Random high throughput sequencing of a human osteoclast cDNA library was used 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 or 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 hydroxyapatite 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. 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) 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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2 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 protease described in this paper.

2 The abbreviations used are: EST, expressed sequence tag; TESPA, 3-aminopropyltriethoxy silane; PAGE, polyacrylamide gel electrophoresis.
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 and resuspended in cold RPMI. 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 removed by extensive washing. The 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—Cryosection of osteoepthyes and osteoclastoma tissue were processed as described previously (31). The sections were picked off onto 3-aminopropyltriethoxy silane (TESPA)-coated slides and air-dried for at least 15 min. Tissues (human kidney, spleen, liver, lung, heart, skin, and colon) were placed into phosphate-buffered saline. Demineralization was in 0.2N HCl for 20 min, followed by acetylation in 0.25% acetic anhydride, 0.1M triethanolamine. Finally, the sections were washed twice in 2X SSC (20 mM sodium citrate, pH 7.0), dehydrated in 30, 60, 80, 95, and 100% ethanol, and air-dried. Sections were then fixed in 10% formalin for 10 min and then washed in citrate buffer (pH 6.0). The sections were then boiled for 15–30 min in the citrate buffer by microwaving them at high power in a household microwave oven (General Electric, Louisville, KY); the buffer was periodically topped up to prevent the tissue from drying out. After cooling slides to room temperature, the sections were overlaid with either the rabbit polyclonal cathepsin-K reactive primary antibodies, or with nonimmune serum controls, and a standard avidin-biotin alkaline phosphatase method was performed according to the manufacturer's protocol (DAKO, Carpenteria, CA). Positive reactivity was detected using a post-coupling naphthol phosphate method with Fast Red-TR (Sigma) as the coupler; a red precipitate indicated positive reactivity.

Alternatively, cryosections were used to demonstrate the reactivity of the antibodies. Sections (8 µm) of human tissues were cut using a Bright's cryostat (Bright Instrument Co., Huntingdon, United Kingdom) equipped with a tungsten-tipped steel knife (ARP, Cheshire, United Kingdom). The sections (undecalified adult osteophytic bone and rheumatoid synovium from osteoarthritic and rheumatoid femoral heads, respectively; post-mortem specimens of human kidney, spleen, liver, lung, heart, skin, and colon) were placed onto TESA-coated slides and air-dried for at least 15 min. Tissues were fixed in 10% formalin for 10 min and then washed 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 osteodentral 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 osteodental cDNA library. For example, in cDNA libraries from tissues containing greater than 1000 ESTs, cathepsin K was represented in only 0.011%, placenta, 0.0016% white adipose, 0.06% retina, 0.06% colon, 0.037% epididymus, 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 osteodental library. Only two ESTS (0.036%) for cathepsin B were identified from the osteodental 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 osteodental cDNA library.

Expression of Cathepsin K mRNA—Northern blot analysis of osteodental 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 osteophyte. Cathepsin K was abundantly and selectively expressed in osteoclasts and a discrete population of mononu-
Cathepsin K Is Abundantly Expressed in Human Osteoclasts

Fig. 1. In situ hybridization. Sections were hybridized to the probes indicated, followed by methylene blue counterstain (original magnification, \( \times 20 \)). 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.

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 mRNA (not shown).

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 pro- and 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 opposed to the surface of bone (Fig. 2, C 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. 2, C 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-CHN\(_2\)) 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 respon-
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

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**Table I**

Characterization of cathepsin K protein and mRNA expression in a panel of human tissues by immunocytochemistry and in situ hybridization

<table>
<thead>
<tr>
<th>Human tissue</th>
<th>Anti-cathepsin K antibody (SR-1)</th>
<th>Anti-cathepsin K antibody (C2)</th>
<th>mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone (6)</td>
<td>+++b</td>
<td>+++b</td>
<td>+++b</td>
</tr>
<tr>
<td>GCT (6)</td>
<td>+++b</td>
<td>+++b</td>
<td>+++b</td>
</tr>
<tr>
<td>Lung (3)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Kidney (2)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Heart (2)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Spleen (4)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Liver (5)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Skin (2)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Colon (1)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Rheumatoid synovium (5)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Number in parentheses indicates number of patient samples screened.

---

**Table II**

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

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>Cathepsin B</th>
<th>Cathepsin S</th>
<th>Cathepsin L</th>
<th>Cathepsin K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone osteoclasts</td>
<td>Negative&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Negative</td>
<td>Negative</td>
<td>+++</td>
</tr>
<tr>
<td>GCT osteoclasts</td>
<td>Negative&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Negative</td>
<td>Negative</td>
<td>+++</td>
</tr>
<tr>
<td>Cartilage chondroclasts</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>+++</td>
</tr>
<tr>
<td>Spleen</td>
<td>+++c</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+++c</td>
<td>Negative</td>
</tr>
<tr>
<td>Liver</td>
<td>+++c</td>
<td>ND</td>
<td>+++c</td>
<td>Negative</td>
</tr>
<tr>
<td>Kidney</td>
<td>+++c</td>
<td>ND</td>
<td>+++c</td>
<td>Negative</td>
</tr>
</tbody>
</table>

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

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

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

<sup>d</sup> ND, not done.
related to cathepsins S and L (25). A striking finding was the high frequency of ESTs for this enzyme in the osteoclast library and its relative lack of expression in other libraries, suggesting that this enzyme may be expressed selectively in osteoclasts. Surprisingly, ESTs for other cysteine proteases were nearly absent from the osteoclast library. To determine whether frequency of ESTs in the osteoclast library reflects expression levels in vivo, in situ hybridization studies on human tissues were performed. The results confirm that cathepsin K mRNA is highly abundant in osteoclasts and is not detectable in cells from other human tissues. These studies also confirmed that cathepsins S, B, and L are either absent or expressed at very low levels in osteoclasts.

In addition, specific antibodies to cathepsin K were used to demonstrate for the first time expression of the protein. Western blotting showed expression of the enzyme in extracts of osteoclastoma as well as normal bone tissues, as demonstrated by immunoreactivity in fetal rat humerus. The mobility of cathepsin K on SDS-PAGE suggests that the enzyme is expressed as a 38-kDa proenzyme and that it is processed to a 27-kDa mature form (Fig. 3). Studies with purified cathepsin K have demonstrated that the 38-kDa proenzyme is inactive, and protease activity correlates with the appearance of the 27-kDa mature enzyme (34). Immunohistochemistry confirmed the abundant expression of cathepsin K selectively in osteoclasts. Furthermore, the subcellular localization of cathepsin K at the osteoclast surface adjacent to the bone further supports a role of the enzyme in the bone resorption process. Thus, the abundant, selective expression of cathepsin K, coupled with the apparent lack of other cysteine proteases, strongly suggests that this enzyme plays a key role in osteoclast-mediated bone resorption.

Although previous studies have shown remarkable agreement that a cathepsin(s) is involved in bone resorption, the identification of the protease(s) has been a difficult problem, since osteoclasts are very rare cells and no appropriate osteoclast cell model has been identified. These previous studies have attempted to identify the cathepsin involved in bone resorption by immunolocalization (11-15, 17) or histochemically (16, 18-19). Contrary to our observations, these studies have suggested that cathepsins B and L are expressed by osteoclasts. However, the approaches taken in these studies necessarily relied on reagents for previously known cathepsins. Because cathepsin K is highly homologous to cathepsins L and B and is similar in size, cross-reactivity with cathepsin K by the antibodies used in earlier studies is possible. In addition, since cathepsin K may have similar enzymatic properties and substrate preferences as other cathepsins, interpretation of biochemical data is also difficult.

Another approach that has been taken to identify the relevant protease(s) involved in bone resorption has been purification of the 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, chondroclasts 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 a population 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 indicated 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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Institute for Genomic Research (TIGR), Smithkline Beecham, and Human Genome Sciences (HGS) using established EST methods (28, 29). These clones are part of a larger EST project (30). We thank Dr. R. H. Rothman (Rothman Institute, Philadelphia, PA) for supplying bone samples, Dr. R. D. Lackman (Jefferson Hospital, Philadelphia, PA) for supplying osteoclastomas, and the Anatomical Gift Foundation (Palkson, GA) for providing tissue samples. We also thank Frank McCabe for technical assistance, Drs. John Lee and Jeremy Bradbeer for critical review of the manuscript, and Drs. Brian Metcalf and Martin Rosenberg for continued support. We thank scientists at the sequencing facilities of HGS and TIGR, and the Bioinformatics staff at Smithkline Beecham. We would also like to thank Dr. Alan Barrett, Chair of the Advisory Panel on Peptidase Nomenclature to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, for promptly responding to our request to resolve the nomenclature conflict.

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