Mouse cathepsin K: cDNA cloning and predominant expression of the gene in osteoclasts, and in some hypertrophying chondrocytes during mouse development

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Abstract **We have constructed cDNA clones covering the entire coding region of mouse, human and rabbit preprocathepsin K mRNA for studies on bone turnover. The clone pMCatK-1 for mouse cathepsin K shares 87% nucleotide homology with the corresponding human and rabbit sequences. Analysis of a panel of mouse tissues for tissue distribution of cathepsin K mRNA revealed the highest levels in musculoskeletal tissues: bone, cartilage and skeletal muscle. In situ hybridization of developing mouse embryos was performed to identify the cellular source of cathepsin K mRNA. The strongest mRNA signal was detected in osteoclasts of bone, identified in serial sections by positive TRAP staining. Cathepsin K mRNA was also observed in some hypertrophic chondrocytes of growth cartilages. Association of cathepsin K production with degradation of bone and cartilage matrix suggests that this enzyme and its mRNA levels could serve as markers for matrix degradation in diseases affecting these tissues.**

Key words: Cathepsin; Cartilage; Bone; Osteoclast; Mouse; mRNA

1. Introduction

Continuous remodeling of extracellular matrix by concomitant resorption and new bone formation is a characteristic feature of bone. Normally bone resorption and formation are coupled and balanced so that no net change of bone mass occurs. The balance is regulated through a complex interplay of hormones, growth factors and other affector molecules [1–3]. Osteoporosis is a common disease in which an imbalance between bone resorption and bone formation results in loss of net bone mass and in bone fragility. Biochemically, analysis of bone turnover is challenging as changes in the concentrations of bone components are difficult to measure. In experimental systems molecular biologic hybridization methods have demonstrated that mRNA levels for bone components, e.g. type I collagen, reflect the synthesis rate of new bone matrix [4–6]. Molecular biologic analyses of bone resorption through changes in osteoclast gene expression have proceeded more slowly. Active osteoclasts produce an acidic extracellular microenvironment (Howship's lacuna) and secrete various proteolytic enzymes responsible for the degradation of bone extracellular matrix proteins [7]. These proteases belong to at least two different gene superfamilies, the matrix metalloproteinases (MMPs) and the cysteine proteases (cathepsins). Previous immunohistochemical and in situ hybridization data have demonstrated expression of cathepsins B, D, E, L, OC-2 [8–10] and MMP-9 (92 kDa gelatinase) [11] in osteoclasts. Cathepsins B, D and L, as well as most MMPs, are also produced in a variety of other tissues, whereas recent data suggest that OC-2, a novel cysteine protease, and MMP-9 are predominantly expressed in osteoclasts [10,11]. Therefore, these enzymes are likely to play a role in osteoclastic bone resorption and could serve as markers for bone degradation.

The cDNA for an osteoclast specific cathepsin was first cloned for the rabbit as OC-2 [10], later also for the human as cathepsin K [12]. At the same time, another group cloned the same cDNA and named it cathepsin O [13]. However, as another completely different sequence isolated from human breast carcinoma had already been cloned as cathepsin O cDNA [14], we decided to use the name cathepsin K for this enzyme. Later, this same cDNA has been cloned as human cathepsin O2 [15] and human cathepsin X [16].

The need for mouse specific cDNA and genomic clones is obvious. Characterization of the mouse genome and development of methods for its manipulation have provided researchers with tools to generate mouse models for an increasing number of human diseases. Already several transgenic mice harboring different mutant transgenes are known where the balance between bone formation and resorption is affected. Osteoporosis, for example, has been observed in mice overexpressing the interleukin-4 [17] and TGF-β2 genes [18]. Metaphyseal osteopenia has also been discovered in transgenic mice harboring a dominant negative mutation in the cartilage specific type II collagen gene [5]. On the other hand, knockout mutation of the interleukin-6 gene makes mice resistant to ovariectomy-induced osteoporosis [19]. Targeted inactivation of the c-*fos* and c-*src* genes inhibits bone resorption and shifts the balance towards bone formation resulting in osteopetrosis [20,21]. These and other models demonstrate the suitability of mice for research on bone turnover and osteoporosis. Molecular biologic characterization of these phenotypes requires mouse specific hybridization probes. Therefore, we decided to construct a cDNA clone for mouse preprocathepsin K for specific detection of the corresponding mRNA in various models of skeletal turnover, and for future production of the proenzyme under different promoters in transgenic mice.

2. Materials and methods

2.1. RNA isolation

Total RNA was extracted from several mouse tissues. After dissec-

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The mouse cathepsin K sequence described in this paper has been submitted in GenBank/EMBL Data Library under accession number X94444.

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tion the samples were immediately frozen in liquid nitrogen, stored at −70°C, pulverized under liquid nitrogen and homogenized in guanidinium isothiocyanate followed by ultracentrifugation through a $CsCl_2$ gradient as described earlier [22].

2.2. cDNA synthesis

Single stranded cDNA was synthesized for RNAs isolated from rabbit articular cartilage, human bone and newborn mouse calvarial bone using Moloney murine leukemia virus reverse transcriptase under conditions suggested by the supplier (Life Technologies, Gaithersburg, MD). Both oligo(dT) and random hexamers were used as primers. Aliquots of cDNA were used for amplification by the polymerase chain reaction (GeneAmp, Perkin-Elmer, Foster City, CA) using two oligonucleotide primers: ATGTGGGG(G/A)CTCAAGGTTCT as the 5′-sense primer, and TCACATCTT(G/A)GGGAAGCTGG as the 3′-antisense primer. The reactions were cycled by denaturing at 94°C for 1 min, annealing at 50°C for 2 min and extension at 72°C for 2 min. After 30 amplification cycles aliquots of the reactions were fractionated by electrophoresis on 1.0% agarose gels. Specific fragments of expected size were purified and cloned into the pGEM-T vector as suggested by the supplier (Promega, Madison, WI). Ligation products were transformed into competent *E. coli* JM109 cells. Plasmids containing appropriate inserts were sequenced on both strands using the Sanger dideoxy method (Sequenase kit, USB, Cleveland, OH) and automated sequencer (ABI, Perkin Elmer). The sequences were stored and analyzed using Lasergene software (DNAStar, Madison, WI). The new clone for mouse preprocathepsin K cDNA reported here was named pMCatK-1.

2.3. Northern hybridizations

For Northern analyses 10 μg aliquots of total RNA were denatured with formamide, fractionated on 0.8% agarose gels and transferred by blotting onto Pall Biodyne nylon membranes as recommended by the supplier (Pall Europe, Portsmouth, UK). Equal loading of the gels was ascertained by measurement of the rRNAs fractionated on a duplicate gel stained with ethidium bromide, and by hybridization of the filters with a probe for 28S rRNA [23]. The filters were prehybridized at 42°C overnight. After hybridizations with the ^{32}P-dCTP-labeled probes, the filters were washed and the bound probe detected by autoradiography and quantified by laser densitometry.

2.4. In situ hybridizations and staining for tartrate resistant acid phosphatase (TRAP)

For the preparation of RNA probes the pMCatK-1 cDNA clone was digested with *Ava*II restriction enzyme and the resulting 385 bp fragment was cloned into pBS plasmid vector (Stratagene, La Jolla, CA) and assigned pMCatK-7. The clone pMCatK-7 was linearized with *Bam*HI (antisense) and *Hin*dIII (sense) restriction enzymes. The [^{35}S]uridine triphosphate (1000 Ci/nmol, Amersham Life Science, Amersham, UK) labeled RNA probes were transcribed using a transcription kit (Promega). The labeled probes were fractionated on Sephadex G-50 columns (Pharmacia, Uppsala, Sweden) and dissolved in hybridization buffer, and used at 15000–20000 cpm/μl. Details of the technique used have been published earlier [24]. The samples were fixed in phosphate buffered formaldehyde and decalcified in 5% EDTA for 2 days, dehydrated in graded alcohols, defatted in xylene and embedded in paraffin. For in situ hybridization, the sections were pretreated with proteinase K and HCl, and acetylated. The hybridizations were performed for 18 h at 52°C, followed by high stringency washes. Slides were dipped in autoradiographic emulsion (Kodak NTB-3, Rochester, NY) and exposed for 5–28 days at 10°C. After developing the film the sections were stained with hematoxylin. For specific detection of osteoclasts, adjacent serial sections were stained for TRAP activity using leukocyte acid phosphatase detection kit (Sigma, St. Louis, MO) as recommended by the supplier.

3. Results

3.1. Sequence analysis of the cDNA clone for mouse cathepsin K

Existing human and rabbit sequences were used to design primers for amplification of the entire coding region of mouse preprocathepsin K cDNA. The same primers were also used for amplification of the corresponding human and rabbit cDNA. Nucleotide sequencing of the latter cDNAs, named pHCatK-1 and pKCatK-1, respectively, confirmed their identity. Nucleotide sequencing of the 990 bp insert in the mouse cathepsin K clone pMCatK-1 demonstrated 87% sequence homology with both the human and the rabbit sequences (Fig. 1). At the amino acid level the similarities were 87% and 90%, respectively. A homology search against protein sequence database revealed that mouse cathepsin K shares 50% sequence similarity with rat cathepsin S, 46% with mouse cathepsin L, 18% with mouse cathepsin B and 15% with mouse cathepsin D at the amino acid level. Sequence homology analysis indicates that the clone pMCatK-1 is the mouse counterpart to rabbit OC-2 and that it is closely related to cathepsins S and L.

Fig. 1. Nucleotide and amino acid sequence of cDNA clone pMCatK-1 for the entire coding region of mouse preprocathepsin K mRNA. The complete nucleotide (line m) and deduced amino acid sequence (line M) of the clone are compared with the corresponding nucleotide sequences of the human (line h) and rabbit (line r) cDNA, and with the deduced amino acid sequences of the rabbit (line R) and human (line H) cathepsin K. The mouse sequences corresponding to the oligonucleotide primers are in italics. The dots in the human and rat sequences denote nucleotide and amino acid identity. The arrows denote the putative cleavage sites of the signal peptide and the propeptide of the preprocathepsin K molecule. Consensus sequences conserved among all cathepsins that code for amino acids in the active site are underlined. Potential *N*-glycosylation sites are in bold face.

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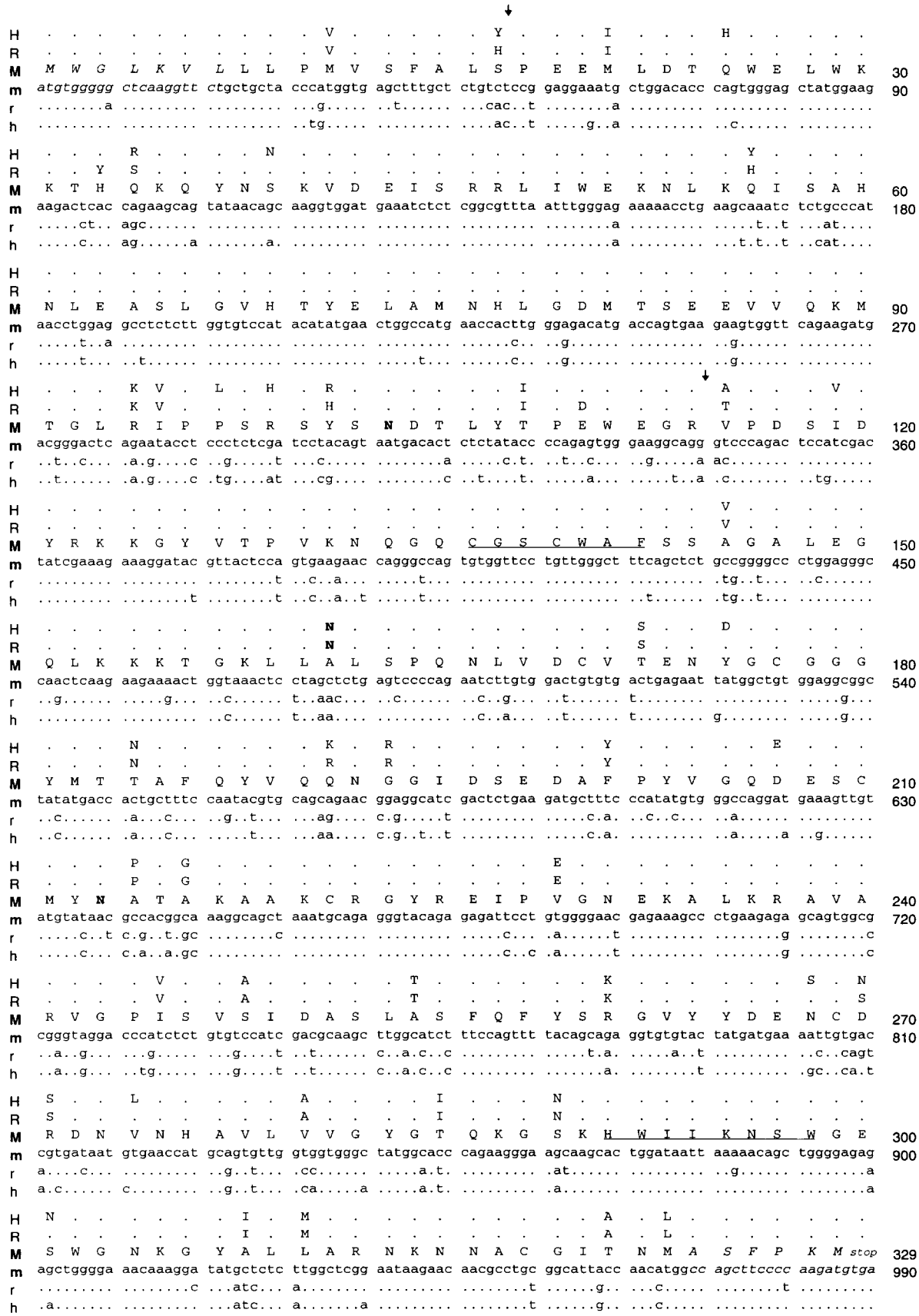
3.2. Tissue distribution of cathepsin K mRNA

We first analyzed the overall distribution of cathepsin K mRNAs in mouse tissues by hybridizing a filter containing a panel of total RNA isolated from 17 mouse tissues with the [^{32}P]dCTP-labeled pMCatK-1 probe. The highest levels of cathepsin K mRNA were observed in skeletal tissues. Upon extended exposure low levels of cathepsin K mRNA were also detected in numerous other tissues (Fig. 2). A panel of RNAs were harvested from the limbs of developing mouse embryos. Low levels of the mRNA were detected in the limbs of 16.5 day embryos and intermediate levels in 18.5 day embryos (data not shown). RNAs were also isolated from the long bones of newborn and adult mice to further characterize the relative abundance of the cathepsin K transcripts both temporally and spatially (Fig. 3). The limbs of newborn mice were dissected under a preparation microscope, all soft tissues were removed and total RNA was isolated from bony diaphyses and cartilaginous epiphyses separately. Cartilaginous epiphyses of the newborn mouse contained intermediate levels of the cathepsin K mRNA whereas an extensive signal was detected in bony diaphyses and calvarial bone (Fig. 3). Total RNA from adult mouse long bones was harvested from the epiphyses (containing articular surface, subchondral bone and the growth plate), metaphyseal corticocancellous bone, diaphyseal cortical bone, and from bone marrow. In adult mouse long bones the highest signal for cathepsin K transcripts was found in metaphyseal bone; lower levels were seen in epiphyses and in diaphyseal cortical bone. In bone marrow RNA the signal was at background level (Fig. 3).

3.3. Localization of cathepsin K mRNA in the developing mouse skeleton

To identify the cell types responsible for cathepsin K pro-



duction in cartilage and bone the corresponding mRNA was localized by in situ hybridization of histologic sections of developing mouse embryos. The strongest signal was detected in multinuclear, apparently osteoclastic and chondroclastic cells of various calcified tissues, e.g. calvaria, mandible, ribs, vertebrae and long bones of 16.5 and 18.5 day embryos and of newborn mice (Fig. 4). No signal was detected in the limbs of 14.5 day embryos (data not shown). TRAP staining was performed on adjacent serial sections to identify osteoclasts: positive TRAP staining co-localized with the hybridization signals on histologic sections (Fig. 5A,B). Specificity of the hybridization was verified by hybridizations performed with the sense control probe of adjacent sections (Fig. 4F).

Analysis of the growth plates of newborn mouse long bones revealed the presence of cathepsin K mRNA in some hypertrophic chondrocytes close to the osteochondral junction (Fig. 5E). On some occasions using extended exposures for 28 days, distinct hybridization signals were also detected over individual hypertrophying chondrocytes in central regions of the epiphyseal heads of the long bones of newborn mice (Fig. 5E). The signal was also detected in some chondrocytes which had recently undergone mitosis (Fig. 5D).

4. Discussion

Previous analyses of rabbit and human tissues have shown the expression of the cathepsin K (OC-2) gene to occur predominantly in osteoclasts and some other cells of the monocyte/macrophage lineage [10,12,13]. By Northern analysis, cathepsin K transcripts have been detected at low levels in several other human and rabbit tissues but the exact cellular source of these mRNAs is not known. The results of our Northern analyses of 17 different mouse tissues for cathepsin K mRNA agree mostly with the earlier observations in human and rabbit tissues [10,12,13]. Although the highest levels of the mRNA in the mouse were clearly seen in the skeletal tissues

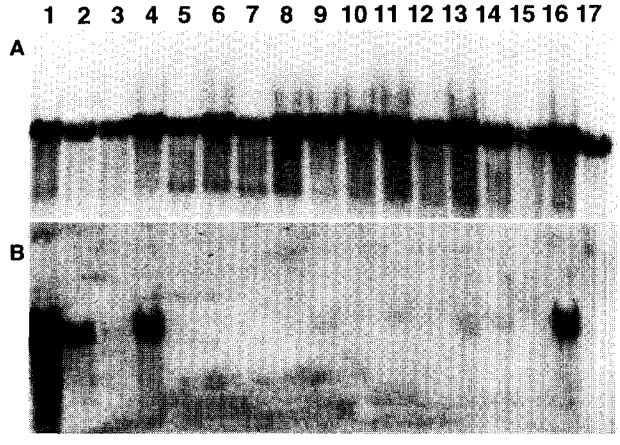


Fig. 2. Northern analysis of mouse tissues for cathepsin K mRNA. Total RNA was isolated from the following mouse tissues: calvarial bone (lane 1), articular cartilage (2), auricular cartilage (3), nasal cartilage (4), pancreas (5), thymus (6), spleen (7), liver (8), kidney (9), brain (10), testis (11), heart (12), intestine (13), skin (14), lung (15), muscle (16), and eye (17). Denatured RNAs were electrophoresed, transferred by blotting onto the hybridization membrane and hybridized with the pMCatK-1 probe (A) and with a probe for 28S rRNA (B). The levels of the 28S rRNA (B) demonstrate that some variation also existed in the amount of total RNA loaded in each lane.

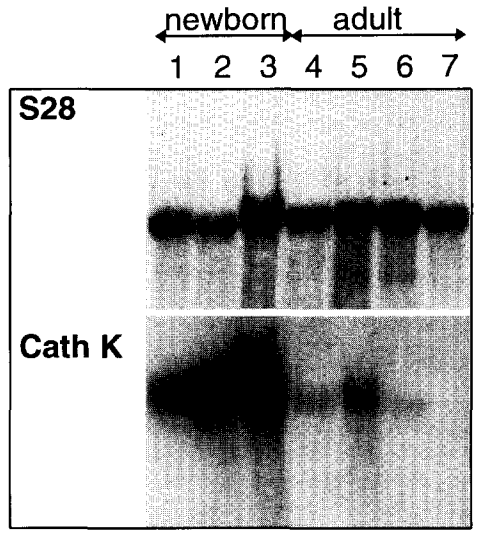


Fig. 3. Northern analysis of mouse skeletal tissues for cathepsin K mRNA. Total RNA was isolated from newborn (lanes 1–3) and adult mouse (4–7) long bones. The samples are as follows: cartilaginous epiphysis (1), diaphysis (2), calvarial bone (3), epiphysis (4), metaphysis (5), diaphysis (6), and bone marrow (7). Denatured RNAs were electrophoresed, transferred by blotting onto the hybridization membrane and hybridized with the pMCatK-1 probe and with a probe for 28S rRNA.

(bone, cartilage and skeletal muscle), some differences were also seen between the three species in the distribution and relative abundance of cathepsin K mRNA. In our Northern analyses calvarial bone and long bones of the newborn mice exhibited the highest cathepsin K mRNA levels while in the rabbit the signal in calvaria appeared low [10]. We do not know at present whether these discrepancies reflect differences between the species studied or the developmental stages of the tissues analyzed.

Remodeling of extracellular matrix is an essential part of bone and cartilage metabolism. Bone remodeling is essentially a surface-based phenomenon and in the skeleton there are several types of bone surfaces available. It has been estimated that in humans cortical bone comprises three quarters of the total bone mass. However, there is approximately 10 times more surface per mass ratio available for bone remodeling in the cancellous bone than in the cortical bone surfaces [1]. Our results on the quantification of the relative abundance of cathepsin K mRNA levels in adult mouse long bones reflect the amounts of available surface for bone remodeling. In the adult mouse long bones, the mRNA levels for cathepsin K mRNA were highest in metaphyseal corticocancellous bone where most of the remodeling surfaces are located. Convenient and sensitive molecular biologic hybridization methods are currently available for determination of bone specific mRNAs which reflect the synthesis rates of these proteins in different experimental systems and species [4–6]. Demonstration of cathepsin K mRNA in cells at sites of bone and cartilage degradation suggests that determination of the mRNA levels might provide a similar molecular biologic tool for studies on bone and cartilage resorption. Osteoclasts have been found to contain several proteolytic enzymes, but many of these are also found in other tissues [7–9]. In addition to tartrate resistant acid phosphatase, the 92 kDa gelatinase (MMP-9) has also been shown to be highly enriched in mouse

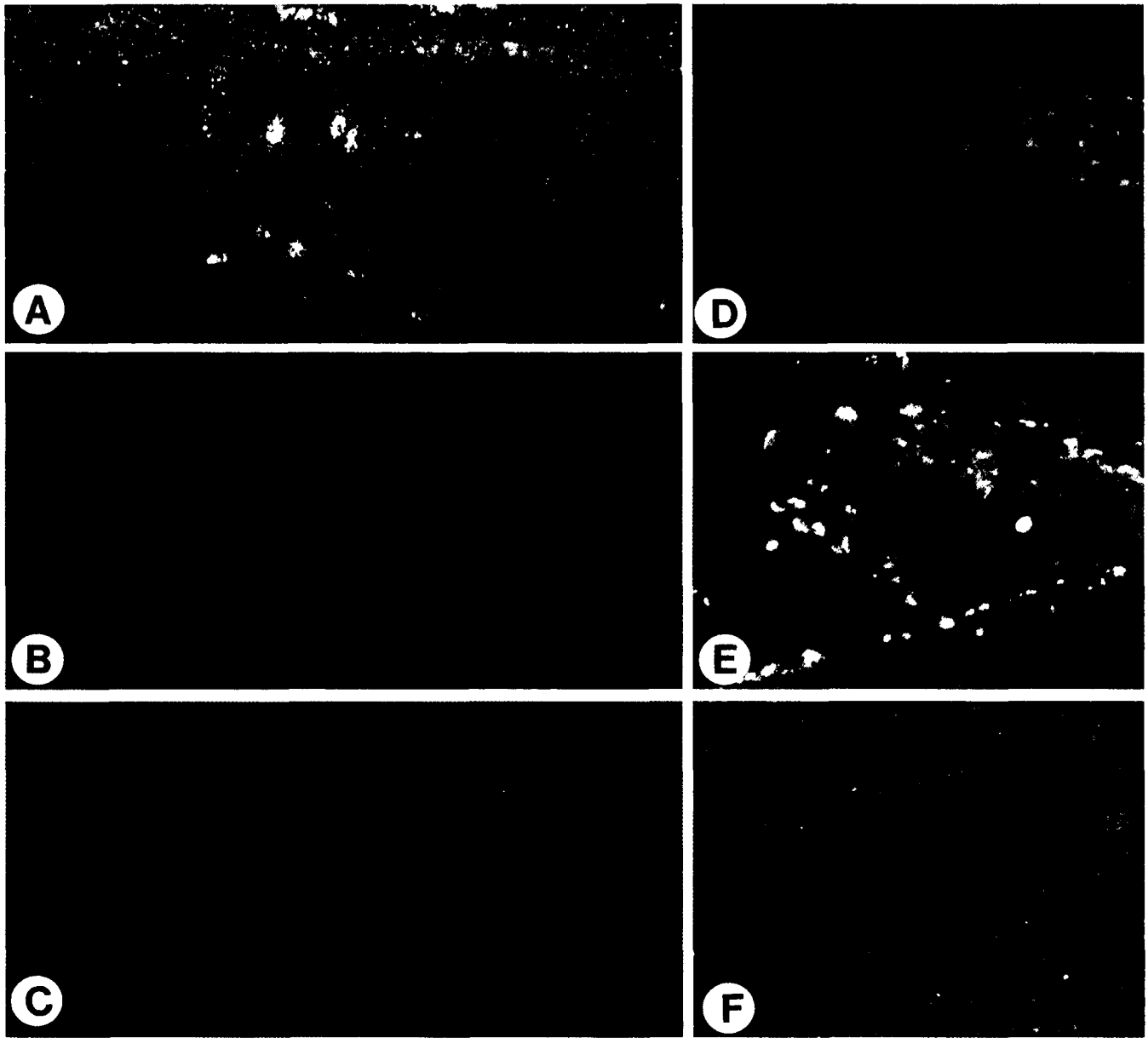


Fig. 4. Expression of cathepsin K mRNA during endochondral ossification. Histological sections of mouse limbs were hybridized with antisense (panels A–E) and sense (panel F) ^{35}S-labeled RNA probes of clone pMCatK-7. The bound probe was detected with autoradiography. As an example of the initial stage of ossification a metatarsal bone of an 18.5 day embryo was sectioned for in situ hybridization (A–C). A dark field view (in A) shows the positive hybridization signal for cathepsin K mRNA in central parts of the primary ossification center. B shows a low power view of the same section stained with hematoxylin, and C a magnification of the area boxed in B illustrating the autoradiographic grains in primitive multinuclear cells of bone. As an example of ongoing endochondral ossification femoral bones of 16.5 day embryos (D) and newborn mice (E and F) were used. Low power dark field view of 16.5 day femoral bones shows positive hybridization signals on trabecular bone surfaces and in calcifying growth cartilage (D); in newborn mouse femurs the signal is seen on endosteal and trabecular bone surfaces (E). Sections hybridized with the sense probe showed no positive hybridization signal (F). The bar in C (for C–E) corresponds to 25 μm. The same distance in panels A and B corresponds to 40 μm.

osteoclasts [11] and it could also serve as a marker for bone degradation.

As we were particularly interested in the cellular origin of cathepsin K in skeleton, we performed in situ hybridizations and focused our attention on limbs of the developing mouse embryos. Although limbs of 14.5 day embryos are actively producing cartilage components (Savontaus et al., unpublished), no sign of calcified cartilage or bone was detected in the sections analyzed; neither did we detect any positive signal for cathepsin K mRNA. In the limbs of 16.5 day old embryos, primary ossification centers were well developed and extensive positive signal for cathepsin K mRNA was seen in the center of the bone marrow within the cells lining cancellous bone surfaces. This is also the time by which blood forming cells have invaded the primary bone marrow. As blood formation in the bone marrow requires a lot of space, the primitive bone and previously formed calcified cartilage need to be removed. The association of cathepsin K mRNA with multinuclear bone lining cells and its co-location with the positive TRAP staining confirms that this enzyme plays a role in osteoclastic bone and cartilage degradation.

Demonstration of cathepsin K mRNA in hypertrophic

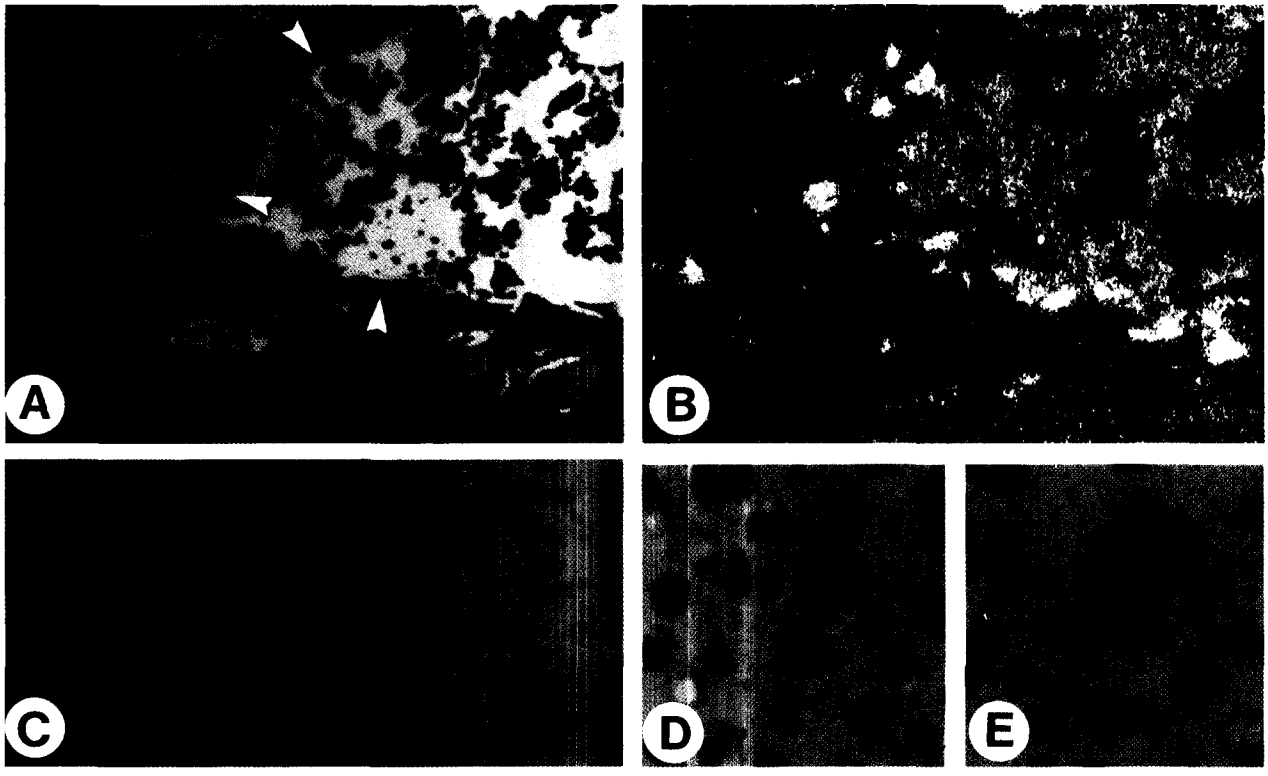


Fig. 5. TRAP staining and in situ hybridization of newborn mouse skeletal tissues for cathepsin K transcripts. A low power view of positive TRAP staining on an endosteal surface of newborn mouse femur counterstained with hematoxylin (A). The arrowheads in A demonstrate a positive reaction for TRAP activity. A dark field view of an adjacent serial section shows the autoradiographic grains in the same locations (B). High power view of osteochondral junction stained with hematoxylin demonstrates autoradiographic signal for cathepsin K in a hypertrophic chondrocyte at the interface between bone and cartilage (C). High power views of proliferating (D) and hypertrophying (E) chondrocytes from the epiphyseal heads of newborn mice demonstrate positive hybridization signal for cathepsin K mRNA after extended exposure for 28 days. The bar in C corresponds to 25 μm. The same distance in A and B corresponds to 100 μm, in D to 500 μm, and in E and F to 250 μm.

chondrocytes of epiphyseal and growth cartilages is a novel observation and clearly demonstrates that the expression of the gene is not unique to osteoclasts and other cells of the monocyte/macrophage lineage. In a previous study on metacarpal bones of newborn rabbits no cathepsin K/OC-2 mRNA was observed in the hypertrophic chondrocytes in the growth cartilages [10]. Human osteoarthritic hip bones have been reported to contain variable levels of cathepsin K mRNA, but the report made no mention about the possible presence of articular cartilage in the samples analyzed [12]. Further studies are needed to determine if the cathepsin K mRNA in osteoarthritic samples is merely of osteoclastic origin or whether chondrocytes play a role in local production of cathepsin K in osteoarthritic cartilage.

Chondrocytes within epiphyseal heads and growth plates are surrounded by a rigid cartilaginous matrix rich in collagens. During the time that chondrocytes are hypertrophying, the cells enlarge to about five times their previous size. Before cell division or hypertrophy the chondrocytes must create space for the increase in cell size. In growth plates the dividing and hypertrophying cells create space by concomitantly synthesizing new matrix in the longitudinal direction resulting in long bone growth [25]. Thus the increase in cellular volume is not solely dependent on matrix removal since space is also created by longitudinal growth. Actual reduction of the amount of cartilaginous matrix is predominantly seen at the lowermost zones of hypertrophic chondrocytes when the cartilaginous septa are resorbed [26]. For chondrocytes within epiphyseal cartilage any increase in their volume, whether by hypertrophy or division, is dependent on prior removal of the surrounding matrix by production and secretion of proteolytic enzymes. Other proteolytic enzymes have also been detected in hypertrophying chondrocytes [27]. We therefore interpret the results to indicate that local production and activation of cathepsin K by hypertrophying chondrocytes is related to degradation of the cartilaginous extracellular matrix analogous to degradation to bone matrix by the osteoclast derived enzyme.

The degradation rates of cartilage and bone matrices are clearly dependent of the developmental stage of the skeleton. With the cDNA probe constructed here these possibilities can now be tested not only by a systematic analysis of normal mouse development and growth but by analyzing the different mouse models of osteoporosis, osteopetrosis and osteoarthrosis. The availability of the entire preprocathepsin K coding sequence also makes it possible to modulate cathepsin K production in transgenic animals by expressing the cDNA under different tissue specific promoters.

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References

[1] Kanis, J.A. (1994) Osteoporosis, pp. 22–55. Blackwell Science, Oxford.
[2] Raisz, L.G. (1988) New Engl. J. Med. 318, 818–828.
[3] Manolagas, S.C. and Jilka, R.L. (1995) New Engl. J. Med. 332, 305–311.
[4] Turner, R.T. and Spelsberg, T.S. (1991) Am. J. Physiol. 261, E348–353.
[5] Hiltunen, A., Metsäranta, M., Virolainen, P., Aro, H.T. and Vuorio, E. (1994) Dev. Dyn. 200, 340–349.
[6] Virolainen, P., Perälä, M., Vuorio, E. and Aro, H.T. (1995) Clin. Orthop. 317, 263–272.
[7] Väänänen, H.K. (1993) Ann. Med. 25, 353–359.
[8] Goto, T., Kiyoshima, T., Moroi, R., Tsukuba, T., Nishimura, Y., Himeno, M., Yamamoto, K. and Tanaka T. (1994) Histochemistry 101, 33–40.
[9] Yoshimine, Y., Tsukuba, T., Isobe, R., Sumi, M., Akamine, A., Maeda, K. and Yamamoto, K. (1995) Cell Tissue Res. 281, 85–91.
[10] Tezuka, K., Tezuka, Y., Maejima, A., Sato, T., Nemoto, K., Kamioka, H., Hakeda, Y. and Kumegawa, M. (1994) J. Biol. Chem. 269, 1106–1109.
[11] Reponen, P., Sahlberg, C., Munaut, C., Thesleff, I. and Tryggvason, K. (1994) J. Cell Biol. 124, 1091–1102.
[12] Inaoka, T., Bilbe, G., Ishibashi, O., Tezuka, K., Kumegawa, M. and Kokubo T. (1995) Biochem. Biophys. Res. Commun. 206, 89–96.
[13] Shi, G.P., Chapman, H.A., Bhairi, S.M., DeLeeuw, C., Reddy, V.Y. and Weiss, S.J. (1995) FEBS Lett. 357, 129–134.
[14] Velasco, G., Ferrando, A.A., Puente, X.S., Sánchez, L.M. and López-Otín, C. (1994) J. Biol. Chem. 269, 27136–27142.
[15] Brömme, D. and Okamoto, K. (1995) Biol. Chem. Hoppe-Seyler 376, 379 384.
[16] Li, Y-P., Alexander, M., Wucherpfennig, A.L., Yelick, P., Chen, W. and Stashenko, P. (1995) J. Bone Miner. Res. 10, 1197–1201.
[17] Lewis, D.B, Liggitt, H.D., Effmann, E.L., Motley, S.T. Teitelbaum, S.L., Jepsen, K.J., Goldstein, S.A., Bonadio, J., Carpenter, J. and Perlmutter R.M. (1993) Proc. Natl. Acad. Sci. USA 90, 11618–11622.
[18] Erlebacher, A. and Derynck, R. (1996) J. Cell Biol. 132, 195–210.
[19] Poli, V., Balena, R., Fattori, E., Markatos, A., Yamamoto, M., Tanaka, H., Ciliberto, G., Rodan, G.A. and Costantini, F. (1994) EMBO J. 13, 1189–1196.
[20] Soriano, P., Montgomery, C., Geske, R. and Bradley, A. (1991) Cell 64, 693–702.
[21] Wang, Z.Q., Ovitt, C., Grigoriadis, A.E., Möhle-Steinlein, U., Rüther, U. and Wagner, E.F. (1992) Nature 360, 741–745.
[22] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[23] Iruela-Arispe, M.L., Hasselaar, P. and Sage, H. (1991) Lab. Invest. 64, 174–186.
[24] Ylä-Herttuala, S., Rosenfield, M.E., Parthasarathy, S., Glass, C.K., Sigal, E., Witztum, J.L. and Steinberg, D. (1990) Proc. Natl. Acad. Sci. USA 87, 6959–6963.
[25] Sandberg, M. and Vuorio, E. (1987) J. Cell Biol. 104, 1077–1084.
[26] Huntziker, E.B. and Hermann, W (1990) in: Ultrastructure of Skeletal Tissues (Bonucci, E. and Motta, P.M., Eds.) pp. 79–109. Wiley-Liss, New York.
[27] Brown, C.C., Hembry, R.M. and Reynolds, J.J. (1989) J. Bone Joint Surg. 71-A, 580–593.