5hr, nonadherent cells were removed with α-MEM. Half of the medium was replaced every day with fresh medium, and culture was continued for 10 days.

Mature Osteoclast-like Cell Activity Assay

After 6 days of culture, cell creeping silces were removed and dried at room emperature. The cells were fixed in2.5% (v/v) glutaraldehyde at 4℃ for 10 min. TRAP staining fluid was added, and the plates were incubated at 37℃ for an additional 60 min. After removal of the TRAP solution, the plates were washed three times with distilled water and air-dried. The slices were sealed with glycerogelatin. Osteoclast morphology, including cellular configuration, size, and nuclei, was observed under a microscope. TRAP staining was also performed 10 days after cell isolation.

After 10 days of culture with osteoclast cells as described above, the bone slices were removed and cleaned ultrasonically three times for 10 min each in a 0.25M NH_4OH solution to remove adherent cells. They were then rinsed with PBS, fixed in 2.5% (v/v) glutaraldehyde, postfixed in 1% osmiumtetroxide, dehydrated with an alcohol series, and replaced in alcohol with tertiary butanol. Finally, the bone slice samples were subjected to CO_2 critical-point drying and gold staining, and the resorptive lacunae were observed by scanning electronicmicroscopy (SEM, Jeol model 100S, Japan) operated at 20 kV.

Experimental Protocols

Cells derived from the OVX group were randomly and equally divided into the OVX control group and OVX experimental group. After 24 hr of incubation, culture plates (6 well) derived from the OVX experimental group were placed in the center of plastic shelves (mimicking the dimensions of the field) within an incubator, and the cultures were treated with 5% RMO-containing serum as OVX + RMO group, E2-containing serum as OVX + E2 group for 3 days. RANK and CA II mRNA assays were subsequently performed at the end of time points. The OVX control group and the sham group of cells that grew in another identical incubator add 5% FBS, and their RANK and

CA II mRNA levels were assayed.

Real-Time Polymerase Chain Reaction Analysis

Total RNA was isolated using the Trizol reagent (Invitrogen, USA), then dissolved in DEPC (diethylprocarbonate)-treated water, quantitated by spectrometry at 260 nm. The absorption ratio of OD260/OD280 was between 1.8 and 2.0. The concentration of RNA (ug/ul) = OD260 × dilution factor × 40/1000. Complementary DNAs (cDNA) were synthesized by reverse transcription kit (TOYOBO). Housekeeping gene β-actin was used as an internal standard. Primers were designed from the published cDNA sequences and were synthesized by Invitrogen Biotechnology (China, Table1).

The PCR reaction mixture was filled up with FastStart Universal SYBR Green Master (Roche, Germany) and distilled water to a final volume of 50ul. PCR reaction were carried out in areal-time PCR cycler (ABI7500, USA) and analyzed using system (SDS Documents). The program was optimized and performed finally as denaturation 10min at 95℃, and then 15s at 95℃, 60s at 60℃ for 40 cycles. For analysis of relative gene expression data, $2^{-\Delta\Delta_{et}}$ method was used. Target gene = $2^{-\Delta\Delta Ct} \times$ Control, $\Delta\Delta Ct$ = ($Ct_{target\ gene}$ $Ct_{reference\ gene}$) treat group-($Ct_{target\ gene}$ - $Ct_{reference\ gene}$) control group[16]

Table 1 Primer sequences, product size, and condition for real-time RT-PCR analysis

Target gene	Primer sequences (5'-3')	Product size (bp)
RANK	TTATGAGCATCTCGGACGGTGTT GGTAGCCAGCCGTGCAAG	196
CAII	GCT CTG AGC ACA CCG TGA ACA GGA AGA AGG GAG CAA GGA TCA	244
β-actin	GAACCCTAAGGCCAACCGTG AGGCATACAGGGACAACACAGC	104

Statistical Analysis

All values were expressed as mean ± SD. One-way ANOVA followed by the LSD multiple comparison procedure was performed using a SPSS 11.0 Statistical package. $P < 0.05$ was considered significant.

REAULTS

Serum sex hormones

Levels of serum E2 in the sham group were significantly higher than those in the OVX group (62.93 ± 23.81 pg/ml vs 24.69 ± 9.10 pg/ml) ($P < 0.01$).

Identification of Osteoclast Cells

There were noosteoclast-like cells found in the bone marrow cells immediately after plating out. Osteoclast-like cells first appeared in the culture at day 3 and increased in number until the end of the culture period. Cell suspensions producing osteoclast cells contained osteoclast-like cells, red cells, mononuclear phagocytic systems, and fibroblast cells. After nonadherent cells were washed out, the cytoplasms of the osteoclast cells with three or more nuclei appeared to have pseudofoot-like activity (Figure 1). The number of multinucleated, TRAP-positive osteoclast-like cells was observed (Figure 2). After 10 days, the osteoclast cells were still observed, but the TRAP staining appeared faintly red. In bone resorption studies, the bone slices cultured with bone marrow cells showed an excavated pit on the surface formed by osteoclast cells in vitro at day 10. These lacunae were round, elliptical, or irregular (Figure 3).

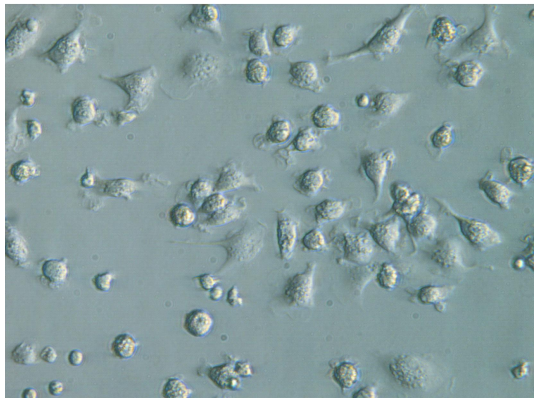

Fig. 1 Inverted phase contrast microscopic appearance of some typical rat osteoclast-like cells with 3 or more nuclei (×200).

Regulation of RANK and CA II mRNA Expression

The quantitative PCR was carried out after the

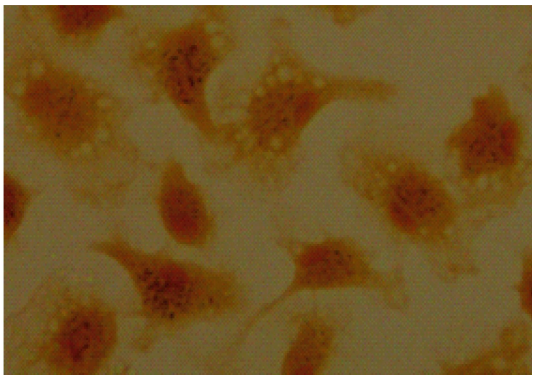

Fig. 2 Inverted phase contrast microscopic appearance of some typical rat osteoclast cells stained with tartrate-resistant acid phosphatase (TRAP) on chamber slides (×400).

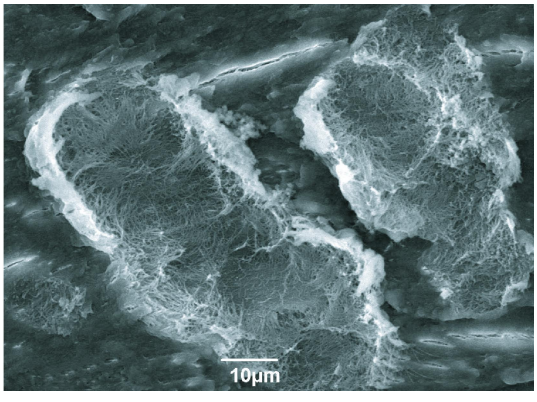

Fig. 3 Scanning electron microscopic photograph of bone slice surface cultured with marrow cells for 10 days. Irregular shape of pit was clearly observed (× 1400).

high purity of the total RNA was confirmed and the primer design and probe synthesis were validated. The Ct values of the samples were determined by comparison with a standard curve. Expression of RANK and CAII mRNA in the OVX control group was significantly higher than that in sham group ($p < 0.05$). Morinda officinalis-containing serum and E2 decrease the the expression of RANK and CAII mRNA compared with OVX control group (Figure 4 and Figure 5)

DISCUSSION

The theory of traditional Chinese medicine believes that bones are governed and dominated by the kidney, which means that the kidney plays an important role in formation and growth of bones. Therefore, Strong "kidney" can nourish bone and make it flourish, but

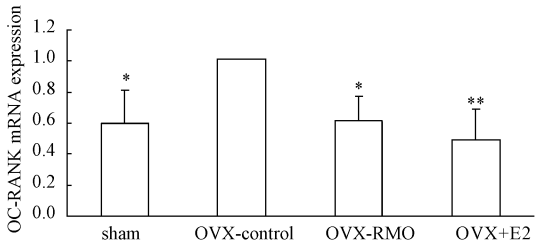

Fig. 4 Effect of RMO and E2 on expression of RANK genes in rat osteoclast-like cells. The expression levels of RANK mRNA was determined with real-time PCR. mRNA expression is shown as relative mRNA expression defining the control as 1, for this reason no standard deviation could be calculated. RANK mRNA expression was increased in OVX control group. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. OVX-control group. (n = 12 in sham group and n = 36 in other three groups).

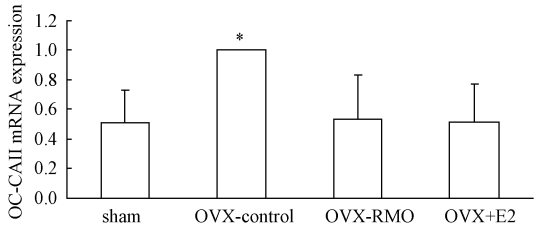

Fig. 5 Effect of RMO and E2 on expression of CAII genes in rat osteoclast-like cells. The expression levels of CAII mRNA was determined with real-time PCR. mRNA expression is shown as relative mRNA expression defining the control as 1, for this reason no standard deviation could be calculated. CAII mRNA expression was increased in OVX control group. The asterisk represents significant increase ($P < 0.05$) compared with sham, OVX-RMO and OVX-E2 ($n = 12$ in sham group and $n = 36$ in other three groups).

the weak "kidney" makes bone perish[17,18]. Based on above theory and researches, a Serum pharmacological testing method was employed to address the effects of RMO on the mRNA expression of RANK and CAII in Ovariectomized Rat Osteoclast Cell. "Serum pharmacology" was first put forward by Tashino[19] and has widely been utilized in Southeast Asian countries, by which the serum from animals administered herbal medicines could retain effective components of these medicines and also potentially active metabolites of the components[20]. Therefore, serum pharmacology has

extensively been used to study effects and mechanisms of traditional Chinese drugs in vitro. Moreover, the serum from animals treated with drugs was usually called drugs-containing serum.

RANKL is a member of the TNF superfamily and is expressed by osteoblasts. The cognatereceptor for RANKL, RANK, is a member of the TNF receptor superfamily and is expressed on osteoclasts and osteoclast precursors. RANK/RANKL complex is able to recruit some adaptor proteins such as TRAF-6, TAB-2, IRAK 1-3 and Src that activate Akt, AP-1 and NF-κB[21]. CAII affects osteoclast function and gives rise to metabolic bone disease. Dissolution of the inorganic phase of bone precedes matrix degradation. CAII and vacuolar H^+-ATPases are involved in the extracellular acidification caused by osteoclasts. CAII generates H^+ and HCO^{3-} by the hydration of CO_2, and the H^+ are transported through the apical ruffled border of the osteoclasts to the resorption zone by avacuolar H^+-ATPase[22]. The result issecretion of HCl into the resorptive microenvironment, producing a pH of 4.5[23]. This acidic milieu first mobilizes bone mineral; subsequently, the demineralize dorganic component of bone is degraded by a lysoso malprotease, cathepsin K, and MMP-9[24]. Thus, CAII Plays an important role in polarized osteoclastic resorption[25,26].

In our present study, the level of serum E2 was significantly decreased in the OVX group compared to that in the sham group. This indicated that the ovaries were totally excised. The rat ovariectomy (OVX) is an excellent preclinical animal model that correctly emulates the important clinical feature of the estrogen depleted human skeleton and the response of therapeutic agents[27]. The protective effect of estrogens on bone tissue is believed to result primarily from their antiresorptive action. Rapid postmenopausal osteoporosis occurring in female rats following ovariectomy is characterized by a decrease in trabecular density and a deterioration of bone architecture.

The osteoclast obtained from the long bones of SPF rats are most likely to resemble the characteristics of osteocytes in the physiological condition, which display various morphological appearances: most had irregular or elliptical configurations. It is suitable for us to make this experiment. Studies have also demonstrated that RMO can inhibit bone loss[10] and can be applied in clinical treatment. Primary bone marrow cells from the OVX experimental group were treated with Premarin or RMO. After 3 days, RANK and CAII gene expression was analyzed by real-time PCR. The current results indicated that RMO had inhibitory effects on osteoclast cells via the path ways of RANK and CAII mRNA expression.

It is well-accepted that bone remodeling is orchestrated by various cytokines and hormones. E2 deficiency in elderly people has directly an effect on the skeleton and can lead to osteoporosis. It has been show that administration of E2 is considered to be effective in preventing bone loss[28]. Mechanism by which E2 act on bones is not well understood yet, but one theory points out the importance of RANKL and OPG[29]. In this study, we found that the levels of RANK and CAIImRNA expression were decreased compared to the OVX-control group. These results suggested that OPG/RANKL/RANK system can be affected by E2. However, the 17β-estradiol treatment appears to increase the risk of breast cancer and endometrial malignancy[30]. All current marketed osteoporosis drugs, none of them offers acomplete cure, there is therefore a need to develop new drugs for this disease without various adverse effects. Previous studies have reported that RMO has estrogen-like effects[31]. But it did not reveal estrogen-like activity on uterus and fat tissue in ovariectomized rats, this lack of uterotrophic activity could be beneficial to reduce the risk of endometrial, breast or ovarian cancer associated with estrogen treatment[10,32-34]. Moreover, in south China, this plant has been developed into various health food, such as "Ba-ji-tian wine". Accordingly, RMO extract inhibits bone loss without side effects of estrogens, which could be a safe and effective drug.

In conclusion, the result demonstrate that RMO might modulate the process of osteoclastogenesis and subsequent bone resorption, at least partially, through RANK and CAII. Therefore, RMO has favorable potency to develop a new anti-osteoporotic agent in clinic. However, its exact mechanism of action on anti-osteoporosis is in need of further clarity.

【 参 考 文 献 】

[1] Goltzman D. Discoveries, drugs and skeletal disorders. Nat Rev Drug Discov, 2002, 1:784-796.

[2] Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation, Nature, 2003, 423:337-342.

[3] BrendanF Boyce, Lianping Xing. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Archives Biochemistry Biophysics, 2008, 473:139-146.

[4] Lee ZH, Kim HH. Signal transduction by receptor activator of nuclear factor kappaB in osteoclasts. Biochem Biophys Res Commun, 2003, 305:211-214.

[5] Hiroshi Takayanagi, Sunhwa Kim, Takako Koga, HiroshiNishina, et al. Inductionand activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Developmental Cell, 2002, 3,889-901.

[6] Takayanagi H, Kim S, Koga T. et al. Induction and activation of the Transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev Cell, 2002, 3:889-901.

[7] Naoko Kumagai, Keita Ohno, Ryusuke Tameshige, et al. Induction of mouse c-src in RAW264 cells is dependent on AP-1 and NF-κB and important for progression to multinucleated cell formation. Biochemical Biophysical Reasearch Communications, 2004, 325:758-768.

[8] Zhang Q, Liang X, Zhu B, et al. Effects of fluid shear stress on mRNA expression of carbon icanhydrase II inpolarized rat osteoclasts. Cell Biol Int, 2006, 30:714-720.

[9] National Pharmacopoeia Commission of P. R. China. Pharmacopoeia of the People's Republic of China, 2005, 1:55.

[10] Nan Li, Lu-Ping Qin, Ting Han et al. Inhibitory effects of morinda officinalis extract on bone loss in ovariectomized rats. Molecules, 2009, 14:2049-2061.

[11] Wang HM, Wang L, Li N. Effect of Morinda Officinalis on the expression of cbfα1 during the differentiation from BMSCs to the osteoblast. Chine J Trad Med, 2004, 12:22-27.

[12] Li Nan, Wang Heming, Guo Suhua, et al. Effect of radix morindae officinalis polysaccharide on expression of Cbfα1mRNA in osteoblast cultured in vitro. China journal of traditional chinese medicine and pharmacy, 2007, 22(8):517-519.

[13] Li Nan, Wang Heming, Guo Suhua, et al. Effect of morinda root polysaccharide and its extract on the activity of osteoblast cultured in vitro. Journal of clinical rehabilitative tissue engineering research, 2007, 11(23):4570-4572.

[14] Lin N, Wang HM, Lin X, et al. Experimental study on effects of Radix morinda officinalis on biological specialty of osteoblast. Zhong Guo Yi Yao Xue Bao, 2004, 19:726-728.

[15] Chambers TJ, Magnus CJ. Calcitonin alters behaviour of isolated osteoclasts. Pathol, 1982, 136:27-39.

[16] Kenneth JL, Thomas DS. Analysis of relative gene expression data using Real-Time Quantitative PCR and the 2-△△ct method. Methods, 2001, 25:402-408.

[17] Qin LP, Han T, Zhang QY, et al. Antiosteoporotic chemical constituents from Er-Xian Decoction, a traditional Chinese herbal formula. Ethnopharmacol, 2008, 118:271-279.

[18] Luo ZG, Zhao Y, Wang A-T. Effect of serumcontaining Chinese herb of strengthening kidney on proliferation and differentiation of human osteoblast. Bone, 2006, 8:14.

[19] Tashino S. "Serumpharmacology" and "serum pharmaceutical chemistry": from pharmacology of Chinese traditional medicines to start a new measurement of drug concentration in blood. Ther Drug Monit Res, 1988, 5:54-64.

[20] Bochu W, Liancai Z, Qi C. Primary study on the application of serum pharmacology in Chinese traditional medicine. Colloid Surf B Biointerf, 2005, 43:194-7.

[21] Ye H, Arron JR, Lamothe B, et al. Distinck molecular mechanism for initiating TRAF6 signalling. Nature, 2002, 25:443-447.

[22] Teitelbaum SL. Bone resorption by osteoclasts. Science, 2000, 289:1504-1508.

[23] Mundlos S, Otto F, Mundlos C, et al. Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell, 1997, 89:773-779.

[24] Teitelbaum, Andersen T, delCarmenOvejero M, et al. A scrutiny of matrix metalloproteinases in osteoclasts: evidence for heterogeneity and for the presence of MMPs synthesized by other cells. Bone, 2000, 35:1107-1119.

[25] Zelzer E, Olsen BR. The genetic basis for skeletal diseases. Nature, 2003, 423:343-348.

[26] Mahon CM, Will A, Hu P. Carbonic anhydrase II deficiency: phenotype, genotype and marrow transplantation. Br J Haematol Suppl, 2001, 113(Suppl. 1):34.

[27] Walter F. Boron. Evaluating the role of carbonicanhydrases in the transport of HCO3-related species. Biochimica et Biophysica Acta, 2010, 1804:410-421.

[28] Turner RT, Riggs BL, Spelsberg TC. Skeletal effects of estrogen. Endocr Rev, 1994, 15:275-300.

[29] Marsden J. The menopause, hormone replacement therapy and breast cancer. J Steroid Biochem Mol Biol, 2002, 83:123-132.

[30] Eriksen EF. Cellular mechanisms of bone remodeling, Rev Endocr MeTab Disord, 2010, 11:219-227.

[31] Zhang ZQ, Li Y, Yang M, et al. The effect of morinda officinalis how a Chinese traditional medicinal plant, on the DRL 72-s schedule in rats and the forced swimming test in mice. Pharmacol Biochemi Behavi, 2002, 72:39-43.

[32] Mokbel K. Risk-reducing strategies for breast cancer—a review of recent literature. Int J Fertil Womens Med, 2003, 48: 274-277.

[33] Smith RE. A review of selective estrogen receptor modulators and national surgical adjuvant breast and bowel project clinical trials. Semin Oncol, 2003, 30:4-13.

[34] Beck V, Rohr U, Jungbauer A. Phytoestrogens derived from red clover: an alternative to estrogen replacement therapy. Steroid Biochem Mol Biol, 2005, 94:499-518.

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巴戟天含药血清对原代破骨细胞RANK和CA II mRNA表达的影响

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