Review Article

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

Shigeru Kotake, Toru Yago, Manabu Kawamoto, and Yuki Nanke

Institute of Rheumatology, Tokyo Women's Medical University, 10-22 Kawada-cho, Shinjuku, Tokyo 162-0054, Japan

Correspondence should be addressed to Shigeru Kotake, skotake@ior.twmu.ac.jp

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. However, in addition to these cytokines, other factors expressed in synovial tissues may play a role in regulating bone resorption. In 2009, we demonstrated that novel peptides from T-cell leukemia translocation-associated gene (TCTA) protein expressed in synovial tissues from patients with RA inhibit human osteoclastogenesis, preventing cellular fusion via the interaction between TCTA protein and a putative counterpart molecule. Only a few studies on the role of TCTA protein have been reported. 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. In the current paper, we reviewed the roles of TCTA protein in lung cancer cell lines and human osteoclastogenesis.

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, M_r 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(l;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 PGE_2 on these cells; activation of EP4 receptors inhibits actin ring formation and activation of EP3 receptors increases the number of lamellipodia [17]. Thus, PGE_2 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 $1,25(OH)_2D_3$, PTH, and prostaglandin E_2 (PGE_2) [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 PGE_2 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 inhibit 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. Geranylgeranylacetone Inhibits the Formation and Function of Human Osteoclasts. The antiulcer drug geranylgeranylacetone (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 ovariectomized 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 menatetrenone.

We hypothesized that GGA also has an inhibitory effect on osteoclastogenesis both *in vitro* and *in vivo*. GGA in pharmacological concentrations directly inhibited osteoclastogenesis from human monocytes induced by soluble RANKL. In addition, GGA induced the degradation of actin rings in mature osteoclasts, which was reversed by adding geranylgeranylpyrophosphatase. Moreover, GGA increased the bone mineral density of the total femur, proximal metaphysis, and diaphysis of femur in ovariectomized rats. GGA also prevented bone loss induced by hindlimb unloading in tail-suspended rats. These results indicate that GGA prevents bone loss by maintaining a positive balance of bone turnover through suppression of both the formation and activity of osteoclasts. In addition, in 2009, we also reported that GGA induces cell death in fibroblast-like synoviocytes from patients with rheumatoid arthritis [34]. Thus, GGA could be used to prevent and improve osteoporosis, especially in patients with RA.

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 osteoclastogenesis. 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-dependently 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, **GQN**GSTPDGSTHFPSWEMAANEPLKTHRE and

GQNGSTPDGSTHFPSWEMAAN, 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 $1.6\,\mu$M. Osteoclastogenesis induced by soluble RANKL (sRANKL) was not inhibited by adding $1.6\,\mu$M of a scrambled peptide, SPFTGTKGSWNETAHPDHGNEERQAPMSL (MW, 3182.4), randomly designed from the sequence of peptide A. We finally synthesized 3 more peptides from a sequence of TCTA protein, also using "PeptideCutter": "peptide B": **GQN**GSTPDGSTHF, "peptide C": PGLG**GQN**GS-TPDGSTHF, and "peptide D": GFYGNTVTGLYHRPG-LG**GQN**GSTPDGSTHFPSWEMAANEPLKTHRE, which is the whole of the human extracellular domain (Figure 1). Sequences of both peptide B and peptide C are included in a human/mouse identical sequence of TCTA protein (Figure 1).

Both peptide B and peptide C showed a weak inhibitory effect on human osteoclastogenesis from human peripheral monocytes. Peptide D did not show inhibitory activity. On the other hand, we also examined the effects of these peptides on mouse osteoclastogenesis; however, in contrast to our expectation, peptides B and C, which are included in the mouse sequence, did not inhibit ddY mouse osteoclastogenesis from mouse bone marrow cells stimulated by sRANKL and M-CSF. Peptides A and D did not show the inhibitory effects on mouse cells as we expected. Peptide A did not show cytotoxicity on human monocytes or cell proliferation on human monocytes [11].

4.5. Peptide A Failed to Inhibit the Expression of Protein and mRNA of NFATc1, and mRNA of Cathepsin K.

We then examined the effect of peptide A on the expression of both protein and mRNA of NFATc1, a key regulator of osteoclastogenesis under the signals of RANK-RANKL, in osteoclasts. Peptide A appeared to weakly inhibit the expression of protein of NFATc1 in osteoclasts in immunohistological staining for NFATc1. Peptide A (0.8–$3.2\,\mu$M) also weakly, but not significantly, inhibited the expression of mRNA of NFATc1 in osteoclasts. In addition, peptide A did not inhibit the expression of mRNA of cathepsin K using the scrambled peptide as a control [11].

4.6. Peptide A Inhibits Pit Formation of Mature Human Osteoclasts in the Culture of 3 Days, but Not 24 h.

We then examined the effect of peptide A on pit formation of mature human osteoclasts on Osteologic. Mature osteoclasts formed from monocytes in culture with RANKL and M-CSF for 14 days were removed from plates. The mature osteoclasts then cultured on Osteologic discs with peptide A or a scrambled peptide in the presence of RANKL and M-CSF for 24 h or 3 days. Peptide A as well as a scrambled peptide did not inhibit pit formation for 24 h. However, peptide A significantly inhibited pit formation in the culture of 3 days, although a scrambled peptide did not inhibit pit formation

at all. Thus, peptide A showed inhibitory activity on mature human 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 formed than osteoclasts cultured with sRANKL and peptide A. Huge 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 immunohistologically detected in cultured fibroblast-like synoviocytes using polyclonal anti-TCTA antibodies #1. TCTA protein was also immunohistologically 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
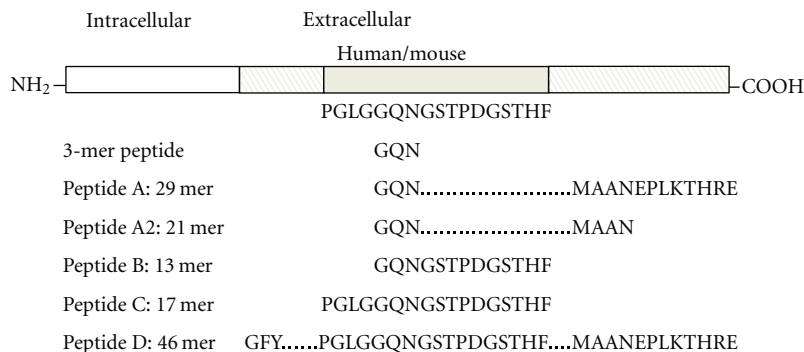
FIGURE 1: Structure of TCTA and sequences of peptides. Structure of TCTA showing both intra-cellular (white bar) and extra-cellular (meshed bar) domains. Black bar shows the human/mouse identical sequence in the extra-cellular domain.
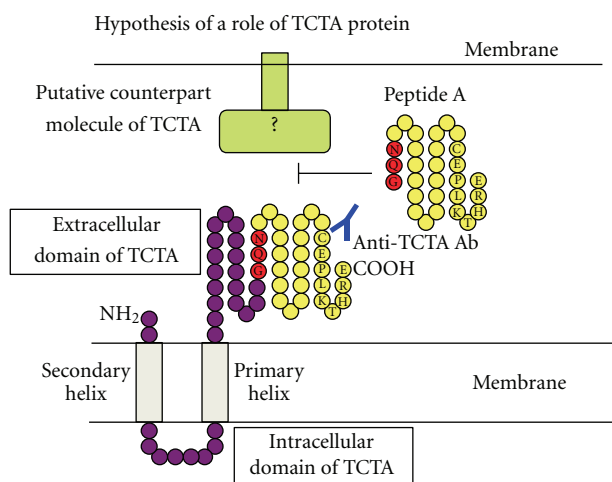

FIGURE 2: Possible mechanism of human osteoclastogenesis by peptide A and antibodies against TCTA protein. (Structure of TCTA is derived from SOSUI; http://bp.nuap.nagoya-u.ac.jp/sosui/sosui_submit.html). TCTA protein plays an important role in cellular fusion in human osteoclastogenesis from monocytes 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].

was significantly detected in human monocytes by flow cytometry using polyclonal anti-TCTA antibody no. 1 [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-STAMP) [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 upregulating 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 cell cancer. Thus, a novel therapy for small cell lung cancer with bone metastases may be developed using peptide A from TCTA protein.

Acknowledgment

References

[1] N. J. Horwood, V. Kartsogiannis, J. M. W. Quinn, E. Romas, T. J. Martin, and M. T. Gillespie, "Activated T lymphocytes support osteoclast formation in vitro," *Biochemical and Biophysical Research Communications*, vol. 265, no. 1, pp. 144–150, 1999.

[2] T. Kawai, T. Matsuyama, Y. Hosokawa et al., "B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease," *American Journal of Pathology*, vol. 169, no. 3, pp. 987–998, 2006.

[3] K. Kobayashi, N. Takahashi, E. Jimi et al., "Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction," *Journal of Experimental Medicine*, vol. 191, no. 2, pp. 275–285, 2000.

[4] S. Kotake, K. Sato, K. J. Kim et al., "Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation," *Journal of Bone and Mineral Research*, vol. 11, no. 1, pp. 88–95, 1996.

[5] S. Kotake, N. Udagawa, N. Takahashi et al., "IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis," *Journal of Clinical Investigation*, vol. 103, no. 9, pp. 1345–1352, 1999.

[6] S. Kotake, N. Udagawa, M. Hakoda et al., "Activated human T cells directly induce osteoclastogenesis from human monocytes: possible role of T cells in bone destruction in rheumatoid arthritis patients," *Arthritis and Rheumatism*, vol. 44, no. 5, pp. 1003–1012, 2001.

[7] S. Kotake, Y. Nanke, M. Mogi et al., "IFN-γ-producing human T cells directly induce osteoclastogenesis from human monocytes via the expression of RANKL," *European Journal of Immunology*, vol. 35, no. 11, pp. 3353–3363, 2005.

[8] H. Takayanagi, K. Ogasawara, S. Hida et al., "T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ," *Nature*, vol. 408, no. 6812, pp. 600–605, 2000.

[9] T. Yago, Y. Nanke, M. Kawamoto et al., "IL-23 induces human osteoclastogenesis via IL-17 in vitro, and anti-IL-23 antibody attenuates collagen-induced arthritis in rats," *Arthritis Research and Therapy*, vol. 9, no. 5, article R96, 2007.

[10] T. Yago, Y. Nanke, N. Ichikawa et al., "IL-17 induces osteoclastogenesis from human monocytes alone in the absence of osteoblasts, which is potently inhibited by anti-TNF-α antibody: a novel mechanism of osteoclastogenesis by IL-17," *Journal of Cellular Biochemistry*, vol. 108, no. 4, pp. 947–955, 2009.

[11] S. Kotake, Y. Nanke, M. Kawamoto et al., "T-cell leukemia translocation-associated gene (TCTA) protein is required for human osteoclastogenesis," *Bone*, vol. 45, no. 4, pp. 627–639, 2009.

[12] S. Kotake, T. Yago, M. Kawamoto, and Y. Nanke, "The role of T-cell leukemia translocation-associated gene (TCTA) protein in human osteoclastogenesis," *Japanese Journal of Clinical Immunology*, vol. 32, no. 6, pp. 466–471, 2009.

[13] P. D. Aplan, B. E. Johnson, E. Russell, D. S. Chervinsky, and I. R. Kirsch, "Cloning and characterization of TCTA, a gene located at the site of a t(1;3) translocation," *Cancer Research*, vol. 55, no. 9, pp. 1917–1921, 1995.

[14] J. F. Rual, K. Venkatesan, T. Hao et al., "Towards a proteome-scale map of the human protein-protein interaction network," *Nature*, vol. 437, no. 7062, pp. 1173–1178, 2005.

[15] C. Luxenburg, D. Geblinger, E. Klein et al., "The architecture of the adhesive apparatus of cultured osteoclasts: from podosome formation to sealing zone assembly," *PLoS ONE*, vol. 2, no. 1, article e179, 2007.

[16] K. Fuller, B. Kirstein, and T. J. Chambers, "Regulation and enzymatic basis of bone resorption by human osteoclasts," *Clinical Science*, vol. 112, no. 11-12, pp. 567–575, 2007.

[17] P. Sarrazin, J. A. Hackett, I. Fortier, M. A. Gallant, and A. J. De Brum-Fernandes, "Role of EP3 and EP4 prostaglandin

receptors in reorganization of the cytoskeleton in mature human osteoclasts," *Journal of Rheumatology*, vol. 31, no. 8, pp. 1598–1606, 2004.

[18] N. Takahashi, T. Akatsu, N. Udagawa et al., "Osteoblastic cells are involved in osteoclast formation," *Endocrinology*, vol. 123, no. 5, pp. 2600–2602, 1988.

[19] N. Udagawa, N. Takahashi, T. Akatsu et al., "Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 18, pp. 7260–7264, 1990.

[20] T. Suda, N. Udagawa, I. Nakamura, C. Miyaura, and N. Takahashi, "Modulation of osteoclast differentiation by local factors," *Bone*, vol. 17, no. 2, supplement, pp. 87S–91S, 1995.

[21] N. Udagawa, S. Kotake, N. Kamatani, N. Takahashi, and T. Suda, "The molecular mechanism of osteoclastogenesis in rheumatoid arthritis," *Arthritis Research*, vol. 4, no. 5, pp. 281–289, 2002.

[22] J. Lam, C. A. Nelson, F. P. Ross, S. L. Teitelbaum, and D. H. Fremont, "Crystal structure of the TRANCE/RANKL cytokine reveals determinants of receptor-ligand specificity," *Journal of Clinical Investigation*, vol. 108, no. 7, pp. 971–979, 2001.

[23] Y. Y. Kong, U. Feige, I. Sarosi et al., "Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand," *Nature*, vol. 402, no. 6759, pp. 304–309, 1999.

[24] N. G. Testa, T. D. Allen, L. G. Lajtha, D. Onions, and O. Jarret, "Generation of osteoclasts in vitro," *Journal of Cell Science*, vol. 47, pp. 127–137, 1981.

[25] K. J. Ibbotson, G. D. Roodman, L. M. McManus, and G. R. Mundy, "Identification and characterization of osteoclast-like cells and their progenitors in cultures of feline marrow mononuclear cells," *Journal of Cell Biology*, vol. 99, no. 2, pp. 471–480, 1984.

[26] B. R. MacDonald, N. Takahashi, L. M. McManus, J. Holahan, G. R. Mundy, and G. D. Roodman, "Formation of multinucleated cells that respond to osteotropic hormones in long term human bone marrow cultures," *Endocrinology*, vol. 120, no. 6, pp. 2326–2336, 1987.

[27] N. Takahashi, H. Yamana, S. Yoshiki et al., "Osteoclast-like cell formation and its regulation by osteotropic hormones in mouse bone marrow cultures," *Endocrinology*, vol. 122, no. 4, pp. 1373–1382, 1988.

[28] G. Hattersley and T. J. Chambers, "Calcitonin receptors as markers for osteoclastic differentiation: correlation between generation of bone-resorptive cells and cells that express calcitonin receptors in mouse bone marrow cultures," *Endocrinology*, vol. 125, no. 3, pp. 1606–1612, 1989.

[29] S. Shimizu, S. Shiozawa, K. Shiozawa, S. Imura, and T. Fujita, "Quantitative histologic studies on the pathogenesis of periarticular osteoporosis in rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 28, no. 1, pp. 25–31, 1985.

[30] S. Kotake, M. Higaki, K. Sato et al., "Detection of myeloid precursors (granulocyte/macrophage colony forming units) in the bone marrow adjacent to rheumatoid arthritis joints," *Journal of Rheumatology*, vol. 19, no. 10, pp. 1511–1516, 1992.

[31] S. Kotake, H. R. Schumacher Jr., and R. L. Wilder, "A simple nested RT-PCR method for quantitation of the relative amounts of multiple cytokine mRNAs in small tissue samples," *Journal of Immunological Methods*, vol. 199, no. 2, pp. 193–203, 1996.

[32] S. Kotake, H. R. Schumacher, C. H. Yarboro et al., "In vivo gene expression of type 1 and type 2 cytokines in synovial

tissues from patients in early stages of rheumatoid, reactive, and undifferentiated arthritis," *Proceedings of the Association of American Physicians*, vol. 109, no. 3, pp. 286–302, 1997.

[33] Y. Nanke, S. Kotake, T. Ninomiya, T. Furuya, H. Ozawa, and N. Kamatani, "Geranylgeranylacetone inhibits formation and function of human osteoclasts and prevents bone loss in tail-suspended rats and ovariectomized rats," *Calcified Tissue International*, vol. 77, no. 6, pp. 376–385, 2005.

[34] Y. Nanke, M. Kawamoto, T. Yago, J. Chiba, H. Yamanaka, and S. Kotake, "Geranylgeranylacetone, a non-toxic inducer of heat shock protein, induces cell death in fibroblast-like synoviocytes from patients with rheumatoid arthritis," *Modern Rheumatology*, vol. 19, no. 4, pp. 379–383, 2009.

[35] S. Kotake, T. Yago, M. Kawamoto, and Y. Nanke, "Effects of NSAIDs on differentiation and function of human and murine osteoclasts—crucial "Human Osteoclastology"," *Pharmaceuticals*, vol. 3, no. 5, pp. 1394–1410, 2010.

[36] S. Kotake, T. Yago, M. Kawamoto, and Y. Nanke, "The role of T-cell leukemia translocation-associated gene (TCTA) protein in human osteoclastogenesis," in *T-Cell Leukemia* , O. Babusikova, Ed., InTech-Open Access Publisher, Rijeka, Croatia, 2011.

[37] C. Saginario, H. Sterling, C. Beckers et al., "MFR, a putative receptor mediating the fusion of macrophages," *Molecular and Cellular Biology*, vol. 18, no. 11, pp. 6213–6223, 1998.

[38] S. J. Choi, N. Kurihara, Y. Oba, and G. D. Roodman, "Osteoclast inhibitory peptide 2 inhibits osteoclast formation via its c-terminal fragment," *Journal of Bone and Mineral Research*, vol. 16, no. 10, pp. 1804–1811, 2001.

[39] F. Ikeda, R. Nishimura, T. Matsubara et al., "Critical roles of c-Jun signaling in regulation of NFAT family and RANKL-requlated osteoclast differentiation," *Journal of Clinical Investigation*, vol. 114, no. 4, pp. 475–484, 2004.

[40] E. Jimi, K. Aoki, H. Saito et al., "Selective inhibition of NF-κB blocks osteoclastogenesis and prevents inflammatory bone destruction in vivo," *Nature Medicine*, vol. 10, no. 6, pp. 617–624, 2004.

[41] T. Kukita, N. Wada, A. Kukita et al., "RANKL-induced DC-STAMP is essential for osteoclastogenesis," *Journal of Experimental Medicine*, vol. 200, no. 7, pp. 941–946, 2004.

[42] T. Yi, H. J. Kim, J. Y. Cho et al., "Tetraspanin CD9 regulates osteoclastogenesis via regulation of p44/42 MAPK activity," *Biochemical and Biophysical Research Communications*, vol. 347, no. 1, pp. 178–184, 2006.

[43] P. Lundberg, C. Koskinen, P. A. Baldock et al., "Osteoclast formation is strongly reduced both in vivo and in vitro in the absence of CD47/SIRPα-interaction," *Biochemical and Biophysical Research Communications*, vol. 352, no. 2, pp. 444–448, 2007.

[44] T. Yago, Y. Nanke, M. Kawamoto et al., "Antibodies against CD47 expressed on monocytes in rheumatoid arthritis patients inhibits human osteoclastogenesis by blocking fusion of monocytes," *Arthritis and Rheumatism*, vol. 50, supplement, p. 1394, 2006, abstract no. S354.

[45] G. Mbalaviele, H. Chen, B. F. Boyce, G. R. Mundy, and T. Yoneda, "The role of cadherin in the generation of multinucleated osteoclasts from mononuclear precursors in murine marrow," *Journal of Clinical Investigation*, vol. 95, no. 6, pp. 2757–2765, 1995.

[46] A. Vignery, "Osteoclasts and giant cells: macrophage-macrophage fusion mechanism," *International Journal of Experimental Pathology*, vol. 81, no. 5, pp. 291–304, 2000.

[47] A. Vignery, "Macrophage fusion: the making of osteoclasts and giant cells," *Journal of Experimental Medicine*, vol. 202, no. 3, pp. 337–340, 2005.

[48] E. Abe, H. Mocharla, T. Yamate, Y. Taguchi, and S. C. Manolagas, "Meltrin-α, a fusion protein involved in multinucleated giant cell and osteoclast formation," *Calcified Tissue International*, vol. 64, no. 6, pp. 508–515, 1999.

[49] K. Suzuki, B. Zhu, S. R. Rittling et al., "Colocalization of intracellular osteopontin with CD44 is associated with migration, cell fusion, and resorption in osteoclasts," *Journal of Bone and Mineral Research*, vol. 17, no. 8, pp. 1486–1497, 2002.

[50] S. Kotake, Y. Nanke, T. Yago, M. Kawamoto, and H. Yamanaka, "Human osteoclastogenic T cells and human osteoclastology," *Arthritis and Rheumatism*, vol. 60, no. 11, pp. 3158–3163, 2009.

[51] A. D. Johnston, "Pathology of metastatic tumors in bone," *Clinical Orthopaedics and Related Research*, vol. 73, pp. 8–32, 1970.