

A novel peptide from TCTA protein inhibits proliferation of fibroblast-like synoviocytes of rheumatoid arthritis patients

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Abstract

Background: We have demonstrated that a peptide, which we named 'Peptide A', derived from the extracellular domain of T-cell leukemia translocation-associated gene (TCTA) protein, inhibited human osteoclastogenesis.

Objective: In the current study, we examined whether this peptide inhibits the proliferation of rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS) or not.

Material and methods: Fibroblast-like synoviocytes obtained from five RA patients were cultured in the absence or presence of 1, 5, 10 µg/ml of peptide. We used 29-mer scrambled peptide as a control.

Results: The peptide inhibited the proliferation of RA FLS dose-dependently. On the other hand, the scrambled peptide showed no inhibition.

Conclusions: The peptide inhibits both human osteoclastogenesis and the proliferation of RA FLS. Thus, the peptide may be used for the therapy of both osteoporosis and synovitis of RA patients.

This is the first report of the new peptide we discovered, which inhibits both osteoclastogenesis and synovitis. Thus, this new peptide could be a new drug for patients with both osteoporosis and RA.

Key words: osteoclast, arthritis, synovium, translocation-associated gene (TCTA), T-cell leukemia.

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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, M_r 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, which we named 'Peptide A', from TCTA protein expressed in synovial tissues of patients with rheumatoid arthritis (RA) that regulates human osteoclastogenesis [3]. In the study, we therefore purified proteins from synovial tissues of patients with RA, using gel filtration chromatography, reverse-phase 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 from TCTA protein on the proliferation of FLS. The peptide significantly inhibited the proliferation of FLS.

Material and methods

Materials

Synovial tissues were obtained from 5 patients with RA at the time of total knee replacement surgery. All patients gave their informed consent.

Cell proliferation assay

Proliferation of FLS was measured using a Cell Proliferation Assay Kit (XTT baser) (Biological Industries Ltd. Israel). The assay was performed according to the manufacturer's protocol. Cells ($0.6-1.0 \times 10^3$ /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 h, the cells were collected, and cell proliferation was measured using the kit. Experiments were repeated 5 times. All experiments were performed in quadruplicate.

Statistical analysis

Significance was analyzed using the Wilcoxon's rank sum test. The results are expressed as the means \pm SD, and considered significant at a *p*-value of < 0.05 .

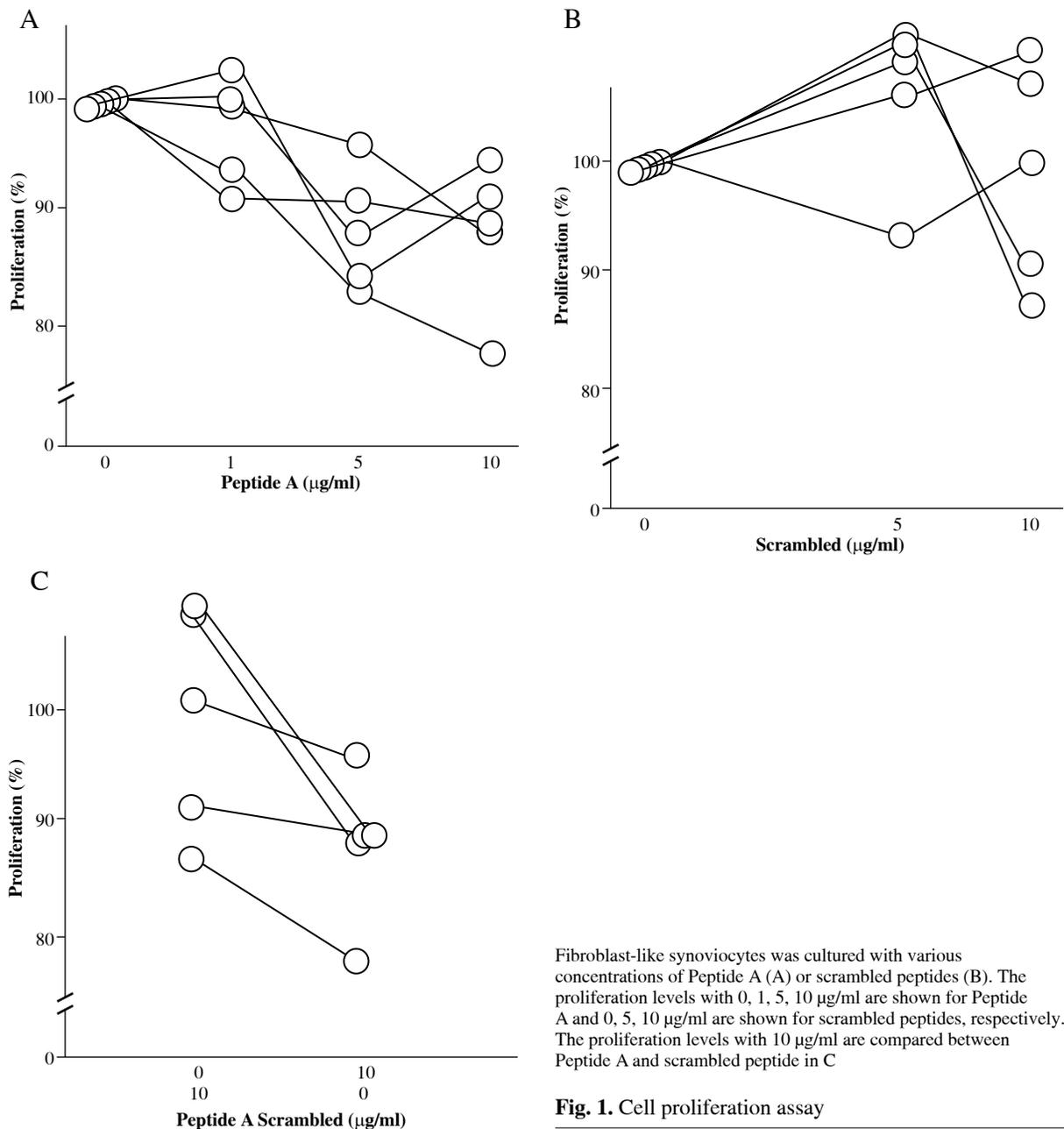


Fig. 1. Cell proliferation assay

Results

Peptide A significantly inhibited the proliferation of fibroblast-like synoviocytes

Peptide A dose-dependently inhibited the proliferation of FLS (Fig. 1A). On the other hand, the scrambled peptide used as a control did not inhibit the proliferation of FLS (Fig. 1B). At 10 µg/ml, Peptide A significantly inhibited the proliferation of FLS compared with the scrambled peptide (Wilcoxon test, $p = 0.031$, Fig. 1C).

Discussion

In the current study, we demonstrated that Peptide A dose-dependently inhibited the cell proliferation of FLS from RA patients. On the other hand, the scrambled peptide as a control did not inhibit the proliferation of FLS. 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 synovitis in RA patients, although the mechanism of the inhibition remains to be elucidated.

Peptide A may be useful for patients with osteoporosis as well, because we have demonstrated that Peptide A inhibits the formation and function of osteoclasts [3]. Osteoclastogenesis and activity of osteoclasts are triggered and/or supported by proinflammatory cytokines overproduced in RA patients. Therefore, these patients are prone to both local and systemic osteoporosis [4]. In the current study, we demonstrated that Peptide A inhibited the proliferation of FLS. Thus, Peptide A is a novel strategy for RA patients with synovitis and osteoporosis.

We have also reported that Peptide A significantly inhibited the cell proliferation of a small-cell lung carcinoma cell line, RERF-LC-MA [5]. In addition, TCTA protein was detected in RERF-LC-MA by immunohistological staining [5]. Recently, it has been reported that breast cancer cell lines induce osteoclastogenesis in a paracrine manner [6]. Thus, the peptide may provide a novel therapeutic strategy for patients with small-cell lung carcinoma, especially with bone metastasis [5].

Interestingly, synovitis of RA patients and cancer metastasis/invasiveness have some similar behavior. For example, the regulation of cell motility, proliferation and apoptosis, as well as stimulation of angiogenesis and remodeling of the extracellular matrix are important in both pathological conditions [7]. The hallmark of metastatic activity of tumor cells and the invasive behavior of synovial fibroblasts in RA appear similar. Thus, it is intriguing that Peptide A inhibits the proliferation of both FLS and RERF-LC-MA.

In conclusion, Peptide A inhibited the cell proliferation of FLS from RA. Since Peptide A inhibits human osteoclastogenesis as we previously reported [3], Peptide A may be useful for the treatment of RA with osteoporosis.

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Ethics approval: The study received ethical approval from the Institute of Rheumatology, Tokyo Women's Medical University.

The authors declare no conflict of interest.

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