**Steroid and Xenobiotic Receptor SXR Mediates Vitamin K₂-activated Transcription of Extracellular Matrix-related Genes and Collagen Accumulation in Osteoblastic Cells**

Vitamin K₂ is a critical nutrient required for blood coagulation. It also plays a key role in bone homeostasis and is a clinically effective therapeutic agent for osteoporosis. We previously demonstrated that vitamin K₂ is a transcriptional regulator of bone marker genes in osteoblastic cells and that it may potentiate bone formation by activating the steroid and xenobiotic receptor, SXR. To explore the SXR-mediated vitamin K₂ signaling network in bone homeostasis, we identified genes up-regulated by both vitamin K₂ and the prototypical SXR ligand, rifampicin, in osteoblastic cells using oligonucleotide microarray analysis and quantitative reverse transcription-PCR. Fourteen genes were up-regulated by both ligands. Among these, tsukushi, matrilin-2, and CD14 antigen were shown to be primary SXR target genes. Moreover, collagen accumulation in osteoblastic MG63 cells was enhanced by vitamin K₂ treatment. Gain- and loss-of-function analyses showed that the small leucine-rich proteoglycan, tsukushi, contributes to vitamin K₂-mediated enhancement of collagen accumulation. Our results suggest a new function for vitamin K₂ in bone formation as a transcriptional regulator of extracellular matrix-related genes, that are involved in the collagen assembly.

From the §Division of Gene Regulation and Signal Transduction, Research Center for Genomic Medicine, Saitama Medical School, Saitama 350-1241, Japan, the ‡Department of Developmental and Cell Biology, University of California, Irvine, California 92697-2300, and the †Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan

Received for publication, January 30, 2006, and in revised form, March 28, 2006. Published, JBC Papers in Press, April 10, 2006. DOI 10.1074/jbc.M600896200

Tomoe Ichikawa†, Kuniko Horie-Inoue‡, Kazuhiro Ikeda§, Bruce Blumberg‡, and Satoshi Inoue†‡

This article was selected as a Paper of the Week.

© 2006 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.
**EXPERIMENTAL PROCEDURES**

**Cell Culture**—MG63 human osteosarcoma cells, 293T, and COS1 cells were grown in Dulbecco’s modified Eagle’s medium supplement with 10% fetal bovine serum (FBS), 50 units/ml penicillin, and 50 µg/ml streptomycin. Prior to vitamin K2 treatment, cells were cultured in phenol red-free media containing 10% dextran-charcoal-stripped FBS.

**Cloning and Construction of cDNAs**—Human SXR (pcDG-SXR), human SXR containing the VP16 activation domain upstream to SXR (VP16-SXR), and tk-(3A4)-Luc containing three-copy SXR response elements from human cytochrome P-450 (CYP) 3A4 promoter were described previously (14–16). N-terminally FLAG-tagged pcDNA3 described previously (14–16) was inserted in-frame to FLAG-tagged pcDNA3 at EcoRI and XhoI sites with 10% fetal bovine serum (FBS), 50 units/ml penicillin, and 50 µg/ml streptomycin.

**Experimental Procedures**—Total RNA was isolated from each sample with the OligotexTM-dT30 Super mRNA purification kit (Takara Bio, Kyoto, Japan) and converted into double-stranded cDNA using the cDNA synthesis kit (SuperScript Choice, Invitrogen) with a special oligo(dT)20 primer containing a T7 polymerase promoter site added 3′ of the poly(T) tract (Amersham Biosciences). After second-strand synthesis, labeled cDNA was generated from the cDNA sample by an in vitro transcription reaction using the bioarray high yield RNA transcript labeling kit (Enzo Life Sciences, Farmingdale, NY) supplemented with biotin-CTP and biotin-UTP (Enzo Life Sciences). The labeled cDNA was purified using RNeasy spin columns (Qiagen). Twenty µg of each cDNA sample was fragmented by mild alkaline treatment, at 94°C for 35 min in fragmentation buffer (200 mM Tris acetate, pH 8.1, 500 mM potassium acetate, 150 mM magnesium acetate) and then used to prepare 400 µl of master hybridization mix (0.1 mg/ml herring sperm DNA (Promega), 0.5 µg/ml of acetylated bovine serum albumin in hybridization buffer containing 100 mM MES, 1 M Na[+], 20 mM EDTA, 0.01% Tween 20).

**Oligonucleotide Array Hybridization and Scanning**—Before hybridization, total RNA samples were heated to 99°C for 5 min, equilibrated to 45°C for 5 min, and clarified by centrifugation (15,000 rpm) at room temperature for 5 min. Aliquots of each sample (10 µg of cRNA in 200 µl of the master mix) were hybridized to U133A GeneChip arrays at 45°C for 16 h in a rotisserie oven set at 60 rpm. After this, the arrays were washed with non-stringent wash buffer (5 X saline/sodium phosphate/EDTA, 0.01% Tween 20) and stringent wash buffer (100 mM MES/0.1 M Na[+], 0.01% Tween 20), stained with streptavidin-phycocerythrin (Molecular Probes), washed again, and read using a microarray scanner G2500A (Affymetrix) with the 570-nm long-pass filter. Data analysis was performed using Affymetrix Microarray Suite software. For comparing arrays, normalization was performed using data from all probe sets.

**Reverse Transcription-PCR Analysis**—MG63 cells were treated with 10 µM RIF, 10 µM MK-4, or vehicle for indicated times. Total RNA was isolated using the ISOGEN reagent (Nippon Gene, Tokyo, Japan). First strand cDNA was generated from RNase-free DNase I-treated total RNA by using the SuperScript II reverse transcriptase (Invitrogen) and oligo(dT)20 primer. For PCR amplification, the primer sequences were: human TSK, 5′-CTTGAGCCACGATGCAGCTTCGGA-3′; human MATN2, 5′-ACAGATCTCCTTGGCTGTCAGTG-3′; human CD14, 5′-GACTGATGGCGGCTCCTTGTG-3′; human CYP4A9, 5′-TTGACGCCCCCTTTCATATTT-3′; human GAPDH, 5′-GCCGCTGACCAAATGC-3′; and 5′-GAGTTGGCGTGGGCAAATG-3′. PCRs were performed using SYBR green PCR master mix (Applied Biosystems) and the ABI Prism 7000 system (Applied Biosystems) based on SYBR Green I fluorescence. The evaluation of relative differences of PCR product amounts among the treatment groups was carried out by the comparative cycle threshold (Ct) method, using GAPDH as an external control (17).
The experiments were independently repeated at least three times, each performed in triplicate. For cycloheximide treatment, cells were preincubated with the compound (10 μg/ml) 2 h prior to the stimulation by SXR ligands.

**RNA Interference**—Small interfering RNA (siRNA) duplexes to target human SXR and TSK were synthesized by Qiagen (Qiagen, Tokyo, Japan). The siRNA target sequences were: SXR, 5’-GGCCACTTGCTCTACTCCCT-3’ (18) and TSK, 5’-CTCTGAACACACCACCTCCTCA-3’. The siRNA specific to the luciferase gene (Luciferase GL2 Duplex, Dharmaco, Lafayette, CO) and nonspecific control VII (Dharmacon) was used as control. Cells were transfected with siRNA (70 nM) using GeneSilencer reagent (Genlantis, San Diego, CA) for 48 h, and further maintained in the culture medium containing 10% dextran-charcoal-stripped FBS with or without ligand stimulation for indicated times.

**Collagen Accumulation Assay by Sirius Red Staining**—Cells were cultured until confluence (day 0), and the medium was replaced by the osteoblast differentiation medium (α-minimal essential medium containing 10% FBS, 2 mM glutamine, 50 μg/ml ascorbic acid, and 5 mM β-glucosylan). Cells were fixed with Bouin’s fluid (8.3% formaldehyde and 4.8% acetic acid in saturated aqueous picric acid) for 1 h at room temperature, rinsed with water, and stained with 1 mg/ml of sirius red dye (Direct Red 80) (Sigma) in saturated aqueous picric acid for 1 h. Cells were treated with 0.01N HCl, and then the stain was extracted by 0.1N NaOH. The absorbance of the dye solution was measured at 550 nm (19). In experiments with warfarin [3-(α-acetylbenzyl)-4-hydroxycoumarin, Sigma] treatment, cells at confluence were pretreated with vehicle or warfarin at 5 μM or 25 μM for 1 day, then treated with vehicle or vitamin K2 (1 μM) for another 3 days in the presence of warfarin (final concentration; 25 μM or 12.5 μM). In siRNA treatment experiments, cells were treated with the siRNA twice, 2 days before day 0 and on day 0.

**Statistical Analysis**—Differences between two groups were analyzed using two-sample, two-tailed Student’s t test. A p value less than 0.05 was considered to be significant. All data are presented in the text and figures as the mean ± S.D.

**RESULTS**

**Construction of SXR Expression Vectors and Generation of Osteoblastic Cells Stably Expressing SXR**—Our previous studies showed the direct effect of vitamin K2 on bone marker expression in osteoblastic cells. Although SXR is endogenously expressed in osteoblastic cells, it has been shown that the expression level is lower than that in cells derived from the intestine. Therefore, to identify vitamin K2 and SXR target genes in osteoblastic cells, we generated MG63 cells stably expressing SXR constructs. SXR protein expression in MG63/FLAG-SXR clones #2 and #3 and MG63/FLAG-VP16C-SXR clones #15 and #17 was confirmed by Western blotting (WB) using anti-SXR antibody.

**Identification of Genes Up-regulated by SXR Ligand in Osteoblastic Cells by GeneChip Analysis**—To identify dual up-regulated genes by vitamin K2 and RIF treatment in osteoblastic cells, we prepared biotin-labeled cRNA samples from MG63 cells expressing FLAG-VP16C-SXR treated with vitamin K2, RIF, or vehicle. The Affymetrix U133A GeneChip array represents more than 18,000 human transcripts from ~14,000 genes. Analysis of the MG63 samples was performed by hybridizing aliquots of cRNA to the GeneChip arrays. Seventy-seven transcripts were induced 2-fold or greater by vitamin K2, whereas 100 transcripts were induced by RIF. Eighteen transcripts were up-regulated by both SXR ligands. Therefore, we infer that these are potential SXR target genes.

Table 1 shows the list of 18 transcripts from 14 distinct genes up-regulated by vitamin K2 and RIF. It is notable that a prototypical SXR-responsive gene ATP-binding cassette subfamily B or multidrug resistance 1 (MDR1) (20) was most significantly up-regulated by either vitamin K2 or RIF. Among these SXR target molecules, we were particularly interested in three genes due to their putative bone-related functions. There were a small leucine-rich proteoglycan named tsukushi...
**Vitamin K₂ Activates SXR Target Genes in Osteoblastic Cells**

**TABLE 1**

Dual up-regulated genes by 48-h treatment with vitamin K₂ (10 μM) or Rif (10 μM) in MG63/FLAG-VP16C-SXR cells identified by GeneChip analysis

| Probe set ID   | Ensemble ID | Gene symbol | Description | -Fold increase over control |
|----------------|-------------|-------------|-------------|-----------------------------|
| 209994_s_at    | ENSG00000085563 | ABCB1      | ATP-binding cassette, subfamily B (MDR/TAP), member 1 | Vitamin K₂ | Rif |
| 209993_at      | ENSG00000085563 | ABCB1      | ATP-binding cassette, subfamily B (MDR/TAP), member 1 | 6.06 | 4.29 |
| 205357_s_at    | ENSG00000144891 | AGTR1      | Angiotensin II receptor, type 1 | 2.83 | 2.46 |
| 212798_at      | ENSG000000142156 | COL6A1     | Collagen, type VI, α1 | 2.64 | 2.14 |
| 201743_at      | ENSG000000710458 | CD14       | CD14 antigen | 2.46 | 4.92 |
| 209771_s_at    | ENSG000000185275 | CD24       | CD24 antigen (small cell lung carcinoma cluster 4 antigen) | 2.46 | 2.30 |
| 211839_s_at    | ENSG000000143871 | CSF1       | Colony-stimulating factor 1 (macrophage) | 2.46 | 2.30 |
| 212937_at      | ENSG000000142156 | COL6A1     | Collagen, type VI, α1 | 2.46 | 2.14 |
| 216379_at      | ENSG000000185275 | CD24       | CD24 antigen (small cell lung carcinoma cluster 4 antigen) | 2.46 | 2.30 |
| 202550_s_at    | ENSG00000132561 | MATN2      | Matrilin-2 | 2.30 | 6.96 |
| 203632_s_at    | ENSG000000167191 | GPRC5B     | G protein-coupled receptor, family C, group 5, member B | 2.30 | 5.28 |
| 211653_x_at    | ENSG000000151632 | AKR1C2     | Aldo-keto reductase family 1, member C2 | 2.30 | 3.73 |
| 218245_at      | ENSG000000182704 | TSK        | Likely ortholog of chicken tsukushi | 2.30 | 2.64 |
| 218854_x_at    | ENSG00000118187 | SART2      | Squamous cell carcinoma antigen recognized by T cells 2 | 2.30 | 5.28 |
| 210002_at      | ENSG000000141448 | GATA6      | GATA-binding protein 6 | 2.14 | 2.14 |
| 216594_x_at    | ENSG000000187134 | AKR1C1     | Aldo-keto reductase family 1, member C1 | 2.14 | 3.73 |
| 204151_x_at    | ENSG000000187134 | AKR1C1     | Aldo-keto reductase family 1, member C1 | 2.00 | 3.48 |
| 212268_at      | ENSG00000021355 | SERPINB1   | Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1 | 2.00 | 2.83 |

Gene annotation was determined based on the probe set ID by the Array Finder on the Affymetrix web site.

(TSK), an extracellular matrix protein matrilin-2 (MATN2), and CD14 antigen.

**Ligand-dependent Induction of SXR Target Genes in Osteoblastic Cells**—We next validated whether mRNA expression levels for these three genes could be modulated by vitamin K₂ and Rif in MG63 cells ectopically expressing either FLAG-VP16C-SXR or FLAG-SXR using quantitative real-time RT-PCR analysis (Fig. 2). All of the three SXR target genes, TSK, MATN2, and CD14, were up-regulated by SXR ligands. The time-dependent expression profiles of those genes in FLAG-VP16C-SXR and FLAG-SXR-expressing cells were quite similar, although the amplitude of induction was different in these cells. In both cell types, Rif generated stronger induction of mRNA expression than vitamin K₂. Nevertheless, the maximal induction by vitamin K₂ was greater than 2-fold for all three genes in both cell types.

**Transcriptional Regulation of SXR Target Genes in Osteoblastic Cells**—We next asked whether the induction of SXR target genes was dependent on direct activation of transcription or required ongoing protein synthesis. MG63 cells overexpressing FLAG-SXR were treated with vitamin K₂ or Rif in the presence or absence of cycloheximide. The ligand-dependent up-regulation of the three SXR target genes, including TSK, MATN2, and CD14, was not affected by cycloheximide treatment, indicating that the transcriptional regulation of those genes was independent of protein synthesis (Fig. 3A).

To further demonstrate the requirement for SXR in the regulation of TSK, MATN2, and CD14, we investigated the effects of siRNA on the ligand-dependent induction of gene expression. Forty-eight hour treatment with a specific siRNA duplex against SXR (siRNA-SXR), but not with a control siRNA directed against luciferase (siRNA-Luc), reduced the SXR protein level by more than 60% in MG63/SXR clone #3 (Fig. 3B). The effectiveness of the SXR-specific siRNA was confirmed as the vitamin K₂-induced up-regulation of CYP3A4 mRNA expression was diminished by the SXR siRNA in MG63/SXR clone #3 (Fig. 3C). In that cell system, the SXR siRNA significantly reduced either vitamin K₂- or Rif-activated mRNA expression for TSK, MATN2, and CD14 (Fig. 3D).

We next examined whether the SXR siRNA duplex reduced the endogenous expression of SXR protein (Fig. 4). The endogenous level of SXR protein in parental MG63 cells was barely detected in Western blot analysis (Fig. 4A). Thus, we immunoprecipitated MG63 cell lysates with a polyclonal antibody against the hinge and a part of ligand-binding domain of SXR (H-160) and immunodetected SXR protein by another polyclonal antibody against the SXR N terminus (N-16). The enrichment of SXR protein in immunoprecipitated fraction was also confirmed in COS1 cells transiently transfected with FLAG-SXR (Fig. 4A). Based on this evaluation system, we could show that the SXR siRNA reduced the level of endogenous SXR protein in MG63 (Fig. 4B).

Since we confirmed that the SXR siRNA duplex was effective to inhibit the endogenous expression of SXR protein, we next analyzed whether the SXR siRNA reduced the expression of the SXR target genes in parental MG63 cells. The SXR siRNA at 14 or 70 nM could significantly reduce endogenous SXR mRNA levels in natural MG63 cells (Fig. 4C). The expression of TSK, MATN2, and CD14 was all up-regulated by either vitamin K₂ or Rif, indicating that the three genes were bona fide SXR targets in parental MG63 cells (Fig. 4D). This ligand-dependent induction of all three genes was significantly reduced by the SXR siRNA transfection in parental MG63 cells (Fig. 4D).

**Vitamin K₂ and TSK Stimulate Collagen Accumulation in Osteoblastic Cells**—TSK was recently identified as a bone morphogenetic protein-binding protein that belongs to the small leucin-rich proteoglycan family (21), which is implicated as an extracellular matrix component. Because small leucine-rich proteoglycans such as biglycan and decorin are known to interact with matrilin-1 in the cartilage extracellular matrix (22), TSK and matrilin-1-related MATN2 are likely to be involved in the assembly of extracellular matrix, including collagens, in osteoblastic cells.

We next asked whether vitamin K₂ promoted collagen production or stabilized collagen levels. We evaluated collagen amounts by staining cells with a strong anionic dye Sirius red, which reacted with basic groups present in collagens via its sulfonic acid groups. It has been reported that type I and III collagens are well stained by Sirius red (19). Four-day treatment with vitamin K₂ exhibited significantly more intense staining by Sirius red compared with vehicle in MG63 cells under conditions favoring osteoblast differentiation (Fig. 5A). We also examined collagen accumulation in murine MC3T3-E1 cells, one of the cell lines with a close-to-normal osteoblast phenotype. Four-day treatment with vitamin K₂ increased collagen accumulation by 15.0% in this cell line. Note that Rif (1 μM) also increased collagen accumulation by 13.6% in MG63 cells after 4-day treatment. Moreover, the vitamin K₂-stimulated collagen accumulation in MG63 cells was not affected by warfarin treatment, suggesting that the γ-carboxylase-dependent vitamin K₂ action may not be involved (Fig. 5B).
To determine whether TSK plays a role in the vitamin K2-stimulated collagen accumulation, MG63 cells stably expressing FLAG-tagged TSK were generated. Two TSK-overexpressing clones were isolated, as confirmed by Western blot analysis (Fig. 5C). MG63 clones overexpressing TSK showed significantly enhanced collagen accumulation in 7-day culture under differentiation conditions compared with vector-transfected cells (Fig. 5D). During the 7-day culture, the growth of MG63 clones expressing TSK and vector was almost stationary, and there was no significant difference in proliferation between the two groups as determined by the proliferation assay using WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) reagent (Nacalai Tesque, Kyoto, Japan; Ref. 23) (data not shown).

We further investigated whether SXR or TSK loss-of-function affected collagen accumulation in MG63 cells. A siRNA duplex against TSK (70 nM) reduced the target mRNA levels by more than 40% in parental MG63 cells, verifying its efficiency (Fig. 6A). The SXR- and TSK-specific siRNA significantly reduced the vitamin K2-stimulated
accumulation of collagen in parental MG63 cells compared with luciferase siRNA (Fig. 6, B and C).

Taken together, our results indicate that vitamin K2 promotes collagen accumulation in osteoblastic cells via the SXR signaling pathway.

DISCUSSION

In the present study, we identified novel SXR target genes that are up-regulated by both vitamin K2 and RIF in osteoblastic cells using oligonucleotide microarrays. The effectiveness of vitamin K2 and RIF treatment was evident by their ability to up-regulate mRNA levels for the well known SXR target gene MDR1. SXR-dependent induction of TSK, MATN2, and CD14 has not been previously reported. Functional analyses indicated that vitamin K2 can enhance collagen accumulation in osteoblastic cells and that SXR may play a role in the collagen assembly mechanism. Taken together, these results provide important evi-
Vitamin $K_2$ Activates SXR Target Genes in Osteoblastic Cells

The vitamin $K_2$-stimulated collagen accumulation through the activation of SXR signaling may be beneficial to decrease bone fractures. Since bone collagen content is reduced in aged and osteoporotic bones (24), the amount and quality of collagen fibrils may be important for maintaining bone strength. Therefore, in addition to its role as an enzymatic cofactor that facilitates $\gamma$-carboxylation of bone Gla proteins, vitamin $K_2$ may serve as a critical factor regulating bone matrix formation.

The identification of new SXR-mediated vitamin $K_2$ target genes in bone cells has implications for bone homeostasis. Human TSK is an ortholog of chicken TSK, which was recently identified as a bone morphogenic protein-binding protein that plays a role in the development of primitive streak and Hensen's node formation during chick gastrulation (21). TSK, like other small leucine-rich proteoglycans, may play a role in bone formation. Small leucine-rich proteoglycans such as biglycan, decorin, and chondroadherin have been characterized as collagen-binding proteins in bone tissues (25–28). Biglycan-deficient mice exhibit reduced bone mass (29), and biglycan/decorin double-deficient mice show a more severe phenotype of osteoporosis (30).

MATN2 is expressed in various osteoblastic cells as well as mouse primary osteoblasts (31, 32), and it was shown to interact with collagen I (33). The involvement of matrilin proteins together with small leucine-rich proteoglycans in the collagen assembly is exemplified by the complex of matrilin-1 and biglycan/decorin that act as a linkage between the collagen II and collagen VI fibrils (22).

The CD14 antigen is a lipopolysaccharide-binding protein expressed in monocytes where it initiates the innate immune response to bacterial invasion (34). The soluble form of CD14 is an inducer of B-lymphocyte growth and differentiation (35), and B-lymphocyte lineage cells regulate osteoclastogenesis by expressing receptor activator of NF-$\kappa$B ligand (RANKL) and serving as osteoclast progenitor cells (36). This suggests a role for CD14 in osteoclastogenesis through B-lymphocyte lineage cells. A role for CD14 in bone formation is also suggested by a report showing that the antigen was up-regulated during the differentiation of mouse primary osteoblasts (37). Because osteoclastic resorption and osteoblast formation are coupled in the bone remodeling process, CD14 may play a role as a “coupling factor” between the two functions. In this context, it is interesting that CD24 was identified as an up-regulated gene by both vitamin $K_2$ and RIF in osteoblastic cells in our microarray analysis (38). This suggests a role for CD24 as an up-regulated gene by both vitamin $K_2$ and RIF in osteoblastic cells in our microarray analysis because CD24 is also a cell surface antigen predominantly expressed in B-cell lineage cells and it has been implicated in both activation and differentiation of B lymphocytes (38).

In summary, we conclude that SXR mediates vitamin $K_2$-activated transcription of extracellular matrix-related genes as well as cell surface markers of B-lymphoid lineage cells that may be involved in both osteoblastogenesis and osteoclastogenesis. These results would provide new insight into vitamin $K_2$ and SXR action on bone homeostasis and osteoporosis treatment and further support the idea that vitamin $K_2$ acts as a transcriptional mediator of gene expression in bone cells, in addition to its well known role as an enzymatic cofactor.

Acknowledgments—We thank T. Suzuki and R. Nozawa for their technical assistance.

REFERENCES

1. Akedo, Y., Hosoi, T., Inoue, S., Ikegami, A., Minuno, Y., Kaneki, M., Nakamura, T., Ouchi, Y., and Orimo, H. (1992) Biochem. Biophys. Res. Commun. 187, 814–820
2. Akiyama, Y., Hara, K., Tajima, T., Murota, S., and Morita, I. (1994) Eur. J. Pharmacol. 263, 181–185
3. Hara, K., Akiyama, Y., Nakamura, T., Murota, S., and Morita, I. (1995) Bone (N.Y.) 16, 179–184
Vitamin K$_2$ Activates SXR Target Genes in Osteoblastic Cells

4. Koshihara, Y., and Hoshi, K. (1997) J. Bone Miner. Res. 12, 431–438
5. Koshihara, Y., Hoshi, K., Okawara, R., Ishibashi, H., and Yamamoto, S. (2003) J. Endocrinol. 176, 339–348
6. Shiraki, M., Shiraki, Y., Aoki, C., and Miura, M. (2000) J. Bone Miner. Res. 15, 515–521
7. Booth, S. L., Tucker, K. L., Chen, H., Hannan, M. T., Gagnon, D. R., Cupples, L. A., Wilson, P. W., Ordovas, J., Schaefer, E. J., Dawson-Hughes, B., and Kiel, D. P. (2000) Am. J. Clin. Nutr. 71, 1201–1208
8. Plaza, S. M., and Lamson, D. W. (2005) Altern. Med. Res. 10, 24–35
9. Price, P. A., and Baukol, S. A. (1980)
10. Vergnaud, P., Garnero, P., Meunier, P. J., Breart, G., Kamihagi, K., and Delmas, P. D. (1997) J. Clin. Endocrinol. Metab. 82, 719–724
11. Luo, G., Ducy, P., McKee, M. D., McKee, M. D., and Karsenty, G. (1997) Nature 386, 78–81
12. Mushed, M., Schinke, T., McKee, M. D., and Karsenty, G. (2004) J. Cell Biol. 165, 625–630
13. Tabb, M. M., Sun, A., Zhou, C., Grun, F., Errandi, J., Romero, K., Pham, H., Inoue, S., Mallick, S., Lin, M., Forman, B. M., and Blumberg, B. (2003) J. Biol. Chem. 278, 43919–43927
14. Blumberg, B., Sabbagh, W., Jr., Juguilon, H., Bolado, J., Jr., van Meter, C. M., Ong, E. S., Sabbagh, W., Jr., Juguilon, H., Bolado, J., Jr., van Meter, C. M., Ong, E. S., and Evans, R. M. (1998) Genes Dev. 12, 3195–3205
15. Wright, S. D., Ramos, R. A., Tobias, P. S., Ulevitch, R. J., and Mathison, J. C. (1990) J. Immunol. 145, 1431–1433
16. Akira, S., and Julius, M. (2001) Science 294, 1431–1433
17. Filipp, D., Alizadeh-Khiavi, K., Richardson, C., Palma, A., Paredes, N., Takeuchi, O., Akira, S., and Mathison, J. C. (1999) J. Immunol. 163, 2625–2631
18. Rosman-Roman, S., Garcia, T., Jackson, A., Theilhaber, J., Rawadi, G., Connolly, T., Spinella-Jaergel, S., Kawai, S., Courtois, B., Bushnell, S., Auberval, M., Mall, K., and Baron, R. (2003) Bone (N.Y.) 32, 474–482
19. Kay, R., Rosten, P. M., and Humphries, R. K. (1991) J. Immunol. 147, 1412–1416