

# Expression analysis of plectin isoforms in osteoclast

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## Abstract

Bone homeostasis is maintained by osteoclasts and osteoblasts. Regulation of bone homeostasis is important for orthodontic treatments, oral implant, treatment for periodontal disease, osteonecrosis of the jaw and so on. We reported an intermediate filament linking protein Plectin plays important role in osteoclastic bone resorption. Plectin is a large molecule >500 kDa and have 13 isoforms. Individual Plectin isoforms have different roles. To reveal the roles of Plectin in osteoclasts, we examined the expression levels of Plectin isoforms and found the isoforms regulating actin organization are highly expressed in osteoclasts.

## Introduction

Osteoclasts are one of the major player for bone resorption and regulate bone remodeling to maintain bone homeostasis [1,2]. Activation of osteoclastic bone resorption in comparison with bone formation by osteoblasts results in bone metabolic diseases such as osteoporosis and periodontal disease [3-5]. Moreover, the function of osteoclasts is important in orthodontic treatments, oral implant treatment and pathogenesis of osteonecrosis of the jaw [6-9]. Thus, regulation of osteoclasts is one of the serious themes for improvement of various dental treatments.

Osteoclasts are differentiated from hematopoietic stem cells with macrophage colony stimulating factor (M-CSF) and Receptor activator NF- $\kappa$ B ligand (RANKL) stimulation [1,2]. Attachment and sealing of bone matrix by differentiated osteoclasts is essential for bone resorption [10,11]. Actin, tubulin and intermediate filaments are dynamically organized and forms actin ring that is consistent with actin dots called podosomes in cell periphery for attachment and sealing [12-15]. It is well known that the mice defect actin ring formation by knocking out a tyrosine kinase Src are osteopetrosis because of decrease of bone resorbing activity [16,17]. This indicates Src is one of the key molecules to regulate bone resorption.

We identified a cytolinker protein Plectin as a partner of Src and organize actin ring formation in osteoclasts [13]. Plectin was highly expressed in brain, heart, skeletal muscle and TRAP(+) MNCs [13,14,18,19]. Plectin has 13 isoforms and there role is a little different each other [18]. This suggests Plectin variants plays different roles in each tissue. Here, we explore the important isoforms of Plectin in osteoclasts.

## Materials and methods

### Animals

4 weeks old C57BL/6N mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). Spleen and femoral muscle were obtained from these mice. Spleen cells were differentiated to osteoclast like cells with M-CSF and sRANKL described as previously [13,15]. Femoral muscle was mashed with Biomasher II purchased from Nippi (Tokyo, Japan) and obtained mRNA described below.

## Real-time quantitative PCR

Total RNA of the biceps muscle of the thigh, spleen cell derived osteoclasts as described previously [15]. rRNAs were isolated with FastGene™ RNA Basic Kit from Nippon genetics Co., Ltd. (Tokyo, Japan). cDNA was synthesized from 1  $\mu$ g of total RNA by using ReverTra Ace from TOYOBO (Tokyo, Japan). Real-time quantitative PCR was performed by incubating cDNA, PowerUp SYBR™ Green Master Mix from Thermo Fisher Scientific and primers indicated at below in a QuantStudio 3 Real-time PCR system purchased from Thermo Fisher Scientific (San Jose, CA). All reactions were performed in triplicate and analyzed. The expression level was normalized by Gapdh expression.

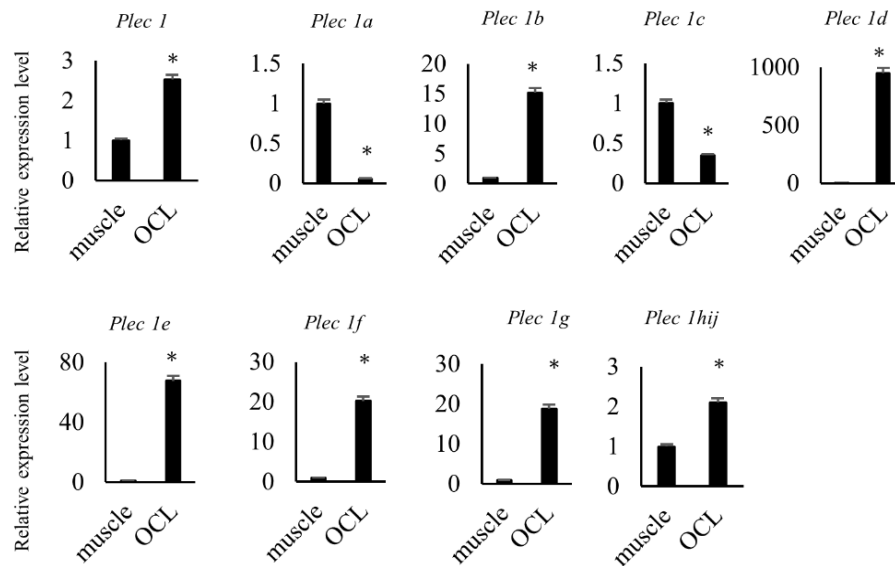
The primer sequences were: *Gapdh*, 5'-aacttggcattgtggaagg-3' (forward) and 5'-acacattggggttaggaaca-3' (reverse); *Plec1*, 5'-ggagcct-gccccagccacag-3' (forward); *Plec1a*, 5'-gggcaagaagatgaacag-3' (forward); *Plec1b*, 5'-cggcaggcaaggatgagcaag-3' (forward); *Plec1c*, 5'-ggc-cgtcatccgcattgcag-3' (forward); *Plec1d*, 5'-ccccaatgaagatcgtgcccg-3' (forward); *Plec1e*, 5'-agcagagatcagctccctcaag-3' (forward); *Plec1f*, 5'-aggaggtgctgggagaagtacaaag-3' (forward); *Plec1g*, 5'-ctctacgggcaactgtgtgtag-3' (forward); *Plec1hij*, 5'-atgaacgagaccgtgtgcagaag-3' (forward); *Plec1* common, 5'-cctgagaggactccagcag-3' (reverse); *Src*, 5'-gttgcttcg-gagaggtgtggat-3' (forward) and 5'-caccagttctcgtgcctcagt-3' (reverse). The experiments were individually performed at least twice and obtained representative data.

## Data analysis and statistics

The experiments were performed at least twice. Differences between groups were analyzed by the Student's t-test to determine statistical significance. A value of  $P < 0.01$  was considered statistically significant.

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**Figure 1.** High expression level of Plectin mRNA in osteoclast like cells

mRNA was harvested from femur muscle (described as muscle) and osteoclast like cells (described as OCL) derived from spleen cells stimulated with 30 ng/mL of M-CSF and 100 ng/mL of sRANKL for 7 days. Expression level of Plectin isoforms' mRNA was measured by quantitative RT-PCR

## Results

To explore what isoform is expressed in osteoclasts, we performed real-time quantitative PCR analysis compared with skeletal muscle where what isoform is working [20] (Figure 1). Expression level of Plectin 1, 1b, 1d, 1e, 1f and 1g in TRAP(+) MNCs was higher than in skeletal muscle (Figure 1). Plectin 1h, 1i and 1j were not distinguished each other due to primer design. On the other hand, Plectin 1a and 1c were less in TRAP (+) MNCs.

## Discussion

Plectin is a large protein which is involving various intracellular signaling such as actin organization, microtubule organization, IF organization and so on [18]. Plectin has 13 transcriptional variants in its amino terminal domain. These variants may control Plectin localization and part of binding proteins. Plectin is expressed in various tissues such as brain, heart, skeletal muscle and TRAP (+) MNCs [13]. The detected molecular weight of Plectin was different by tissues. This suggests Plectin variants plays different roles in each tissue. Plectin 1, 1b, 1d, 1e, 1f, and 1g were higher expressed in osteoclasts than in skeletal muscle in this study. Plectin 1, 1d 1e, 1f and 1g were localized in podosomes, and 1e and 1f were localized in focal adhesion of SW480 colon carcinoma cells [21]. These results suggest that the Plectin variants that needs for actin ring formation and attachment to bone matrix is highly expressed in osteoclasts. Further investigation for individual Plectin isoform analysis needs for understanding the mechanisms of osteoclastic bone resorption.

## Conclusion

Plectin 1, 1b, 1d, 1e, 1f, and 1g were higher expressed in osteoclasts and may play important role in osteoclastic bone resorption.

## Author's contributions

T.M. designed this study and performed most of the experiments. M.U. performed mRNA isolation from osteoclasts. S.K. helped with qPCR data analysis. W.A. performed literature review.

## Conflicts of interest

All authors state that they have no conflicts of interest.

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