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Osteoclastogenic activity of translationally-controlled tumor protein (TCTP) with reciprocal repression of p21

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ABSTRACT

Translationally-controlled tumor protein (TCTP) plays a role in a number of cellular processes, but there is limited information about its function in cell differentiation. Previous observations of a twofold induction of TCTP mRNA during osteoclast differentiation prompted us to investigate its involvement in osteoclast differentiation. The osteoclastogenicity of TCTP gradually expressed during osteoclast differentiation was confirmed in mouse and human cells using loss-of-function studies and TCTP heterogeneous mice and transgenic mice. Higher expression ratios of TCTP to p21 could represent TCTP-mediated phenotypic induction of osteoclast differentiation accompanied by p21 down-regulation, attenuating the proliferation of osteoclast precursor cells.

1. Introduction

Highly conserved translationally-controlled tumor protein (TCTP or TPT1) is ubiquitously and abundantly expressed in all eukaryotic tissues, suggesting that it has a role in a number of important cellular processes and may be a basic housekeeping protein. It is also called IgE-dependent histamine-releasing factor, anti-apoptotic fortilin, and growth-related protein p23 depending on its functional role [1–4]. Especially in view of its role in cell differentiation, proteomic analysis revealed an association between TCTP expression and differentiation of Caco-2 cells [5,6] and down-regulation of TCTP after differentiation of mouse embryonic stem cells [7]. Nonetheless, information about its functional involvement in cell differentiation is limited.

Various cell differentiation pathways can regulate homeostasis in the body, but abnormalities in these pathways can lead to disorders. For examples, menopause represents one of the natural events triggering changes in bone homeostasis. Bones are constantly renewed through osteoblastic bone formation and osteoclastic bone resorption. This process, termed 'bone remodeling', is important not only for achieving normal bone mass, but also for mineral homeostasis. However, over-activation or increased numbers of osteoclasts due to accelerated differentiation, including that caused by estrogen deficiency, can trigger a decrease in bone mineral density (BMD), thereby inducing several bone-related diseases such as osteoporosis, rheumatoid arthritis, periodontal disease, and tumor metastasis.

Macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-kB ligand (RANKL) are the key cytokines that induce osteoclast differentiation (or osteoclastogenesis) via several signaling molecules such as mitogen-activated protein

Abbreviations: TCTP, translationally-controlled tumor protein; BMD, bone mineral density; M-CSF, macrophage colony-stimulating factor; RANKL, receptor activator of nuclear factor-kB ligand; BMMs, bone marrow-derived macrophages; TRAP, tartrate-resistant acid phosphatase; MNC, multinucleated osteoclast cells; BV/TV, bone volume/tissue volume; Tb.N, number of trabeculae; Tb.Sp, trabecular separation

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(MAP) kinase and Akt. In our previous study to identify new differentially expressed genes, the microarray analysis revealed, and the real-time PCR confirmed, a RANKL-mediated twofold induction of TCTP mRNA during osteoclast differentiation (data not shown). Since the RANKL-mediated induction of TCTP has not been previously reported and its functional involvement in osteoclast differentiation has not yet been studied, the relevance of TCTP in osteoclast differentiation was investigated and is reported here.

2. Materials and methods

2.1. Preparation of mouse osteoclast precursor cells and their differentiation

Animals were cared for according to the guide for the Institutional Animal Care and Use Committee of the Korea Research Institute of Chemical Technology (Protocol ID No. 7D-M1, Approved No. 2014-7D-04-02). Mouse bone marrow-derived macrophages (BMMs) were obtained and differentiated into osteoclasts as described previously [8]. Then, mature osteoclasts were visualized by staining with tartrate-resistant acid phosphatase (TRAP), a biomarker of osteoclast differentiation. TRAP-positive multinucleated osteoclast cells (MNC; nuclear $\geqslant 3$) were counted.

2.2. TCTP knockdown experiment

For *TCTP* knockdown, the pLKO.1-sh*TCTP* plasmid was constructed by inserting the mouse *TCTP* cDNA (the following underlined basepairs) into pLKO.1 at AgeI and EcoRI restriction sites (5′-CCG GTG AAG GTA CCG AAA GCA CAG TAC TCG AGT ACT GTG CTT TCG GTA CCT TCA TTT TTG-3′), (3′-ACT TCC ATG GCT TTC GTG TCA TGA GCT CAT GAC ACG AAA GCC ATG GAA GTA AAA ACT TAA-5′) [9]. Generation of lentiviral sh*TCTP* and infection of BMMs were performed as previously described [8]. In brief, lentiviral packaging was performed by transfecting HEK293FT cells with control/sh*TCTP*, Δ 8.9 and VSVG using Lipofectamine® 2000 (Invitrogen™). Lentiviral supernatant was collected from the culture media 2 days after transfection. BMMs were incubated with viral supernatant in the presence of polybrene (10 μg/ml). After infection, BMMs were cultured overnight, detached using StemPro® Accutase® Cell Dissociation Reagent (Invitrogen™), and further cultured with M-CSF (30 ng/ml) and puromycin (2 μg/ml) for 2 days. Puromycin-resistant BMMs were used for down-regulation of sh*TCTP*.

2.3. Proliferation assay

BMMs and sh*TCTP*-infected BMMs were plated in triplicate in 96-well plates at a density of 1×10^4 cells/well. Cells were then incubated for 1–3 days in the presence of mouse M-CSF (30 ng/ml) and mouse RANKL (10 ng/ml), and cell viability was measured using CCK-8 according to the manufacturer's protocol.

2.4. Western blotting

Western blotting analysis was performed as described previously [8]. Anti-TCTP antibody was made in rabbits after injection of the recombinant full-length TCTP proteins. Antibodies against actin, p21, CDK2, and HRP-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology.

2.5. TCTP knockout/transgenic mice

TCTP knockout (*TCTP*$^{+/-}$) and transgenic mice (*TCTP-TG*) were generated as described previously. Briefly, *TCTP-TG* were prepared using the pCAGGS-TCTP cDNA that contains chicken β-actin promoter and CMV-IE as previously described [10]. Mice heterozygous for the deleted allele of *TCTP*, *TCTP*$^{+/-}$ were kindly provided by Hsin-Fang Yang-Yen (Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan) [11]; after the construction and electroporation of targeting vector into the R1 ES cells, the positive clones were transiently transfected with cytomegalovirus promoter-driven Cre expression vector to get the subclones either the floxed or deleted allele of *TCTP*. The ES cell clones so obtained were microinjected into mouse blastocysts and were backcrossed to generate *TCTP*$^{+/-}$ mice [11]. All animal studies were performed according to the guidelines of the Institutional Animal Ethics Committee of Ewha Womans University, Korea (IACUC No. 2013-01-064).

2.6. Micro-computed tomography (CT) and histological analysis

Bone histomorphometric analyses were performed in Genoss Co. (Korea) with a μCT scanner (SkyScan1173, Bruker Corporation) and the images were obtained using DataViewer (SKYSCAN). All histomorphometric parameters were described in accordance with standard criteria [12]. Femurs were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) overnight, decalcified in 12% EDTA, hydrated, paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E) and TRAP solution kit (Sigma–Aldrich).

2.7. Human bone marrow cell (hBMCs) cultures and their differentiation

According to the Ethics Guidelines for Wonkwang University Hospital, after approval by the Institutional Review Board (IRB; 201310-HRBR-014), hBMCs were isolated by density gradient centrifugation using Ficoll Histopaque® (Sigma–Aldrich) and cultured with human M-CSF (50 ng/ml) for 3 days. hBMCs were incubated for 7 days in the presence of human RANKL and human M-CSF (50 ng/ml) to differentiate them into osteoclasts.

2.8. Statistical analysis

All quantitative values are presented as the mean ± standard deviation (S.D.). Statistical differences were analyzed using Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. TCTP induction during osteoclast differentiation

During differentiation of BMMs into osteoclasts, TCTP was gradually induced by RANKL (Fig. 1A). In addition, because TCTP did not affect the osteoblast differentiation in its in vitro gain-of-function study (SI Fig. 1A–C) and in the in vivo condition of TCTP deficiency (SI Fig. 1D and E), we focused to identify the functional role of TCTP in osteoclast differentiation in the following study.

3.2. shTCTP-mediated inhibition of osteoclast differentiation

To validate the functional involvement of TCTP in the process of osteoclast differentiation, a loss-of-function study was carried out. *TCTP* knockdown strongly attenuated the RANKL-mediated induction of TCTP protein levels (Fig. 1A). The RANKL-induced formation of TRAP-positive multinucleated osteoclast cells (TRAP$^+$-MNCs) was significantly inhibited by *TCTP* knockdown (Fig. 1B and C).