Opa1-mediated mitochondrial dynamics is important for osteoclast differentiation

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Abstract

Opatic atrophy 1 (Opa1) is a mitochondrial GTPase that regulates mitochondrial fusion and maintenance of cristae architecture. Osteoclasts are mitochondrial rich-cells. However, the role of Opa1 in osteoclasts remains unclear. Here, we demonstrate that Opa1-deficient osteoclast precursor cells do not undergo efficient osteoclast differentiation and exhibit abnormal cristae morphology. Thus, Opa1 is a key factor in osteoclast differentiation through regulation of mitochondrial dynamics.

Figure 1. Expression of opatic atrophy 1 (Opa1) and its knockout effect on osteoclastogenesis.
We generated and genotyped $Opa1^{floxflo}x$ and $RANK^{Cre^+}$ mice as previously described (Maeda et al., 2012; Zhang et al., 2011). $Opa1^{+/+}$, $Opa1^{floxflo}$ and $Opa1^{floxflo}$ littermate mice that did not carry the Cre recombinase were used as controls.

**Methods**

**Mice and bone analysis**

We generated and genotyped $Opa1^{floxflo}$ and $RANK^{Cre^+}$ mice as previously described (Maeda et al., 2012; Zhang et al., 2011). $Opa1^{+/+}$, $Opa1^{floxflo}$ and $Opa1^{floxflo}$ littermate mice that did not carry the Cre recombinase were used as controls.
Following their birth, all mice were maintained under specific pathogen-free conditions. All animal experiments were approved by the Institutional Animal Care and Use Committee of both Doshisha University and Osaka University. All the strains featured a C57BL/6 background. Two-week-old sex-matched mice were used in the experiments. Animals were randomly included in the experiments based on the genotyping results.

**Cell culture**

*In vitro* osteoclast differentiation was performed as previously described (Iwamoto *et al.*, 2016; Nishikawa *et al.*, 2013; Nishikawa *et al.*, 2015). Briefly, bone marrow-derived cells cultured with 10 ng/ml M-CSF (Miltenyi Biotec) for 2 days were used as osteoclast precursor cells and BMMs, and were further cultured with 50 ng/ml RANKL (PeproTech) in the presence of 10 ng/ml M-CSF for 3 days. TRAP-positive MNCs (TRAP+ MNCs) having more than three nuclei were counted.

**Transmission electron microscopy**

BMMs cultured on Cell Desk polystyrene cover slip (Sumitomo Bakelte Co., Ltd., Japan) were fixed for 24 hrs at 4°C in 2% formaldehyde and 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH7.4) containing 0.01% calcium chloride. Each sample was washed for 5 min in 0.1M cacodylate buffer (pH7.4) containing 7% sucrose for three times. Cells were post-fixed for 1h with 1% osmium tetroxide and 0.5% potassium ferrocyanide in 0.1M cacodylate buffer (pH7.4), dehydrated in a graded ethanol series, and embedded in Epon812 (TAAB Co. Ltd., UK). Ultrathin sections (80 nm) were stained with saturated uranyl acetate and lead citrate solutions. Electron micrographs were obtained using a JEM-1400 plus electron microscope (JEOL, JP) at 80 kV.

**Immunoblot analysis**

Immunoblot analysis was performed as described previously (Nishikawa *et al.*, 2010b). Briefly, the cell lysates were subjected to immunoblot analysis using antibodies specific for Opa1 (Abcam, ab157457), NFATc1 (Santa Cruz Biotechnology, sc-7294), TRAP (Santa Cruz Biotechnology, sc-30833), Ctsk (Daichi Finechemical, F-95) and Lmnb (Santa Cruz Biotechnology, sc-6217). Whole-cell extracts were prepared by lysis in a radioimmunoprecipitation assay buffer.

**Single-cell RNA-sequencing analysis**

Gene expression data of scRNA-seq (GSE147174) obtained from NCBI’s Gene Expression Omnibus ([https://www.ncbi.nlm.nih.gov/geo/](https://www.ncbi.nlm.nih.gov/geo/)) were processed and analyzed using the Seurat R package (v.4.0.6) as described previously (Tsukasaki *et al.*, 2020). Briefly, cells expressing less than 200 genes and more than 5% of mitochondrial genes were defined as poor-quality data and excluded. After normalization and scaling, the top 2,000 variable genes were selected by directly modelling the mean-variance relationship inherent in single-cell data. We performed dimensionality reduction using principal-component analysis (PCA) and visualized single cells on a uniform manifold approximation and projection (UMAP) plot according to gene expression.

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