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A Novel Peptide from T-Cell Leukemia Translocation-Associated Gene (TCTA) Protein Inhibits Proliferation of a Small-Cell Lung Carcinoma

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ABSTRACT

In 2009, we demonstrated that a peptide, which we named "Peptide A", 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. Here, we examined the effect of Peptide A on the cell proliferation of cell lines of small-cell lung carcinoma, breast cancer, and prostate cancer: RERF-LC-MA, MCF-7, and PC-3, respectively. Peptide A inhibited the proliferation of RERF-LC-MA, but not MCF-7 or PC-3. TCTA protein was immunohistologically detected in RERF-LC-MA and MCF-7. Thus, Peptide A may provide a novel strategy for the therapy of the patients with small-cell lung carcinoma, especially with bone metastasis. In addition, Peptide A may be useful for the treatment of various cancer patients with bone metastasis.

Keywords: Osteoclast; Small-Cell Lung Carcinoma; TCTA

1. Introduction

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, designating this gene as TCTA [1]. 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. 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 [1]. On the other hand, in 2005, it was 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, line 6175 of Ref. [2]); however, the function of TCTA has not been clarified.

In 2009, we identified a novel peptide expressed in synovial tissues of patients with RA that regulates human osteoclastogenesis. We therefore purified proteins from synovial tissues of patients with RA, using gel filtration chromatography, reverse-aspect HPLC, and mass spectrometry [3]. We finally demonstrated that a peptide derived from the extracellular domain of TCTA protein inhibited both RANKL-induced human osteoclastogenesis and pit formation of mature human osteoclasts [3].

In the current study, we investigated the effect of a peptide form TCTA protein on the proliferation of RERF-LC-MA, a small-cell lung carcinoma cell line. The peptide significantly inhibited the proliferation of RERF-LC-MA.

2. Materials and Methods

2.1. Cell Lines

A human small cell carcinoma cell line, RERF-LC-MA, a prostate cancer cell line, PC-3, and a breast cancer cell line, MCF-7, were purchased from Health Science Research Resources Bank (Tokyo, Japan).

2.2. Cell Proliferation Assay

Cell proliferation of cancer cell lines was measured using

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a Cell Proliferation Assay Kit (XTT baser) (BIOLOGICAL INDUSTRES Ltd. Israel). The assay was performed according to manufacturer's protocol. Cells (0.6 - 1.0 × 10E3/well) were cultured in 96-well plates. Before cells were confluent, various concentrations of Peptide A or scrambled peptide as a control were added to the wells. After 24 or 48 hrs, the cells were collected, and cell proliferation was measured using the kit. Experiments were repeated 5 times. All experiments were performed in quadricate.

2.3. RT-PCR for TCTA mRNA in Cell Lines

We detected mRNA of TCTA using RT-PCR in each cell line incubated with Peptide A or the scrambled peptide. Each cell line (1 - 2 ×10E3) was cultured using D-MEM with 10% of FCS in 6-well plates. After 24 h, the medium was exchanged to D-MEM with 1% FCS and 2 mM L-glu. After another 24 h, the medium was changed and peptide A or the scrambled peptide was added. After a further 24 h, total RNA was prepared from the cells cultured as described above. A sense primer and an antisense primer were used under the PCR conditions, as previously described [3].

2.4. Immunohistological Staining

Cell lines were cultured using Lab-Tec chambers (Nunc, Narita, Japan). Immunohistological staining was performed as described previously (4). Anti-TCTA antibody #1 was used as the 1st antibody to detect TCTA protein in cell lines; as previously reported, we obtained 2 polyclonal antibodies against TCTA, #1 and #2 [3]. Rabbit IgG was used as a control antibody. The bound antibodies were visualized as described previously [4]. Stained tissues were examined using one-box microscopy (BZ-9000; Keyence, Osaka, Japan).

3. Results

3.1. Peptide A Significantly Inhibited the Proliferation of RERF-LC-MA

Peptide A dose-dependently inhibited the proliferation of RERF-LC-MA (**Figure 1(a)**). On the other hand, the scrambled peptide as a control did not inhibit the proliferation of RERF-LC-MA (**Figure 1(b)**). At 10 μg/ml, Peptide A significantly inhibited the proliferation of RERF-LC-MA compared with the scrambled peptide (Wilcoxon test, $p = 0.031$, **Figure 1(c)**). Peptide A did not inhibit the proliferation of PC-3 or MCF-7 (data not shown).

3.2. Immunohistological Staining for TCTA Protein

TCTA proteins were immunohistologically detected in RERF-LC-MA and MCF-7 (**Figure 2**). In the current study, it was difficult to detect PC-3 specifically, because non-specific staining was strongly detected (data not shown).

3.3. TCTA mRNA in Cell Lines

Experiments were performed using 5 treatment conditions: 1) None, 2) 10 mg/ml peptide A, 3) 5 mg/ml peptide A, 4) 10 mg/ml scrambled peptide, 5) 5 mg/ml scrambled peptide in each cell lines. TCTA mRNA% expressions were as follows: MCF-7, 1) 100.0, 2) 113.5, 3) 102.4, 4) 109.4, 5) 100.3; PC3, 1) 100.0, 2) 82.9, 3) 106.4, 4) 86.9, 5) 72.3; RERF-LC, 1) 100.0, 2) 114.8, 3) 129.7, 4) 115.1, 5) 108.9. There was no tendency that peptide A changed the level of TCTA mRNA compared with the scrambled peptide.

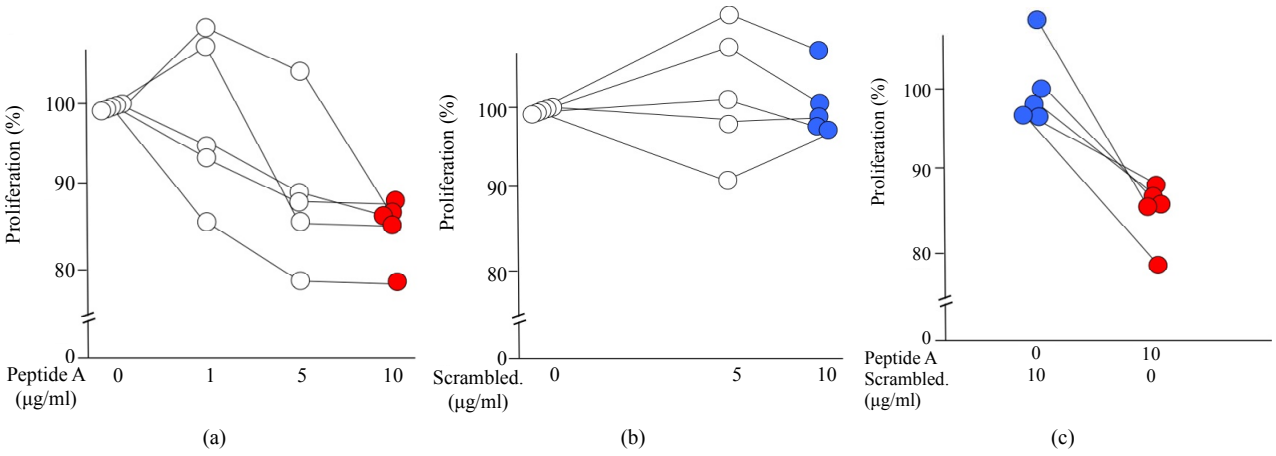

Figure 1. Cell proliferation assay. RERF-LC-MA was cultured with various concentration of Peptide A (a) or scrambled peptides (b). The proliferation levels with 10 μg/ml are shown red and blue for Peptide A and scrambled peptides, respectively. The proliferation levels with 10 μg/ml are compared between Peptide A (red) and scrambled peptide (blue) in (c).

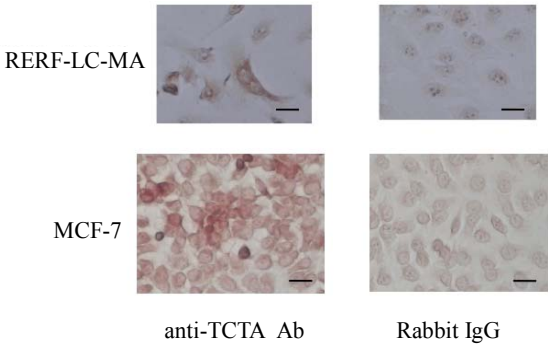

Figure 2. Immunohistological detection of TCTA protein in cell lines. Bar: 10 μm.

4. Discussion

In the current study, we demonstrated that Peptide A significantly inhibited the cell proliferation of a small-cell lung carcinoma cell line, RERF-LC-MA. In addition, TCTA protein was detected in RERF-LC-MA. On the other hand, Peptide A did not change the level of TCTA mRNA in RERF-LC-MA. We previously demonstrated that toxicity is not detected in human cells cultured with peptide A [3]. Thus, our findings suggest that Peptide A is useful in the treatment of patients with small-cell lung carcinoma, although the mechanism of the inhibition remains to be elucidated.

Peptide A may be useful for patients with small lung cell carcinoma showing bone metastasis. We have demonstrated that Peptide A inhibits the formation and function of osteoclasts [3]. In addition, we demonstrated that Peptide A inhibited the proliferation of RERF-LC-MA in the current study. Thus, Peptide A is a novel strategy for late-stage patients with bone metastasis.

Breast cancer shows bone metastasis. Recently, it has been reported that breast cancer cell lines induces osteoclastogenesis in a paracrine manner [5]. In the current study, the proliferation of a breast cell line, MCF-7, was not inhibited by adding Peptide A; however, Peptide A may be useful to treat patients with bone metastasis, because it inhibits human osteoclastogenesis as we previously reported [3].

In conclusion, Peptide A may provide a novel therapeutic strategy for patients with small-cell lung carcinoma, especially with bone metastasis. In addition, Peptide A may be useful for the treatment of various cancer patients with bone metastasis.

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