Caldecrin: A pancreas-derived hypocalcemic factor, regulates osteoclast formation and function

Mineko Tomomura, Akito Tomomura

Caldecrin was originally isolated from the pancreas as a factor that reduced serum calcium levels. This secreted serine protease has chymotrypsin-like activity and is also known as chymotrypsin C; it belongs to the elastase family. Although intravenous administration of caldecrin decreases the serum calcium concentration even when its protease activity is blocked, this effect does require cleavage of caldecrin’s pro-peptide by trypsin, converting it to the mature enzyme. Ectopic intramuscular expression of caldecrin prevented bone resorption in ovariectomized mice. Caldecrin inhibited parathyroid hormone-stimulated calcium release from fetal mouse long bone organ cultures. Furthermore, caldecrin suppressed the formation of osteoclasts from bone marrow cells by inhibiting the receptor activator of nuclear factor-κB ligand (RANKL)-stimulated phospholipase Cγ-calcineurin-nuclear factor of activated T-cells, cytoplasmic 1 pathway. Caldecrin also suppressed the bone resorption activity of mature osteoclasts by preventing RANKL-stimulated Src activation, calcium entry, and actin ring formation. In vivo and in vitro studies have indicated that caldecrin is a unique multifunctional protease with anti-osteoclastogenic activities that are distinct from its protease activity. Caldecrin might be a potential therapeutic target for the treatment of osteolytic diseases such as osteoporosis and osteoarthritis. This mini-review describes caldecrin’s historical background and its mechanisms of action.

Key words: Serine protease; Osteoclasts; Hypocalcemia; Chymotrypsin; Bone resorption; Calcium signaling
kappa B ligand-induced activation of intracellular calcium signaling, thereby reducing osteoclast formation and bone resorption. Caldecrin is a unique multifunctional protease that possesses anti-osteoclastogenic activity, resulting in reduced serum calcium levels.

INTRODUCTION

Calcium homeostasis is controlled by intestinal calcium absorption and calcium resorption in the kidney, as well as by bone formation and resorption. Clinical and experimental observations have also linked the pancreas to calcium homeostasis. Pancreas-derived glucagon[1,2], amylin[3,4] and calcitonin gene-related peptide[5,6] have been shown to regulate calcium homeostasis, while acute and chronic pancreatitis have been shown to associate with hypocalcemia[7]. In the 1960’s, the pioneering work of Takaoka et al[8,9] demonstrated that a porcine pancreatic extract had hypocalcemic activity. In 1992, we first successfully purified a hypocalcemic factor named caldecrin from a pancreatic extract using chromatographic separation techniques including ion exchange, gel filtration chromatography, and high-performance liquid chromatography[10]. To identify caldecrin, each fraction was intravenously administered to overnight-fasted mice and serum calcium concentrations were measured 4 h post-injection. In addition, the samples were assayed for their inhibitory effect on parathyroid hormone-stimulated calcium release from fetal mouse long bone organ cultures. Caldecrin is an anionic protein (pI: 4.5) with a molecular weight of about 28 kDa; it was found to be a serine protease with chymotryptic activity.

In 1995, we isolated rat caldecrin cDNA from pancreatic cDNA expression library by immunoscreening with an anti-caldecrin antibody[11]. A partial amino acid sequence of caldecrin purified from rat pancreas was completely matched with that encoded by the cDNA. The nucleotide sequence was almost identical (except for three nucleotides) to that of a PCR clone referred as elastase IV (ELA4)[12]. Comparison of the amino acid sequences encoded by these two cDNAs indicated that the central region of caldecrin differed from that of ELA4 due to a frame shift caused by this minor nucleotide change (Figure 1). The amino acid sequences of the purified caldecrin fragments, including the central region, were consistent with the deduced amino acid sequence of caldecrin but not with that of ELA4. Over-expression of the ELA4 PCR clone in Sf9 cells caused a complete loss of secretion, low expression levels, and much lower protease activity[13]. Furthermore, the rat genomic DNA sequence matched that of the caldecrin cDNA, but not that of the ELA4 clone[13]. Therefore, the ELA4 PCR clone may be a cloning artifact or represent a mutant caldecrin gene. In 1995, the crystalline structure of bovine chymotrypsinogen C was reported[14-16] and its amino acid sequence was very close to that of rat caldecrin, thereby suggesting a similarity between caldecrin and chymotrypsin C (CTRC). It is now known that CTRC, caldecrin, and ELA4 are the same protein, which is encoded by the CTRC gene and known officially as CTRC (caldecrin), according to the HUGO Gene Nomenclature Committee. Table 1 compares the amino acid sequence of rat caldecrin with that of other members of the rat and human pancreatic chymotrypsin and elastase families. Caldecrin shows a greater similarity with elastase than with chymotrypsin. In addition, expressed recombinant human caldecrin also showed serum calcium-decreasing activity, even following phenylmethylsulfonyl fluoride treatment to abolish its protease activity[17]. In 1996, another research group purified a calcium metabolism-regulating factor from the porcine pancreas by determining its stimulatory effects on proliferation of the osteosarcoma MG-63 cell line and its inhibition of 1, 25 vitamin D3-stimulated calcium release in organ cultures[18]. The terminal sequence of the 28-kDa protein that was isolated corresponded to that of human elastase III B. Recombinant elastase III B decreased interleukin-1-induced hypercalcemia and this effect was dependent on its protease activity. Although both have been isolated from the pancreas, caldecrin and elastase III B were found to be different molecules that exerted their hypocalcemic effects via different mechanisms of action.

Table 1  Amino acid sequence similarity with rat caldecrin

| Species   | Pancreatic protease | Identity (%) | Similarity (%) |
|-----------|---------------------|--------------|----------------|
| Rat       | Caldecrin           | 100          | 100            |
|           | Chymotrypsin B      | 41           | 55             |
|           | Elastase I          | 51           | 67             |
|           | Elastase II A       | 59           | 72             |
|           | Elastase II B       | 57           | 71             |
| Human     | Caldecrin           | 78           | 88             |
|           | Chymotrypsin B      | 41           | 56             |
|           | Elastase II A       | 61           | 74             |
|           | Elastase II B       | 56           | 70             |
|           | Elastase III A      | 57           | 70             |
|           | Elastase III B      | 55           | 69             |
| Cow       | Chymotrypsin A      | 39           | 57             |

Sequence identity: Percent of same amino residues in a sequence alignment between 2 sequences; Sequence similarity: Percent amino acid sequence identity and percent positive substitutions between 2 sequences.

PROTEIN STRUCTURE AND PROTEASE ACTIVITY OF CALDECRIN

The human CTRC gene maps to chromosome 1p36.21. The homologous mouse and rat genes are located on chromosomes 4E1 and 5q36, respectively. The CTRC genes consist of 8 exons in these species. Northern blot...
analysis has indicated that caldecrin is mainly expressed in the pancreas (Figure 2A).

CTRC (caldecrin) is a single protein consisting of 268 amino acids, with a signal peptide (16 amino acids), pro-peptide (13 amino acids), and the mature protein (239 amino acids; Figure 2B). The three-dimensional structure demonstrated that five disulfide bridges were formed at Cys1-Cys125 (according to the chymotrypsin numbering), Cys43-Cys59, Cys139-Cys206, Cys170-Cys186, and Cys196-Cys227 (Figure 2B). CTRC (caldecrin) was shown to have a two-barrel structure, each composed of 6-7 β-sheets and a C-terminal α-helix long tail [14-16] (Figure 2C).

Following tryptic cleavage at Arg13-Val14, the caldecrin pro-peptide remains associated with the mature enzyme via the Cys1-Cys125 disulfide bridge; this generates a structure resembling those of chymotrypsin A and B, as...
Caldecrin is an anti-osteoclastogenic factor

Figure 2 Caldecrin expression and protein structure. A: Caldecrin expression was analyzed by Northern blot. 18S, 28S: 18S, 28S ribosomal RNA; B: Domain structures of caldecrin. Black box: signal peptide; orange box: pro-peptide; blue box: mature protein; red line: disulfide bridges with cysteine number; the H (histidine), D (aspartic acid), S (serine) catalytic triad; C: Ribbon diagram of the crystal structure of human caldecrin (adapted from PDB ID: 4H4F, prepared from [16]). Red line: disulfide bridge; Yellow line: Pro-peptide; Arrow: β-sheet structure; Cylinder: α-helix structure.

Well as elastase II A, but not those of elastase I, III A, and III B, where the pro-peptide is removed from the mature enzyme after trypptic activation[11,14-16].

CRTC (caldecrin) is a serine protease with the characteristic charge-relayed catalytic triad (His58, Asp105, and Ser200), located in the active site cleft between the barrel structures[14-16]. After trypptic activation, caldecrin changes its structure to a substrate-accessible catalytic cleft form. Active caldecrin hydrolyzes the leucyl bond (e.g., in the N-Succinyl-Ala-Ala-Pro-Leu-p-nitroanilide substrate) more efficiently than chymotrypsin A and B; Caldecrin also cleaves the phenylalanyl bond (e.g., in the N-Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide substrate) and the tyrosyl bond (e.g., in the N-Succinyl-Leu-Leu-Val-Tyr-p-nitroanilide substrate)[10,19-21].

The protease activity of caldecrin is inhibited by serine protease inhibitors (phenylmethylsulfonyl fluoride or diisopropyl fluorophosphate), chymotrypsin inhibitor (chymostatin), and the Bowman-Birk trypsin and chymotrypsin inhibitor. The amino acid sequence and protease activity of caldecrin indicate that it is a hybrid of chymotrypsin and elastase.

CALDECRIN AND BONE METABOLISM

Caldecrin produces dose-dependent decreases in serum calcium concentrations[19]. The administration of purified porcine and rat caldecrin via the tail vein of mice decreased their serum calcium concentration dose-dependently and the maximum effect was attained 2-4 h post-injection with 20-100 μg (about 0.7-3.6 nmol)/kg body weight. The hypocalcemic potency of caldecrin was almost equivalent to that of porcine calcitonin (1 nmol/kg body weight, Tomomura et al[10] data not shown). The caldecrin proform (pro calderon), purified from the porcine pancreas in the presence of diisopropyl fluorophosphate, appeared to show time- and concentration-dependent chymotryptic activity following cleavage by trypsin. Administration of activated caldecrin reduced the serum calcium level in mice, even after treatment with the serine protease inhibitor, phenylmethylsulfonyl fluoride, which abolished the chymotryptic activity. However, administration of procaldenin did not decrease serum calcium levels[20]. Recombinant rat[11] and human[17] caldecrin also decreased serum calcium levels. In addition, rat protease activity-deficient caldecrin mutants (with His58Ala or Ser200Ala substitutions) decreased the levels of serum calcium. Therefore, the effect of caldecrin on serum calcium levels in vivo requires its activation by trypsin cleavage. An intramolecular responsive region required for this calcium decreasing activity may therefore be exposed by trypsin activation.

The caldecrin-induced serum calcium decrease occurred concomitantly with a decrease in the serum concentration of hydroxyproline, which is a marker of bone resorption. This observation suggested that this serum calcium decrease may be due to the suppression of bone resorption[10]. The effects of caldecrin on osteoclast function have also been investigated; recombinant wild-type and protease activity-deficient mutant caldecrin produced concentration-dependent suppression of bone resorption in isolated rabbit mature osteoclasts[23].

Osteoclasts execute bone resorption, which is modulated by macrophage colony-stimulating factor and receptor activator of nuclear factor-kappa B (NF-κB) ligand (RANKL), produced by osteoblasts and osteocytes. An imbalance between bone formation and resorption leads to bone diseases, including osteoporosis. Osteoclast differentiation and maturation involves the following three steps: (1) Osteoclast precursor cells are generated from bone marrow cells in response to macrophage colony-stimulating factor; (2) osteoclasts begin to differentiate from the precursor cells following stimulation by RANKL; and (3) at the later stage of differentiation, osteoclasts fuse to become multinucleated giant cells, leading to the cytoskeletal actin ring formation required for bone resorption. These processes are tightly regulated to

Table 1

| Step | Description |
|------|-------------|
| 1    | Osteoclast precursor cells generated from bone marrow cells |
| 2    | Osteoclasts differentiate from precursor cells following RANKL stimulation |
| 3    | Osteoclasts fuse to become multinucleated giant cells |

These processes are tightly regulated to
maintain bone homeostasis, and many molecules are involved in osteoclast differentiation[24-26]. The key molecule involved in osteoclastogenesis is RANKL, which is a member of the tumor necrosis factor superfamily that is expressed by osteoblasts and osteocytes in membrane-bound and secreted forms[27-33]. RANKL induces osteoclast differentiation by activating two signaling pathways: the mitogen-activated protein kinase (MAPK), NF-κB, and c-Fos activation axis and the phospholipase Cγ (PLCγ)-mediated calcium oscillation-calcineurin-nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) axis. Caldecrin did not inhibit macrophage colony-stimulating factor-induced osteoclast progenitor formation from bone marrow cells but did inhibit RANKL-induced osteoclast differentiation, even in the presence of protease activity[34]. Caldecrin inhibited the RANKL-stimulated spleen tyrosine kinase- and PLCγ-induced calcium oscillation, leading to an inhibition of calcineurin and NFATc1 activity (Figure 3). Caldecrin also inhibited the RANKL-mediated actin ring formation in mature osteoclasts, which is associated with RANKL-evoked calcium entry via the transient receptor potential vanilloid channel 4[35]. Caldecrin significantly inhibited RANKL-stimulated phosphorylation of c-Src in association with spleen tyrosine kinase, which is upstream of transient receptor potential vanilloid channel 4 and actin ring formation. On the other hand, caldecrin did not inhibit RANKL-mediated stimulation of MAPK, NF-κB, and c-Fos activation in osteoclast precursors or mature osteoclasts[34,35]. Therefore, caldecrin antagonized the RANKL-stimulated calcium signaling pathway involved in both osteoclast differentiation and activation.

Caldecrin is a therapeutic target in osteoporosis. The ovariectomized mouse provides a model of postmenopausal osteoporosis and exhibits an increased serum calcium level due to elevated bone resorption. This is evidenced by an increase in the bone surface to bone volume ratio, increased trabecular separation, decreased bone volume density, and decreased trabecular thickness and number. Expression of the caldecrin plasmid vector, which harbors the wild-type rat caldecrin cDNA, in the femoral muscle of this mouse model reversed this increase in serum calcium levels and restored bone resorption parameters to normal levels[36].

An important, but unaddressed, question relates to how the caldecrin released from the pancreas targets the bone. Recently, osteocalcin, which is osteoblast-derived, stored in the bone matrix, and then released by osteoclastic bone resorption, was shown to increase insulin secretion from pancreatic islets[37,38]. This activity appears to provide a physiological link between bone and pancreas, in relation to the regulation of energy metabolism. It is possible that some of the caldecrin derived from the pancreas enters the circulation and then inhibits osteoclasts, in order to regulate calcium homeostasis. The physiological activation and functions of caldecrin are not defined; however, considering its obvious effects on serum calcium levels and osteogenesis, caldecrin might be an intrinsic calcium regulating factor. The expression and distribution of caldecrin, peptide fragments of caldecrin, and its binding proteins should be explored in order to determine their physiological roles in bone metabolism.

### OTHER BIOLOGICAL ASPECTS

The CTRC gene modulates risk for pancreatitis. Rosendahl et al. reported that CTRC gene mutations were significantly associated with hereditary chronic pancreatitis. Masson et al. also identified a CTRC mutation in patients with idiopathic chronic pancreatitis. CTRC hydrolyzes the pro-peptide and calcium-binding
loop of the trypsinogens, enhancing their activation and degradation, respectively\(^\text{31-33}\). Loss-of-function CTRC variants increase the risk for chronic pancreatitis. CTRC is also a susceptibility gene for tropical calcific pancreatitis, which is a juvenile form of chronic nonalcoholic pancreatitis that occurs in Asians and Africans and is associated with nearly 90% pancreatic calcium deposition\(^\text{34}\).

It is of clinical interest that five decades ago, Takaoka \textit{et al}\(^\text{35,36}\) administered pancreatic extract to patients diagnosed with myasthenia gravis and muscular dystrophy. The symptoms of the patients treated with the extract improved progressively, suggesting that the hypocalcemic effect of the extract could have contributed to protecting them against the development of muscular dystrophy. The effect of caldecrin was also investigated in the dy/dy muscular dystrophic mouse model\(^\text{36}\). These mice genetically lack M-laminin and exhibit defective muscle basement membranes. Peritoneal administration of caldecrin protein or intramuscular expression of a caldecrin vector inhibited muscular destruction in the dy/dy mice. This indicated that caldecrin was responsible for the effects of the pancreatic extract on muscular dystrophy.

In 2011, Lacruz \textit{et al}\(^\text{47}\) found that CTRC (caldecrin) was expressed by amebolastls and was up-regulated during enamel maturation, suggesting that caldecrin might be involved in tooth development.

CTRC (caldecrin) has been reported to be associated with pancreatic cancer, where its expression is drastically reduced. Individuals with chronic pancreatitis who show low or no activity of caldecrin show an increased risk for pancreatic cancer\(^\text{48}\). Furthermore, Wang \textit{et al}\(^\text{49}\) demonstrated that overexpression of CTRC (caldecrin) downregulated the migration of human pancreatic adenocarcinoma Aspc-1 cells, whereas the knockdown of CTRC (caldecrin) increased cell migration. It would be interesting to explore the potential use of caldecrin in pancreatic cancer diagnosis and treatment. In addition, breast cancer is highly associated with osteolytic metastatic disease. RANKL is important in mammary gland development and also in the progression of metastatic breast cancer cells\(^\text{50,51}\). RANKL may partly contribute to the activation of metastatic breast cancer via the calcineurin/NFAT pathway\(^\text{52}\), which is modulated by caldecrin. It would therefore be interesting to investigate whether caldecrin suppresses RANKL-dependent tumor metastases.

CONCLUSION

The serum calcium-decreasing factor, caldecrin, was discovered in the pancreas. Caldecrin inhibits osteoclast differentiation and bone resorption in mature osteoclasts via inhibition of RANKL-induced intracellular calcium signaling. This effect occurs independently of its inherent protease activity. Therefore, caldecrin might be a potential therapeutic target for the treatment of osteolytic diseases such as osteoporosis and osteoarthritis.

REFERENCES

1. Williams GA, Bowser EN, Henderson WJ. Mode of hypocalcemic action of glucagon in the rat. \textit{Endocrinology} 1969; \textbf{85}: 538-541 [PMID: 5793033 DOI: 10.1210/endo-85-5-537]
2. Stern PH, Bell NH. Effects of glucagon on serum calcium in the rat and on bone resorption in tissue culture. \textit{Endocrinology} 1970; \textbf{87}: 110-117 [PMID: 415590 DOI: 10.1210/endo-77-1-111]
3. Zaidi M, Datta HK, Bevis PJ, Wimalawansa SJ, MacIntyre I. Amylin-amide: a new bone-conserving peptide from the pancreas. \textit{Exp Physiol} 1990; \textbf{75}: 529-536 [PMID: 2223054 DOI: 10.1113/expphysiol.1990.sp003429]
4. Alam AS, Moonga BS, Bevis PJ, Huang CL, Zaidi M. Amylin inhibits bone resorption by a direct effect on the motility of rat osteoclasts. \textit{Exp Physiol} 1993; \textbf{78}: 183-196 [PMID: 8385961 DOI: 10.1113/expphysiol.1993.sp003679]
5. D’Souza SM, MacIntyre I, Girgis SI, Mundy GR. Human synthetic calcitonin gene-related peptide inhibits bone resorption in vitro. \textit{Endocrinology} 1986; \textbf{119}: 59-61 [PMID: 3487444 DOI: 10.1210/endo-119-1-58]
6. Zaidi M, Chambers TJ, Gaines D, Morris HR, MacIntyre I. A direct action of human calcitonin gene-related peptide on isolated osteoclasts. \textit{J Endocrinol} 1987; \textbf{115}: 511-518 [PMID: 3502132 DOI: 10.1677/joe.0.115051]
7. D’Souza A, Flee MH. Calcium metabolism in pancreatic disease. \textit{Am J Clin Nutr} 1973; \textbf{26}: 352-361 [PMID: 4347666]
8. Takaoka Y, Hiwaki C, Ozawa H, Ichinose M, Otsuho Y, Shikaya T. A pancreatic protein anabolic extract. Proposal of a protein anabolic extract from pancreas. I. Preliminary report. \textit{Acta Med Nagasaki} 1966; \textbf{10}: 51-57 [PMID: 5961056]
9. Takaoka Y, Takamori M, Ichinose M, Shikaya T, Igawa N. Hypocalcemic action of a pancreatic factor and its clinical significance on the myasthenic patients. \textit{Acta Med Nagasaki} 1969; \textbf{14}: 28-35 [PMID: 539331]
10. Tomomura A, Fukushige T, Noda T, Noikura T, Saheki T. Serum calcium-decreasing factor (caldecrin) from porcine pancreas has proteolytic activity which has no clear connection with the calcium decrease. \textit{FEBS Lett} 1992; \textbf{301}: 277-281 [PMID: 1577166 DOI: 10.1016/0014-5793(92)80256-G]
11. Tomomura A, Tomomura M, Fukushige T, Akiyama M, Kabota N, Kumaki K, Nishii Y, Noikura T, Saheki T. Molecular cloning and expression of serum calcium-decreasing factor (caldecrin). \textit{J Biol Chem} 1995; \textbf{270}: 30315-30321 [PMID: 8530454 DOI: 10.1074/jbc.270.51.30315]
12. Kang J, Wieand U, Müller-Hill B. Identification of cDNAs encoding two novel rat pancreatic serine proteases. \textit{Gene} 1992; \textbf{110}: 181-187 [PMID: 15375555 DOI: 10.1016/0378-1119(92)90064-7]
13. Yoshino-Yasuda I, Kobayashi K, Akiyama M, Itoh H, Tomomura A, Saheki T. Caldecrin is a novel-type serine protease expressed in pancreas, but its homologue, elastase IV , is an artifact during cloning process. \textit{FEBS Lett} 1990; \textbf{277-281} [PMID: 1577166 DOI: 10.1016/0014-5793(92)80256-G]
14. Tomomura M, Akiyama M, Itoh H, Yoshino I, Tomomura M, Fukushige T, Noda T, Noikura T, Saheki T. Molecular cloning and expression of serum calcium-decreasing factor (caldecrin). \textit{J Biol Chem} 1995; \textbf{270}: 30315-30321 [PMID: 8530454 DOI: 10.1074/jbc.270.51.30315]
15. Tomomura A, Fukushige T, Noda T, Noikura T, Saheki T. Serum calcium-decreasing factor (caldecrin) from porcine pancreas has proteolytic activity which has no clear connection with the calcium decrease. \textit{FEBS Lett} 1992; \textbf{301}: 277-281 [PMID: 1577166 DOI: 10.1016/0014-5793(92)80256-G]
16. Tomomura M, Akiyama M, Itoh H, Yoshino I, Tomomura M, Fukushige T, Noda T, Noikura T, Saheki T. Molecular cloning and expression of serum calcium-decreasing factor (caldecrin). \textit{J Biol Chem} 1995; \textbf{270}: 30315-30321 [PMID: 8530454 DOI: 10.1074/jbc.270.51.30315]
17. Tomomura M, Akiyama M, Itoh H, Yoshino I, Tomomura M, Nishii Y, Noikura T, Saheki T. Molecular cloning and expression of human caldecrin. \textit{FEBS Lett} 1996; \textbf{386}: 26-28 [PMID: 8635596 DOI: 10.10
Tomomura M et al. Caldecrin is an anti-osteoclastogenic factor

18 Izbička E, Yoneda T, Takaoka Y, Horn D, Williams P, Mundy GR. Identification of a novel bone/calcium metabolism-regulating factor in porcine pancreas. J Biol Chem 1996; 271: 23230-23234 [PMID: 8988319 DOI: 10.1074/jbc.271.38.23230]

19 Folk JE. Schirmer EW. Chymotrypsin C. I. Isolation of the zymogen and the active enzyme: preliminary structure and specificities. J Biol Chem 1965; 240: 181-192 [PMID: 14253410]

20 Folk JE. Cole PW. Chymotrypsin C. II. Enzymatic specificity toward several polypeptides. J Biol Chem 1965; 240: 193-197 [PMID: 14253411]

21 Keil-Dlouha V, Paigserová A, Marie A, Kořík B. On subunit II of bovine procarboxypeptidase A. Enzymatic specificity after tryptic activation. Biochim Biophys Acta 1972; 1976: 533-555 [PMID: 4672120 DOI: 10.1016/0005-2744(72)90103-3]

22 Tomomura A, Fukushige T, Tomomura M, Noikura T, Nishiyi Y, Saheki T. Caldecrin proform requires trypsin activation for the acquisition of serum calcium-decreasing activity. FEBs Lett 1993; 335: 213-216 [PMID: 8253199 DOI: 10.1016/0005-2760(93)80732-A]

23 Tomomura A, Yamada H, Fujimoto K, Inaba A, Katoh S. Determination of amino acid sequence responsible for suppression of bone resorption by serum calcium-decreasing factor (caldecrin). FEBs Lett 2001; 508: 454-458 [PMID: 11728471 DOI: 10.1016/S0005-2760(01)03107-6]

24 Suda T, Takahashi N, Martin TJ. Modulation of osteoclast differentiation. Endo Rev 1992; 13: 66-80 [PMID: 1555533]

25 Teitelbaum SL. Bone resorption by osteoclasts. Science 2000; 289: 1504-1508 [PMID: 10968780 DOI: 10.1126/science.289.5484.1504]

26 Asagiri M, Takayanagi H. The molecular understanding of bone resorption in osteoclasts. J Exp Med 1998; 180: 1997-1001 [PMID: 9568710 DOI: 10.1084/jem.180.11.1997]

27 Anderson DM, Marakovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBoise RF, Cosman D, Galilith I. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 1997; 390: 175-179 [PMID: 9367155 DOI: 10.1038/36959]

28 Wong BR, Rho J, Arron J, Robinson E, Orlinick J, Chao M, Kalachikov S, Cayani E, Billingsley WL, Dougall WC, Anderson DM, Takayanagi H, Martin TJ. Modulation of osteoclast differentiation factor is a ligand for osteoprotegerin/ RANKL.

29 Yoshida H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Takamori M, Ichinose M, Tsujihata M, Mori M, Eguchi K, A protein anabolic factor from porcine pancreas and its affects on osteoclastogenesis. Acta Biochim Pol 2012; 208: 1235-1241 [PMID: 21909103 DOI: 10.1016/j.mce.2009.04.004]

30 Masson E, Chen JM, Scotet V, Le Maréchal C, Férec C. Association of rare chymotrypsinogen C (CTRC) gene variations in patients with idiopathic chronic pancreatitis. Hum Genet 2008; 123: 83-91 [PMID: 18172691 DOI: 10.1007/s00439-007-0459-3]

31 Nemoda Z, Sahin-Töhö M. Chymotrypsin C (caldecrin) stimulates autophagocytosis of human cationic trypsinogen. J Biol Chem 2006; 281: 11879-11886 [PMID: 16505482 DOI: 10.1074/jbc.M600124200]

32 Szmola R, Sahin-Töhö M. Chymotrypsin C (caldecrin) promotes degradation of human cationic trypsin: identity with Rinderkerkh’s enzyme Y. Proc Natl Acad Sci USA 2007; 104: 11227-11232 [PMID: 17592142 DOI: 10.1073/pnas.0703714104]

33 Zhou J, Sahin-Töhö M. Chymotrypsin C mutations in chronic pancreatitis. J Gastroenterol Hepatol 2011; 26: 1238-1246 [PMID: 21631589 DOI: 10.1111/j.1440-1746.2011.06791.x]

34 Palival S, Bhaskar S, Mani KR, Reddy DN, Rao GV, Singh SP, Thomas V, Chandak GR. Comprehensive screening of chymotrypsinogen C (CTRC) gene in tropical chronic pancreatitis identifies novel variants. Gut 2013; 62: 1602-1606 [PMID: 22580415 DOI: 10.1136/gutjnl-2012-302448]

35 Tomomura M, Hasegawa H, Suda N, Sakagami H, Tomomura A. Serum calcium-decreasing factor, caldecrin, inhibits receptor activator of NF-κB ligand (RANKL)-mediated Ca2+ signaling and actin ring formation in mature osteoclasts via suppression of Rsc signaling pathway. J Biol Chem 2012; 287: 17963-17974 [PMID: 22461633 DOI: 10.1074/jbc.M112.358796]

36 Ot M, Kido S, Hasegawa H, Fujimoto K, Tomomura M, Kanegae H, Suda N, Tomomura A. Inhibitory effects on bone resorption in postmenopausal osteoporosis mouse model by delivery of serum calcium decreasing factor (Caldecrin) gene. J Meikai Dent Med 2011; 40: 146-154

37 Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Daquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G. Endocrine regulation of energy metabolism by the skeleton. Cell 2007; 130: 456-469 [PMID: 17693256 DOI: 10.1016/j.cell.2007.05.047]

38 Confavreux CB, Levine RL, Karsenty G. A paradigm of integrative physiology, the crosstalk between bone and energy metabolisms. Mol Cell Endocrinol 2009; 310: 21-29 [PMID: 19376193 DOI: 10.1016/j.mce.2009.04.004]

39 Tomomura M et al. Caldecrin is an anti-osteoclastogenic factor
Biophys Sin (Shanghai) 2011; 43: 362-371 [PMID: 21460362 DOI: 10.1093/abbs/gmr022]

50 Schramek D, Leibbrandt A, Sigl V, Kenner L, Psogisilik JA, Lee HJ, Hanada R, Joshi PA, Aliprantis A, Glimcher L, Pasparakis M, Khokha R, Ormandy CJ, Widschwendter M, Schett G, Penninger JM. Osteoclast differentiation factor RANKL controls development of progesterin-driven mammary cancer. Nature 2010; 468: 98-102 [PMID: 20881962 DOI: 10.1038/nature09387]

51 Gonzalez-Suarez E, Jacob AP, Jones J, Miller R, Roudier-Meyer MP, Erwert R, Pinkas J, Branstetter D, Dougall WC. RANK ligand mediates progesterin-induced mammary epithelial proliferation and carcinogenesis. Nature 2010; 468: 103-107 [PMID: 20881963 DOI: 10.1038/nature09495]

52 Quang CT, Leboucher S, Passaro D, Fuhrmann L, Nourieh M, Vincent-Salomon A, Ghysdael J. The calcineurin/NFAT pathway is activated in diagnostic breast cancer cases and is essential to survival and metastasis of mammary cancer cells. Cell Death Dis 2015; 6: e1658 [PMID: 25719243 DOI: 10.1038/cddis.2015.14]

P- Reviewer: Hegardt FG, Vlachostergios PJ S- Editor: Qiu S E- Editor: Lu YJ
