Review Article
The Role of T-Cell Leukemia Translocation-Associated Gene Protein in Human Tumorigenesis and Osteoclastogenesis

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Synovial tissues of patients with rheumatoid arthritis (RA) include factors regulating bone resorption, such as receptor activator NF-κB ligand (RANKL), TNF-α, IL-6, IL-17, and IFN-γ [1–10]. In addition to these cytokines, other factors expressed in synovial tissues may play a role in bone resorption. To identify novel peptides or proteins expressed in synovial tissues of patients with RA that regulate human osteoclastogenesis, we purified proteins from synovial tissues of patients with RA, using gel filtration chromatography, reverse-aspect HPLC, and mass spectrometry. We finally demonstrated that a peptide derived from the extracellular domain of T-cell leukemia translocation-associated gene (TCTA) protein inhibited both RANKL-induced human osteoclastogenesis and pit formation of mature human osteoclasts [11, 12].

1. Introduction
Synovial tissues of patients with rheumatoid arthritis (RA) include factors regulating bone resorption by expressing cytokines such as RANKL, TNFα, IL-6, IL-17, and IFN-γ [1–10]. In addition to these cytokines, however, other factors expressed in synovial tissues may play a role in resorbing bone. To identify novel peptides or proteins expressed in synovial tissues of patients with RA that regulate human osteoclastogenesis, we purified proteins from synovial tissues of patients with RA, using gel filtration chromatography, reverse-aspect HPLC, and mass spectrometry. We finally demonstrated that a peptide derived from the extracellular domain of T-cell leukemia translocation-associated gene (TCTA) protein inhibited both RANKL-induced human osteoclastogenesis and pit formation of mature human osteoclasts [11, 12].

2. The Role of TCTA Protein on Human Small Cell Lung Cancer Cell Lines
In 1995, Aplan et al. cloned and characterized a novel gene at the site of a t(1;3)(p34;p21) translocation breakpoint in T-cell acute lymphoblastic leukemia (ALL), designating this gene as TCTA [13]. TCTA is also reported as T-cell leukemia translocation-altered gene. TCTA mRNA is expressed ubiquitously in normal tissues, with the highest levels of expression in the kidney. TCTA has been conserved throughout evolution in organisms ranging from Drosophila to humans. A short open reading frame encodes a protein of 103 amino acid residues, Mr 11,300, without strong homology to any previously reported proteins.

Aplan et al. reported that a preliminary survey of 44 cases of childhood ALL (9 T-cell- and 35 B-cell-) precursors did not reveal any gross structural alterations of TCTA by Southern blot [13]. Additionally, they sequenced the TCTA gene from the translocated allele in the t(1;3) translocation and found no mutations in the open reading frame [13]; however, it remains possible that more complete studies of a larger series of T-cell ALL patients may reveal a more direct role for TCTA dysregulation in T-cell ALL [13].

Of note, genomic Southern blots demonstrated a reduced TCTA signal in three of four small cell lung cancer cell lines, suggesting the loss of one of the two copies of the gene [13]. On the other hand, in 2005, it has been reported that TCTA interacts with SMA- and MAD-related protein 4
(SMAD4) in a proteome-scale map of the human protein-protein interaction network (Supplementary Table S2 of [14]); however, the function of TCTA has not been clarified.

3. Osteoclast

3.1. Structure and Function of Osteoclasts. Osteoclasts are unique multinucleated cells whose specialized function is to resorb calcified tissues [7]. On the surface of bone, osteoclasts develop a specialized adhesion structure, the "podosome," which subsequently undergoes reorganization into sealing zones [15]. These ring-like adhesion structures, that is, actin rings, seal osteoclasts to the surface of bone. In the sealed resorption lacuna, localized acidification is driven by carbonic anhydrase II and vacuolar H(+)-ATPase in osteoclasts; carbonic anhydrase II produces protons and vacuolar H(+)-ATPase transfers them into the lacuna. In acidified lacuna, cathepsin-K and matrix metalloproteinase-9 (MMP-9) are released from osteoclasts to degrade calcified tissues [16].

Osteoclasts express unique cell adhesion structures called podosomes, which contain actin filaments. Podosomes are organized differently depending on the activity of the osteoclast; in bone-resorbing osteoclasts, podosomes form the actin ring, representing a gasket-like structure, necessary for bone resorption, and in motile osteoclasts, podosomes are organized into lamellipodia (Latin lamella, a thin leaf; Greek pous, foot), the structure responsible for cell movement. Thus, the presence of actin rings and lamellipodia is mutually exclusive [17]. In 2004, Sarrazin et al. showed, using mature human osteoclasts extracted from the femurs and tibias of human fetuses, that osteoclasts have two subtypes of EP receptors, EP3 and EP4, that mediate different actions of PGE2 on these cells; activation of EP4 receptors inhibits actin ring formation and activation of EP3 receptors increases the number of lamellipodia [17]. Thus, PGE2 directly inhibits bone resorption by human osteoclasts.

The cooperation of osteoclasts and osteoblasts is critical to maintain skeletal integrity in normal bones. After bone resorption by osteoclasts on normal bone tissues, osteoblasts subsequently rebuild bone in the lacunae resorbed by osteoclasts; this mechanism is called “bone remodeling”. When the activity or number of osteoclasts is elevated compared with osteoblasts, the bone becomes fragile, that is, “osteoporotic”. In addition, bone remodeling is disrupted in all bone diseases associated with changes in bone mass. Thus, bone remodeling is essential to retain both the structure and strength of normal bone.

3.2. Origin of Osteoclasts. The origin of osteoclasts was unclear until the late 1980s. In 1988, Takahashi et al. established a coculture system using mouse spleen cells and osteoblasts to induce osteoclastogenesis in vitro, demonstrating that the origin of osteoclasts is hematopoietic cells and that osteoblastic cells are required for the differentiation of osteoclast progenitors in splenic tissues into multinucleated osteoclasts [18]. The precursor of osteoclasts was then revealed to be colony-forming unit-macrophage (CFU-M) or CFU-granulocyte/macrophage (CFU-GM) in bone marrow or spleen in mice. In 1990, Udagawa et al. demonstrated that osteoclasts are formed from murine macrophages [19]. From these findings, Suda et al. hypothesized that bone marrow hemopoietic cells differentiate into osteoclasts through the stimulation of “osteoclast-differentiation factor (ODF)” expressed on osteoblasts [20].

Finally, ODF, now termed RANKL, which induces osteoclastogenesis from monocytes or macrophages, was independently cloned by three groups in 1997 [21]. RANKL is a member of the TNF superfamily of cytokines. The protein constructs a trimeric complex to bind its receptor, receptor activator NF-κB (RANK) [22]. A decoy receptor is also cloned, which is designated as “osteoprotegerin (OPG)” [21]. In 2000-2001, we and other groups showed that T cells expressing RANKL induce osteoclastogenesis [1, 6, 23]; in particular, we demonstrated osteoclastogenesis using human cells [6], whereas others used murine cells [1, 23]. In addition, in 2009, we reported that, in human osteoclastogenesis induced by RANKL, T-cell leukemia translocation-associated gene (TCTA) protein is required for cellular fusion [11].

3.3. Development of Culture Systems to Form Osteoclasts In Vitro. Culture systems were developed to form osteoclasts in vitro in 1981–1988. In 1981, Testa et al. first succeeded in forming osteoclast-like multinucleated cells from feline marrow cells in long-term Dexter cultures [24]. In 1984, using this feline marrow culture system, Ibbotson et al. showed that the formation of osteoclast-like cells is greatly stimulated by osteotropic hormones, such as 1,25(OH)2D3, PTH, and prostaglandin E2 (PGE2) [25]. In 1987, MacDonald et al. reported the formation of multinucleated cells that respond to osteotropic hormones in long-term human bone marrow cultures [26]. In 1988, Takahashi et al. and in 1989, Hattersley and Chambers used marrow cells of mice to examine osteoclast-like cell formation from their progenitor cells [27, 28]. Moreover, in 1988, Takahashi et al. established an innovative co-culture system using mouse spleen cells and osteoblasts to induce osteoclastogenesis in vitro [18]. Thus, since 1981, studies using osteoclastogenesis in vitro have been developed, and PGE2 was shown to upregulate murine osteoclastogenesis using the marrow culture system in vitro.

3.4. Role of Osteoclasts in the Pathogenesis of RA. Osteoclasts also play an important role in the pathogenesis of RA. Since 1984, it has been reported that in bone destruction of RA, many activated osteoclasts are detected on the surface of eroded bone in the interface with synovial tissues [29]. In addition, we have demonstrated that osteoclasts are detected in synovial tissues as well as eroded bone from patients with RA [4]. We have also reported that the number of precursor cells of osteoclasts increases in bone marrow adjacent to joints with arthritis [30]. Moreover, the amount of cytokine that induces osteoclastogenesis, such as IL-1, TNF-α, and IL-6, is elevated in synovial tissues of patients with RA, while the amount of cytokine that inhibits osteoclastogenesis, such as IL-4 and IL-10, is decreased [30–32]. Thus, patients with
RA are likely to suffer from joint destruction as well as systemic osteoporosis, in which the number of osteoclasts increases, suggesting that osteoclasts play a critical role in the pathogenesis of RA.

3.5. Geranylgeranylandecke Inhibits the Formation and Function of Human Osteoclasts. The antilucl drug geranylenar-ylacetone (GGA), known as Teprenone, is frequently used with NSAIDs in Japan. In 2005, we demonstrated that GGA inhibits the formation and function of human osteoclasts and prevents bone loss in tail-suspended rats and ovariec-
tomized rats [33]. Vitamin K is also used to protect against osteoporosis. It has been reported that the inhibitory effect of vitamin K2 (menatetrenone) on bone resorption may be related to its side chain. GGA has almost the same chemical structure as the side chain of menatetrene.

We hypothesized that GGA also has an inhibitory effect on osteoclastogenesis both in vitro and in vivo. GGA in pharmacological concentrations directly inhibited osteoclastoge-

4. Role of TCTA Protein in Human Osteoclastogenesis

4.1. Hypothesis. Synovial tissues of patients with RA include factors regulating bone resorption by expressing cytokines such as RANKL, TNFα, IL-6, IL-17, and IFNγ as described in Introduction. In addition to these cytokines, however, other factors expressed in synovial tissues may play a role in resorbing bone. We hypothesized that a novel factor in synovial tissues of patients with RA regulates osteocal-
stasis. To test this hypothesis, we tried to identify novel peptides or proteins expressed in synovial tissues of patients with RA that regulate human osteoclastogenesis since 1996. We purified proteins from synovial tissues of patients with RA, using gel filtration chromatography, reverse-aspect HPLC, and mass spectrometry. We finally demonstrated that a peptide derived from the extracellular domain of T-cell leukemia translocation-associated gene (TCTA) protein inhibited both RANKL-induced human osteoclastogenesis and pit formation of mature human osteoclasts [11, 12, 35, 36]. We would like to present our findings as follows.

4.2. Purification of Proteins from Synovial Tissues of Patients with RA. Sixty-five grams of synovial tissues were obtained from 5 patients with RA at total knee replacement. The crude extract was obtained by homogenization of the synovial tissues. NH3Ac buffer was added to the freeze-dried crude extract. The supernatant was then applied to the column equilibrated with NH3Ac buffer and divided into two fractions, low molecular weight (MW) and high MW by first gel filtration chromatography. The low MW fraction was further applied to the column equilibrated with PBS by the second gel filtration chromatography. Proteins were eluted and protein concentration (A280) was monitored by UV absorption. Each fraction was added to the culture for human osteoclastogenesis to evaluate the activity on the osteoclastogenesis. Two fractions showed the inhibitory activity of osteoclastogenesis. These fractions were then subjected to ion-exchange chromatography. The “flow through” from the column showed the inhibitory activity on the osteoclastogenesis. The “flow through” was then applied to second gel filtration chromatography. Ten fractions from gel filtration chromatography showed the inhibitory activity on osteoclastogenesis. Thus, these fractions were applied to reverse-aspect HPLC. Thirty fractions were obtained by the reverse-aspect HPLC.

After each fraction obtained by reverse-aspect HPLC was concentrated, amino acid sequences were determined using a protein sequencer. Amino acid sequences (3–5 mers) were determined in 8 fractions. In addition, we tried to determine the sequence of each fraction by mass spectrometry; however, the sequences were not determined, although the total molecular weight of each peptide was speculated to be less than 1000 Da. Thus, we synthesized 8 peptides according to the sequences determined using the protein sequencer and evaluated the effect of each peptide on human osteoclastogenesis in vitro.

4.3. A Small Peptide Including GQN Inhibits Human Osteoclastogenesis. We finally revealed that the amino acid sequence of Glycine-Glutamine-Asparagine (Gly-Gln-Asn; GQN) alone in 8 synthesized peptides showed inhibitory activity in human osteoclastogenesis; GQN dose-depend-
etly inhibited human osteoclastogenesis from peripheral monocytes by RANKL (IC50: around 30 μM) [11].

When searching for proteins that include GQN using FASTA search, we found only 2 proteins in human proteins, CDW52 antigen (CAMPATH-1 antigen) and TCTA. In the FASTA search, it was possible to identify 3 mer peptides, but we did not find any other human proteins. Using “PeptideCutter”, by which it is possible to predict the site cleaved by enzymes in peptides, we synthesized 4 peptides from a sequence of CDW52 antigen and one peptide from a sequence of TCTA protein, which included GQN. A peptide from TCTA protein showed inhibitory activity on human osteoclastogenesis, but not peptides from CDW52 antigens.

4.4. Peptide A from TCTA Strongly Inhibits Human, but Not Mouse, Osteoclastogenesis. We then synthesized another 2 peptides, GQNGSTDGSTHPSWEMAANEPLKTHRE and
GQN GSTPDGSTHFPSWEMAAAN, using “PeptideCutter”, shown as “peptide A” (MW 3182.4) and “peptide A2”, respectively, from a sequence of TCTA gene protein (Figure 1). Osteoclastogenesis was more potently inhibited by these peptides than by GQN or GQN GST. Peptide A most strongly inhibited osteoclastogenesis; IC50 level was around 1.6 μM. Osteoclastogenesis induced by soluble RANKL (sRANKL) was not inhibited by adding 1.6 μM of a scrambled peptide, SPFTGTGSKSWNETAHPDHGNEERQAPMSL (MW, 3182.4), randomly designed from the sequence of peptide A. We finally synthesized 3 more peptides from a sequence of TCTA gene protein, also using “PeptideCutter”: “peptide B”: GQN GSTPDGSTHFE, “peptide C”: PGLGQNGSTPDGSTHFE, and “peptide D”: GFYGMVTGGLYHRPGL GGQNGSTPDGSTHFEPSWEMAAANPEPLKTHRE, which is the whole of the human extracellular domain (Figure 1). Sequences of both peptide B and peptide C are included although a scrambled peptide did not inhibit pit formation for 24 h. However, peptide A as well as a scrambled peptide showed inhibitory activity on mature osteoclasts in the culture of 3 days, but not 24 h [11].

4.7. Peptide A Suppresses the Formation of Large Osteoclasts in the Culture of Mature Human Osteoclasts. We then investigated the effects of peptide A on mature osteoclasts cultured with sRANKL and M-CSF for 3 days. In the control well with sRANKL alone in the absence of peptide A, larger osteoclasts were detected in the control well alone, but not in the well cultured with peptide A. Osteoclasts in the well cultured with peptide A showed usual size. The number of huge osteoclasts in the control well cultured without peptide A was significantly higher than in the well cultured with peptide A. Thus, we hypothesized that TCTA plays a role in the fusion of monocytes/macrophages, preosteoblasts, and osteoclasts [11].

4.8. Polyclonal Antibodies against TCTA Inhibit Both Human Osteoclastogenesis from Monocytes and Fusion of Mature Osteoclasts. We constructed indirect competitive enzyme-linked immunosorbent assay (ELISA) using 2 polyclonal antibodies against TCTA protein. Standard curves showed specificity and high affinity of the antibodies against TCTA protein. To investigate our hypothesis further, we cultured monocytes with M-CSF and sRANKL in the presence of the polyclonal antibody, #1 or #2, both of which significantly inhibited human osteoclastogenesis, reducing the size of osteoclasts. In addition, polyclonal antibody #2 inhibited the fusion of mature osteoclasts [11].

4.9. TCTA Protein Is Immunohistologically Detected on Human Osteoclasts and Macrophages. Using antibody no. 1, TCTA protein was immunohistologically detected on human osteoclasts induced by sRANKL and M-CSF. TCTA protein was detected in the central area of cells or in the peripheral area of cells. TCTA protein stained in the peripheral area of cells was observed in both a preosteoclast with 2 nuclei and an osteoclast with 4 nuclei, using fluorescent microscopy. TCTA protein also detected human macrophages cultured with M-CSF [11].

4.10. Other Findings on Peptide A. Peptide A as well as a scrambled peptide failed to disrupt the structure of actin rings of mature osteoclasts in the culture of 24 h and 3 days. The amount of TCTA mRNA was significantly lower in human osteoclasts induced by sRANKL and M-CSF than in human macrophages cultured with M-CSF alone. Peptide A or the scrambled peptide did not reduce the amount of TCTA mRNA in osteoclasts. TCTA mRNA was detected in human osteoclasts, monocytes, fibroblast-like synoviocytes, T cells, and PBMC by RT-PCR. TCTA protein was immunohistochemically detected in cultured fibroblast-like synoviocytes using polyclonal anti-TCTA antibodies #1. TCTA protein was also immunohistochemically detected in synovial tissues using polyclonal anti-TCTA antibodies #1. TCTA protein-positive cells were detected in synovial lining cells, but not in lymphoid follicular with many lymphocytes. TCTA protein...
Intracellular Extracellular

![Intracellular Extracellular Diagram]

**Figure 1:** Structure of TCTA and sequences of peptides. Structure of TCTA showing both intracellular (white bar) and extracellular (meshed bar) domains. Black bar shows the human/mouse identical sequence in the extracellular domain.

**Figure 2:** Possible mechanism of human osteoclastogenesis by peptide A and antibodies against TCTA protein. (Structure of TCTA is derived from SOSUI; http://bp.nuap.nagoya-u.ac.jp/sosui/sosui_submit.html). TCTA protein plays an important role in cellular fusion in human osteoclastogenesis from monocytes and mature osteoclasts. Both peptide A and antibodies block the interaction between TCTA protein and a putative counterpart of TCTA protein. Modified from the figure in [11].

5. A Novel Hypothesis in Human Osteoclastogenesis

We demonstrated that a novel peptide derived from the amino acid sequence of the extracellular domain of TCTA protein inhibited not only RANKL-induced human osteoclastogenesis from monocytes but also fusion of activated mature human osteoclasts [11]. In the culture of mature osteoclasts, peptide A suppressed the formation of large mature osteoclasts after 3 days in the presence of sRANKL. In addition, although peptide A inhibited human osteoclastogenesis from monocytes with sRANKL, peptide A did not show significant inhibition of both protein and mRNA of NFATc1. The inhibitory effects of peptide A were not different among early, middle, and late phases of culture. In addition, the structure of actin rings of mature osteoclasts was not disrupted by peptide A. Thus, taken together, our findings suggest that the extracellular domain of TCTA may play a role in the fusion of osteoclast precursors and mature osteoclasts and that peptide A may block the interaction of TCTA and a putative counterpart of TCTA (Figure 2). Supporting our putative mechanism, Saginario et al. reported that, because the soluble extracellular domain of macrophage fusion receptor (MFR) prevents the fusion of macrophages in vitro, MFR belongs to the fusion machinery of macrophages [37].

Several studies have reported that peptides inhibit osteoclastogenesis [38–40]. Jimi et al. reported that, using a cell-permeable peptide inhibitor of the I-κB-kinase complex, the peptide inhibits RANKL-stimulated NF-κB activation and osteoclastogenesis both in vitro and in vivo. They also showed that this peptide significantly reduces the severity of collagen-induced arthritis in mice by reducing levels of TNFα and IL-1β, abrogating joint swelling and reducing the destruction of bone and cartilage [40]. They used 20 μM of peptides to inhibit RANKL-induced mice osteoclastogenesis, whereas our peptide A inhibited RANKL-induced human osteoclastogenesis at 1.6 μM of IC50. Thus, our peptide more effectively inhibits osteoclastogenesis, although the different efficacy of the peptides may be derived from the different species used, humans and mice.

The reasons why peptides B and C, included in the mouse sequence, did not inhibit the osteoclastogenesis from mouse bone marrow cells remain unclear. We used only 3 peptides, peptide B, peptide C, and mouse “peptide A”, in the culture of mouse cells. The other peptides, including GQN, may inhibit mouse osteoclastogenesis. On the other hand, even if TCTA protein plays a role in fusion as discussed above, TCTA protein in mice may be less important than the other molecules for fusion, such as, dendritic cell-specific transmembrane protein (DC-STEMP) [41], CD9 [42], CD47 [9, 43], macrophage fusion protein (MFR) [37, 43, 44], E-cadherin [45–47], meltrin-α (ADAM12) [48], or CD44 [49]. These findings also underline the difference of differentiation
of osteoclasts between human and mice, supporting the importance of the term, “human osteoclastology” [50].

In conclusion, we demonstrated that peptide A and polyclonal antibody against TCTA protein inhibited not only human osteoclastogenesis from monocytes but also the further maturation of mature human osteoclasts in vitro [11]. Our findings suggest that TCTA protein plays an important role in cellular fusion in human osteoclastogenesis from monocytes and mature osteoclasts. Thus, peptide A may show the same inhibitory function in vivo, offering an effective therapeutic approach for inhibiting bone resorption.

6. Costimulation of TCTA on Human Small Cell Lung Cancer and Human Osteoclastogenesis

Metastatic bone tumors occur at particularly high rates in cancers of the breast, prostate, lung, and kidney. Many patients with lung cancer are in advanced stages of the disease at the time of diagnosis. The 5-year survival rate for patients with lung cancer is 10% to 20%, indicating a poor prognosis. In addition, it is reported that bone metastasis from lung cancer occurs in 14% to 40% of patients [51]. The same side of the ribs and spine are the most common. For lung cancer bone metastases, surgical treatment, radiation therapy, chemotherapy, and the biological treatment are performed; however, the effects are not sufficient.

It was reported that TCTA protein may regulate the tumorigenesis of small cell lung cancer, although the mechanism remains elucidative [13]. We have demonstrated that TCTA protein induces human osteoclastogenesis by up-regulating cellular fusion. Thus, it may be possible to treat lung cancer bone metastases by regulating the function of TCTA protein in both small cell lung cancer and osteoclasts.

7. Conclusion

Aplan et al. demonstrated in 1995 that TCTA protein is associated with the tumorigenesis of ALL and small cell lung cancer. The other functions of TCTA protein had not been revealed until we demonstrated that the protein induces human osteoclastogenesis in 2009. We are now investigating the effect of peptide A on the proliferation of cell lines of human small lung cancer. Thus, a novel therapy for small cell lung cancer with bone metastases may be developed using peptide A from TCTA protein.

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References


