Cathepsin K, but Not Cathepsins B, L, or S, Is Abundantly Expressed in Human Osteoclasts*

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Random high-throughput sequencing of a human osteoclast cDNA library was employed to identify novel osteoclast-expressed genes. Of the 5475 ESTs obtained, approximately 4% encoded cathepsin K, a novel cysteine protease homologous to cathepsins S and L; ESTs for other cathepsins were rare. In addition, ESTs for cathepsin K were absent at low frequency in cDNA libraries from numerous other tissues and cells. In situ hybridization in osteoclastoma and osteophyte confirmed that cathepsin K mRNA was highly expressed selectively in osteoclasts; cathepsins S, L, and B were not detectable. Cathepsin K was not detected by in situ hybridization in a panel of other tissues. Western blot of human osteoclastoma or fetal rat humerus demonstrated bands of 38 and 27 kDa, consistent with sizes predicted for pro- and mature cathepsin K. Immunolocalization in osteoclastoma and osteophyte showed intense punctate staining of cathepsin K exclusively in osteoclasts, with a polar distribution that was more intense at the bone surface. The abundant expression of cathepsin K selectively in osteoclasts strongly suggests that it plays a specialized role in bone resorption. Furthermore, the data suggest that random sequencing of ESTs from cDNA libraries is a valuable approach for identifying novel cell-selective genes.

Bone is composed of a protein matrix in which spindle- or plate-shaped crystals of hydroxapatite are incorporated (1). The matrix is approximately 90% Type I collagen, but also contains a number of non-collagenous proteins such as osteocalcin, osteopontin, and bone sialoprotein. It has been recognized for many years that bone resorption requires both dissolution of the inorganic mineral component (acidic microenvironment) and degradation of the protein matrix (protease activity). This has led to extensive efforts to identify the protease(s) responsible for osteoclast-mediated bone resorption. However, since osteoclasts are very rare cells and no appropriate osteoclast cell model has been identified, standard biochemical approaches for identification of the protease(s) have proven to be very difficult.

A number of studies have suggested that a cysteine protease(s) is involved in bone resorption. For example, several known cathepsins have collagenolytic activity under acidic conditions (2), a property that is predicted to be required for the enzyme(s) secreted from the osteoclast into the acidic resorption lacunae. In addition, classical inhibitors of cysteine proteases, such as leupeptin, Z-Phe-Ala-CHN₂, E-64, and cystatin, have demonstrated activity at preventing osteoclast-mediated bone resorption in vitro models (3–8). Z-Phe-Ala-CHN₂ and leupeptin have also shown activity in vivo in a murine hypercalcemia model of bone resorption (4). Based upon observed substrate and inhibitor preferences, as well as immunological reactivity, several groups have suggested that cathepsins B or L, or a closely related enzyme, are likely to be responsible for osteoclast-mediated resorption (9–21).

Recently a novel member of the papain family of cysteine proteases has been cloned that is most homologous to cathepsins S and L (22–27). Clones for this enzyme were first identified in cDNA libraries of rabbit (22) and human (25, 27) osteoclasts, suggesting that it was selectively expressed in osteoclasts. This novel cathepsin has been referred to as OC2 (22) or cathepsins O (23), K (27), X (26), or O2 (24); we refer to it as cathepsin K.1 The approach that we used to identify cathepsin K was to partially sequence large numbers of randomly chosen clones from an osteoclast cDNA library (25). By comparing homology to known sequences, the expressed sequence tags (ESTs)2 obtained from this technique provide a valuable approach for identification of novel expressed genes (28–30).

In the present study, cellular expression of cathepsin K was examined by in situ hybridization in multiple tissues and compared with expression of cathepsins S, B, and L. In addition, specific anti-cathepsin K antibodies were generated and used to demonstrate expression and cellular localization of cathepsin K protein. The data clearly show that cathepsin K is abundantly and selectively expressed in osteoclasts, and that it displays a cellular localization consistent with an involvement of the enzyme in bone resorption. Furthermore, the data indicate that cathepsins S, B, and L, which had been proposed to be involved in bone resorption, are either expressed at very low levels or are absent in osteoclasts.

MATERIALS AND METHODS

Osteoclast cDNA Library—Fresh osteoclastoma tissue was chopped into small pieces and placed into a sterile 50-ml centrifuge tube. The pieces were disaggregated by incubating at 37 °C for 30 min in serum-free RPMI 1640 medium (Life Technologies, Inc.), supplemented with 3 mg/ml (w/v) type I collagenase (Sigma). A cell suspension was obtained

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1 The Nomenclature Committee of the International Union of Biochemistry and Molecular Biology has assigned the name cathepsin K (EC 3.4.22.38) to the enzyme described in this paper.

2 The abbreviations used are: EST, expressed sequence tag; TESPA, 3-aminopropyltriethoxy silane; PAGE, polyacrylamide gel electrophoresis.

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by gently homogenizing the remaining tissue with a plunger from a 30-ml syringe. The osteoclastoma-derived cells were pelleted by centrifugation (400 x g for 10 min) and resuspended in 6 ml of cold culture medium (RPMI with 10% fetal calf serum, 100 units/ml penicillin, and 50 µg/ml streptomycin; Life Technologies, Inc.) to which was added 3 ml of the anti-β3 antibody, C22. Following a 30-min incubation on ice, the cells were washed twice by centrifugation at 300 x g for 10 min. After the last wash, the cells were resuspended in 10 ml of medium and were enumerated in a hemocytometer. Dynabeads (Dynal Inc., Great Neck, NY), coated with goat anti-mouse IgG, were incubated for 30 min on ice with the cell suspension at a density of 6 beads/osteoclast. The bead-coated osteoclasts were immobilized on a magnet, and the uncoated cells were washed off with extensive washing with cold RPMI. Anti-β3 antibody-coated osteoclast-rich suspension was then resuspended in fresh RPMI medium and seeded into eight T250 tissue culture flasks. The cells were cultured for 3 days prior to the extraction of mRNA using the Invitrogen FastTrack mRNA Isolation Kit. The mRNA was methylmercuric hydroxide denatured prior to cDNA synthesis, and a directional oligo(dT)-primed cDNA library was prepared (Stratagene, La Jolla, CA). cDNA was size fractionated, and fragments greater than 1 kilobase were ligated into the Uni-ZAP XR vector.

In situ Hybridization—Cryostat sections of osteocytes and osteoclast tissue were processed as described previously (31). The sections were picked off onto 3-aminopropyltriethoxysilane (TESPA)-coated glass slides. A cDNA clone (pBluescript SK) containing the coding region of human cathepsin K was obtained from the anti-cathepsin K antibody library. Clones for cathepsins B, S, and L were obtained from stromal cell, pancreatic tumor, and human embryo cDNA libraries, respectively. cDNA templates were linearized and then transcribed from the T3 or the T7 promoter to generate sense and antisense probes, respectively. Riboprobes were prepared using the Promega (Madison, WI) In Vitro transcription kit with [35S]thio-CTP (Amersham Corp.). Following transcription, cDNA templates were digested with RQ1 RNase-free DNase I (Promega), and unincorporated nucleotides were removed by centrifugation through Quick Spin Sephadex G-50 columns (Boehringer Mannheim). RNA transcripts with a specific activity in excess of 10⁸ cpm/mg were used for hybridization.

In situ hybridization was performed by a modification of the method of Zeller and Rogers (32), as follows. Cryosections were fixed in 4% paraformaldehyde in phosphate-buffered saline. Denaturation was in 0.2 N HCl for 20 min, followed by acetylation in 0.25% acetic anhydride, 0.1 M triethanolamine, 50 mM sodium citrate, pH 7.0, dehydrated in 30%, 60%, 80%, 95%, and 100% alcohol, and air-dried. Sections were used immediately for hybridization in buffer consisting of 2 parts hybridization mix B (1.2 M NaCl, 20 mM Tris-HCl, pH 7.5, 4 mM EDTA, 2 x 10⁶ cpm/mg). Following incubation, the sections were dehydrated twice in 2 x SSC, 3 x SSC, 20% ethanol, 50% ethanol, and 70% ethanol. Hybrids were visualized using a Kodak (Rochester, NY) X-Omat AR film at −70°C. Sections were then boiled for 15 min in citrate buffer (pH 6.0) immediately before boiling them in the citrate buffer for 15 min in the microwave. The remainder of the technique was performed as outlined above.

RESULTS

Cathepsin K EST Frequency—Cathepsin K was identified as a novel cysteine protease whose ESTs were highly abundant in an osteoclast library from human osteoclastoma (25). Approximately 4% of all ESTs randomly sequenced from this library encoded cathepsin K (223 ESTs of 5475 total). In contrast, ESTs for cathepsin K were absent in most other libraries sequenced. For cDNA libraries in which ESTs for cathepsin K were found, they were present at much lower frequency than in the osteoclast cDNA library. For example, in cDNA libraries for which greater than 1000 ESTs had been sequenced, the frequency of ESTs for cathepsin K was: placenta, 0.0016%; white adipose, 0.06%; retina, 0.06%; colon, 0.037%; epididymis, 0.09%; gall bladder, 0.03%; testes, 0.006%; tonsils, 0.009%; chondrosarcoma, 0.13%; ovarian cancer, 0.018%; B cell lymphoma, 0.015%; pancreatic tumor, 0.017%; prostate cancer, 0.087%; T-cell lymphoma, 0.036%; and activated monocytes, 0.044%. Thus, data from a number of libraries suggest that cathepsin K is abundant only in osteoclasts.

In contrast to the abundance of ESTs for cathepsin K, ESTs for other cathepsins were rare in the osteoclast library. Only two ESTs (0.036%) for cathepsin B were identified from the osteoclast library, and one EST (0.018%) for cathepsin S was found. No ESTs for cathepsin L were found, and no other ESTs for cysteine proteases were represented in the library. Thus, ESTs for cathepsin K represented greater than 98% of the total cysteine protease ESTs in the human osteoclast cDNA library. Expression of Cathepsin K mRNA—Northern blot analysis of osteoclastoma tissue with a specific probe for cathepsin K demonstrated a single band of approximately 2 kilobases (data not shown). To determine which cells expressed the enzyme, in situ hybridization studies were performed in human osteoclastoma and osteocyte. Cathepsin K was abundantly and selectively expressed in osteoclasts and a discrete population of mononu-
clear cells within human osteophyte and osteoclastoma tissue (Fig. 1, A and D). All other cell types, including stromal cells (of the osteoclastoma), marrow cells, osteoblasts, osteocytes, and chondrocytes, were negative. At sites of cartilage remodeling in the osteophyte, chondroclasts also expressed cathepsin K (Fig. 1, A).

To determine the expression of cathepsin K in other cell types, a panel of human tissues was tested by in situ hybridization. Cathepsin K mRNA was not detected in any of the tissues tested (Table I).

Expression of mRNA for Cathepsins S, L, and B—The EST frequency suggested that cathepsins S, L, and B were expressed at a much lower frequency in osteoclasts than cathepsin K. To confirm this, in situ hybridization studies were performed on osteoclastoma and osteophyte sections with probes specific for these cathepsins. No hybridization was detected in osteoclasts in osteoclastoma or osteophyte with probes for cathepsins S, L, or B (cathepsin B shown as representative; Fig. 1, C and F; Table I). As expected, cathepsins B and L were highly expressed in spleen, liver, and kidney (Table I).

Expression of Cathepsin K Protein—Western blot analysis of osteoclastoma tissue with antibody against either synthetic peptides unique to cathepsin K (antibody C2) or to intact procathepsin K that had been expressed in E. coli (antibody SR1) demonstrated immunoreactive bands of 38 kDa and 27 kDa (Fig. 3, lane A), consistent with the predicted size of the proand mature cathepsin K, respectively. For the antipeptide antibody, addition of an excess of the peptide immunogen prevented the detection of these bands (Fig. 3, lane B); an excess of an unrelated peptide had no effect (data not shown). To determine if cathepsin K was expressed in normal tissue, fetal rat humerus was analyzed by Western blot, and a similar pattern of expression was observed (data not shown).

Immunolocalization of cathepsin K using antibody SR1 in osteoclastoma tissue demonstrated abundant staining in osteoclasts and showed a punctate, granular distribution that was very often localized to a single pole of the osteoclasts (Fig. 2A, large arrowheads). A small population of mononuclear cells (potentially representing an osteoclast precursor population) also demonstrated reactivity (Fig. 2A, small arrowheads). Surrounding stromal cells were negative for cathepsin K. Immunolocalization with antibody C2 demonstrated similar results (data not shown). No staining could be detected in any cells on the nonimmune serum control slides (Fig. 2B).

In osteophyte, a similar pattern of cathepsin K reactivity was detected in osteoclasts apposed to the surface of bone (Fig. 2C and D). The osteoclasts showed a distinct polarity of staining that was more intense toward the apical surface of resorbing osteoclasts. Cathepsin K expression also appeared to be restricted to osteoclasts, since other bone marrow cells, chondrocytes, osteoblasts, osteocytes, and connective tissue cells did not demonstrate reactivity (Fig. 2C and D; Table I).

In contrast to the immunoreactivity observed in osteoclasts, cathepsin K protein expression was not detected in the panel of other human tissues analyzed (Table I).

**DISCUSSION**

Previous studies have consistently demonstrated that inhibitors of cysteine proteases are very effective at preventing osteoclast-mediated bone resorption, and have clearly implicated a cathepsin(s) as a key mediator of this process (3-7). Delaisse et al. (3) tested a series of protease inhibitors in a mouse bone organ culture system and found that inhibitors of cysteine proteases (e.g., leupeptin and Z-Phe-Ala-CHN2) reduced bone resorption, while serine protease inhibitors were ineffective. A follow-up study by the same group showed that E-64 and leupeptin were also effective at preventing bone resorption in vivo, as measured by acute changes in serum calcium in rats on calcium-deficient diets (4). Based upon the activity of the enzyme, this group classified the enzyme as

![Fig. 1. In situ hybridization. Sections were hybridized to the probes indicated, followed by methylene blue counterstain (original magnification, × 20). A, cathepsin K antisense probe in a section of human osteoclastoma tissue. Osteoclasts (large arrowheads) and a small population of mononuclear cells (small arrowheads) demonstrated strong cathepsin K mRNA expression. B, serial section of A probed with the cathepsin K sense strand. C, cathepsin B mRNA expression in section of osteoclastoma. Osteoclasts (large arrowheads) did not demonstrate expression; however, associated mononuclear cells (small arrowheads) demonstrated strong cathepsin B mRNA expression. D, cathepsin K antisense probe in a section of human osteophyte. Osteoclasts resorbing or adjacent to bone (B) demonstrated selective and strong cathepsin K mRNA expression (arrowheads). E, serial section of D probed with the cathepsin K sense strand. F, cathepsin B antisense probe in a section of human osteophyte. Osteoclasts (large arrowheads) resorbing bone demonstrated no cathepsin B mRNA expression.](image-url)
sible as cathepsin B. Cystatin, an endogenous cysteine protease inhibitor, was shown to prevent parathyroid hormone-stimulated bone resorption in mouse calvariae (7). Detailed studies demonstrated that the number and volume of resorption pits were decreased in the presence of cysteine protease inhibitors, while the surface area of the pits was unaffected (5). Hill et al. (6) confirmed these findings on resorption pit parameters and suggested that cathepsins B, L, or S were involved. Thus, data from several studies indicated that inhibitors of cysteine proteases were very effective at preventing bone resorption, and strongly suggested that a cysteine protease(s) plays an essential role in the process.

In the present study, an enriched population of human osteoclasts was used to prepare a cDNA library that was subjected to high throughput random sequencing of clones. Among the genes identified was a novel cysteine protease that is highly

| Human tissue | Anti-cathepsin K antibody (SR-1) | Anti-cathepsin K antibody (C2) | mRNA |
|--------------|----------------------------------|--------------------------------|------|
| Bone (6)     | ++ ++ b                          | ++ ++ b                        | +++ b|
| GCT (6)      | ++ ++ b                          | ++ ++ b                        | +++ b|
| Lung (3)     | Negative                         | Negative                       | Negative |
| Kidney (2)   | Negative                         | Negative                       | Negative |
| Heart (2)    | Negative                         | Negative                       | Negative |
| Spleen (4)   | Negative                         | Negative                       | Negative |
| Liver (5)    | Negative                         | Negative                       | Negative |
| Skin (2)     | Negative                         | Negative                       | Negative |
| Colon (1)    | Negative                         | Negative                       | Negative |
| Rheumatoid synovium (5) | Negative | Negative | Negative |

* Number in parentheses indicates number of patient samples screened.

b Osteoclasts and discrete populations of TRAP-positive mononuclear cells.

Expression of cathepsin B, S, L, and K mRNA in human bone, osteoclastoma (GCT), and a panel of human tissues by in situ hybridization

| TISSUE | Cathepsin B | Cathepsin S | Cathepsin L | Cathepsin K |
|--------|-------------|-------------|-------------|-------------|
| Bone osteoclasts | Negative | Negative | Negative | +++ |
| GCT osteoclasts | Negative | Negative | Negative | +++ |
| Cartilage chondroclasts | Negative | Negative | Negative | +++ |
| Spleen | ++ ++ c | ND | ++ c | Negative |
| Liver | ++ c | ND | ++ c | Negative |
| Kidney | ++ c | ND | <= | Negative |

a Variable (low) levels of cathepsin L and B mRNA were observed in osteoblast (− to +), chondrocyte (+), and marrow cell populations (− to +) in sections of osteophyte.

b Discrete populations of mononuclear cells were positive for cathepsin B mRNA; low levels of cathepsin L mRNA were occasionally observed in stromal cells.

c High and discrete expression in macrophages. Variable expression (+/- to +) was observed in hepatocytes, kidney tubule cells, etc.

d ND, not done.

**FIG. 2. Immunolocalization of cathepsin K.** Sections were probed with the antisera indicated, followed by labeled streptavidin-biotin and staining with Mayer’s hematoxylin. A, osteoclasts (large arrowheads) and a minor population of mononuclear cells (small arrowheads) in osteoclastoma demonstrated strong staining with anti-cathepsin K antibody (SR1). In many of the multinucleated osteoclasts, the staining was polarized to one edge of the cytoplasm (asterisks). Original magnification, × 20. B, no reactivity was detected in a section of osteoclastoma probed with pre-immune serum. Original magnification, × 20. C, strong anti-cathepsin K antibody (SR1) staining was detected on osteoclasts apposed (large arrowheads) to and away from (arrowheads) the bone surface in a section human osteophytic bone. The staining was most intense at the apical surface of the majority of osteoclasts apposed to the bone surface. No reactivity was observed in osteoblasts, osteocytes and the majority of cells within the bone marrow space. Original magnification, × 10. D, higher magnification of C to highlight the polarized staining of SR1 in osteoclasts apposed to the bone surface (arrows). Original magnification, × 40.
Cathepsin K Is Abundantly Expressed in Human Osteoclasts

Another approach that has been taken to identify the relevant protease(s) involved in bone resorption has been purification of protease activity. Delaissé (9) purified protease activity from mouse calvariae and found three main peaks of activity, which they suggested were cathepsins B, L, and an unknown protease with an apparent mass of 70 kDa by gel chromatography. Page et al. (10) used osteoclastoma tissue as an enriched source of osteoclasts for purification. They found six peaks of activity, each of which showed characteristics consistent with cathepsin B. As with the immunolocalization and histochemical studies, however, it is difficult to determine whether these protease activities may have been due to cathepsin K, or even an enzyme derived from cells other than osteoclasts.

Tetzuka et al. (22) cloned the rabbit homolog of cathepsin K, OC-2, from a rabbit osteoclast cDNA library. They demonstrated expression of OC-2 mRNA in the osteoclast by in situ hybridization of bone tissue. This group has also recently reported the sequence of the human enzyme (27). Li et al. (33) have also recently reported cloning of cathepsin K from an osteoclast cDNA library, and Bromme et al. (24) cloned the gene from a human spleen library. Each group indicated that there was abundant expression in osteoclasts, although Bromme et al. (24) also reported expression of cathepsin K mRNA in ovary. Shi et al. (23) also cloned human cathepsin K, but from a human monocyte-derived macrophage library. They demonstrated proteolytic cleavage of fibrinogen when the enzyme was transiently transfected into COS cells. It is of interest that they were unable to detect cathepsin K from freshly isolated monocytes, suggesting that it was the extended culture conditions that led to induction of cathepsin K mRNA. Our inability to detect cathepsin K in rheumatoid synovium, which has high levels of macrophages, is consistent with the lack of expression of cathepsin K in macrophages under normal conditions.

In addition to osteoclasts, our data indicate that cathepsin K was expressed in two other populations of cells. At sites of cartilage remodeling in osteophyte, chondrocytes expressed high levels of cathepsin K. This is not surprising, as these cells are related to or identical to osteoclasts. The data also indicate that cathepsin K is expressed in populations of mononuclear cells within the osteoclastoma tissue. Further characterization of this cell population has demonstrated that these cells possess a number of markers of the osteoclast phenotype, and are capable of forming resorption pits in vitro. Thus, in addition to being highly expressed in mature osteoclasts, the enzyme may represent an excellent marker for the osteoclast precursor population as well.

The ability to sequence large number of clones from an osteoclast library has provided a valuable approach for discovery of novel osteoclast proteins and led to the identification of a novel cathepsin. In addition, the availability of data from multiple human cDNA libraries has allowed us to compare the frequency of ESTs for cathepsin K from a number of cells and tissues. EST frequency suggested abundant osteoclast-selective expression of cathepsin K, and this has been confirmed by both in situ hybridization and immunohistochemistry. The results suggest that cathepsin K may play a specialized, and perhaps essential, role in osteoclast-mediated bone resorption.

Selective inhibitors of cathepsin K may be useful in treatment of diseases of excessive bone loss, such as osteoporosis.

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Fig. 3. Cathepsin K protein expression in osteoclastoma. Human osteoclastoma lysate was separated by SDS-PAGE (12%) and blotted onto nitrocellulose. The blot was probed with an antibody raised against a synthetic peptide from a unique region of the predicted amino acid sequence of human cathepsin K (antibody C-2). In lane A, immunoreactive bands of 38 and 27 kDa are observed. Lane B demonstrates that these immunoreactive bands can be competed with 3 μg/ml peptide antigen.
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REFERENCES

1. Baron, R. (1993) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, pp. 3–9, Raven Press, New York
2. Maciewicz, R. A., and Etherington, D. J. (1988) Biochem. J. 256, 433–440
3. Delaisse, J.-M., Eeckhout, Y., and Vaes, G. (1980) Biochem. J. 192, 365–368
4. Delaisse, J.-M., Eeckhout, Y., and Vaes, G. (1984) Biochem. Biophys. Res. Commun. 125, 441–447
5. Delaisse, J.-M., Boyde, A., Maconnachie, E., Ali, N. N., Sear, C. H. J., Eeckhout, Y., Vaes, G., and Jones, S. J. (1987) Bone 8, 305–313
6. Hill, P. A., Buttle, D. J., Jones, S. J., Boyde, A., Murata, M., Reynolds, J. J., and Meikle, M. C. (1994) J. Cell. Biol. 125, 118–130
7. Lerner, U.-H., and Grubb, A. (1992) J. Bone Miner. Res. 7, 433–439
8. Everts, V., Delaisse, J.-M., Korper, W., Niehof, A., Vaes, G., and Beertsen, W. (1992) J. Cell. Physiol. 150, 221–231
9. Delaisse, J.-M., Ledent, P., and Vaes, G. (1991) Biochem. J. 279, 167–174
10. Page, A. E., Warburton, M. J., Chambers, T. J., Pringle, J. A. S., and Hayman, A. R. (1992) Biochem. Biophys. Acta 1116, 57–66
11. Goto, T., Kiyoshima, T., Moroi, R., Tsukuba, T., Nishimura, Y., Himena, M., Yamamoto, K., and Tanaka, T. (1994) Histochemistry 101, 33–40
12. Goto, T., Tsukuba, T., Aiyata, N., Yamamoto, K., and Tanaka, T. (1992) Histochemistry 97, 13–18
13. Ohsawa, Y., Nitatodi, T., Higuchi, S., Komnini, E., and Uchiyama, Y. (1993) J. Histochem. Cytochem. 41, 1075–1083
14. Goto, T., Tsukuba, T., Kiyoshima, T., Nishimura, Y., Kato, K., Yamamoto, K., and Tanaka, T. (1993) Histochemistry 99, 411–414
15. Sasaki, T., and Ueno-Matsuda, E. (1993) Cell Tissue Res. 271, 177–179
16. Sannes, P. L., Schofield, B. H., and McDonald, D. F. (1986) J. Histochim. Cytochem. 34, 983–988
17. van Noorden, C. J. F., Vogels, I. M. C., and Smith, R. E. (1989) J. Histochem. Cytochem. 37, 617–624
18. Kakegawa, H., Nakawa, T., Tagami, K., Kamioka, H., Sumitani, K., Kawata, T., DrobniKosorok, M., Lenarcic, B., Turk, V., and Katunuma, N. (1993) FEBS Lett. 321, 247–250
19. Rikfkn, B. R., Vernillii, A. T., Kleeckner, A. P., Auszmann, J. M., Rosenberg, L. R., and Zimmerman, M. (1991) Biochem. Biophys. Res. Commun. 179, 63–69
20. Tagami, K., Kakegawa, H., Kamioka, H., Sumitani, K., Kawata, T., Lenarcic, B., Turk, V., and Kutunuma, N. (1994) FEBS Lett. 342, 308–312
21. Kremer, M., Judd, J., Rikfkn, B., Auszmann, J., and Oursler, M. J. (1995) J. Cell. Biol. 125, 273–279
22. Tezuka, K., Tezuka, Y., Maehima, A., Sato, T., Nokami, K., Kamioka, H., Hakeda, Y., and Kumegawa, M. (1994) J. Biol. Chem. 269, 1106–1109
23. Shi, G.-P., Chapman, H. A., Bhaui, S. M., Del, eeuc, C., Reddy, V. Y., and Weis, S. J. (1995) FEBS Lett. 357, 129–134
24. Bromme, D., and Okamoto, K. (1995) J. Biol. Chem. HoppeSeyler 376, 379–384
25. Drake, F., James, I., Connor, J., Riemann, D., Dodds, R., McCabe, F., Bertoldi, D., Bartthiow, R., Hastings, G., and Gowen, M. (1994) J. Bone Miner. Res. 9, 5177
26. Li, Y. P., Alexander, M. B., Wucherpfennig, A. L., Chen, W., Yelick, P., and Stashenko, P. (1994) Mol. Biol. Cell 5, 335a
27. Inada, T., Blabe, G., Ishibashi, O., Tezuka, K., Kumegawa, M., and Kokubo, T. (1995) Biochem. Biophys. Res. Commun. 206, 89–96
28. Adams, M. D., Kelley, J. M., Gocayne, J. D., Dubnick, M., Polymeropoulos, M. H., Xiao, H., Merrill, C. R., Wu, A., Olde, B., Moreno, R. F., Kerlavage, A. R., McCombie, W. R., and Venter, J. C. (1991) Science 252, 1651–1656
29. Adams, M. D., Dubnick, M., Kerlavage, A. R., Moreno, R., Kelley, J. M., Utterback, T. R., Nagle, J. W., Fields, C., and Venter, J. C. (1992) Nature 355, 362–364
30. Adams, M. D., et al. (1995) Nature 377, (suppl.), 3–174
31. Dodds, R. A., Gowen, M., and Bradbeer, J. N. (1994) J. Histochim. Cytochem. 42, 599–606
32. Zeiler, R., and Rogers, M. (1991) in Current Protocols in Molecular Biology, (Jansen, K., ed) pp. 14.3.1–14.3.11, John Wiley & Sons, New York
33. Li, Y.-P., Alexander, M., Wucherpfennig, A. L., Yelick, P., Chen, W., and Stashenko, P. (1995) J. Bone Miner. Res. 10, 1197–1202
34. Bossard, M. J., Tomacek, T. A., Thompson, S. K., Nokami, B. Y., Hanning, C. R., Jones, C., Kurdyka, J. T., McNulty, D. E., Drake, F. H., Gowen, M., and Levy, M. A. (1996) J. Biol. Chem. 271, 12517–12524