Notch 2 signaling promotes osteoclast resorption via activation of PYK2

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Abstract

Notch signaling plays a central role in various cell fate decisions, including skeletal development. Recently, Notch signaling was implicated in osteoclast differentiation and maturation, including the resorption activity of osteoclasts. However, the specific involvement of notch signaling in resorption activity was not fully investigated. Here, we investigated the roles of Notch signaling in the resorption activity of osteoclasts by use of the γ-secretase inhibitor dibenzazepine (DBZ). Attenuating Notch signaling by DBZ suppressed the expression of NFATc1, a master transcription factor for osteoclast differentiation. However, overexpression of a constitutively active form of NFATc1 did not fully rescue the effects of DBZ. DBZ suppressed the autophosphorylation of PYK2, which is essential for the formation of the podosome belt and sealing zone, with reduced c-Src/PYK2 interaction. We further observed increased PYK2 activation by RANKL accompanied by increased NICD2 production. These results confirmed that overexpression of NICD2 rescued DBZ-mediated suppression of resorption activity with promotion of PYK2 autophosphorylation, PYK2/c-Src interaction, and microtubule acetylation. Consistent with the *in vitro* results, DBZ strongly suppressed bone destruction in an interleukin-1-induced bone loss model. Collectively, these results demonstrate that Notch 2 in osteoclasts plays a role in the control of resorption activity via the PYK2-c-Src-microtubule signaling pathway.

Result

Figure 1

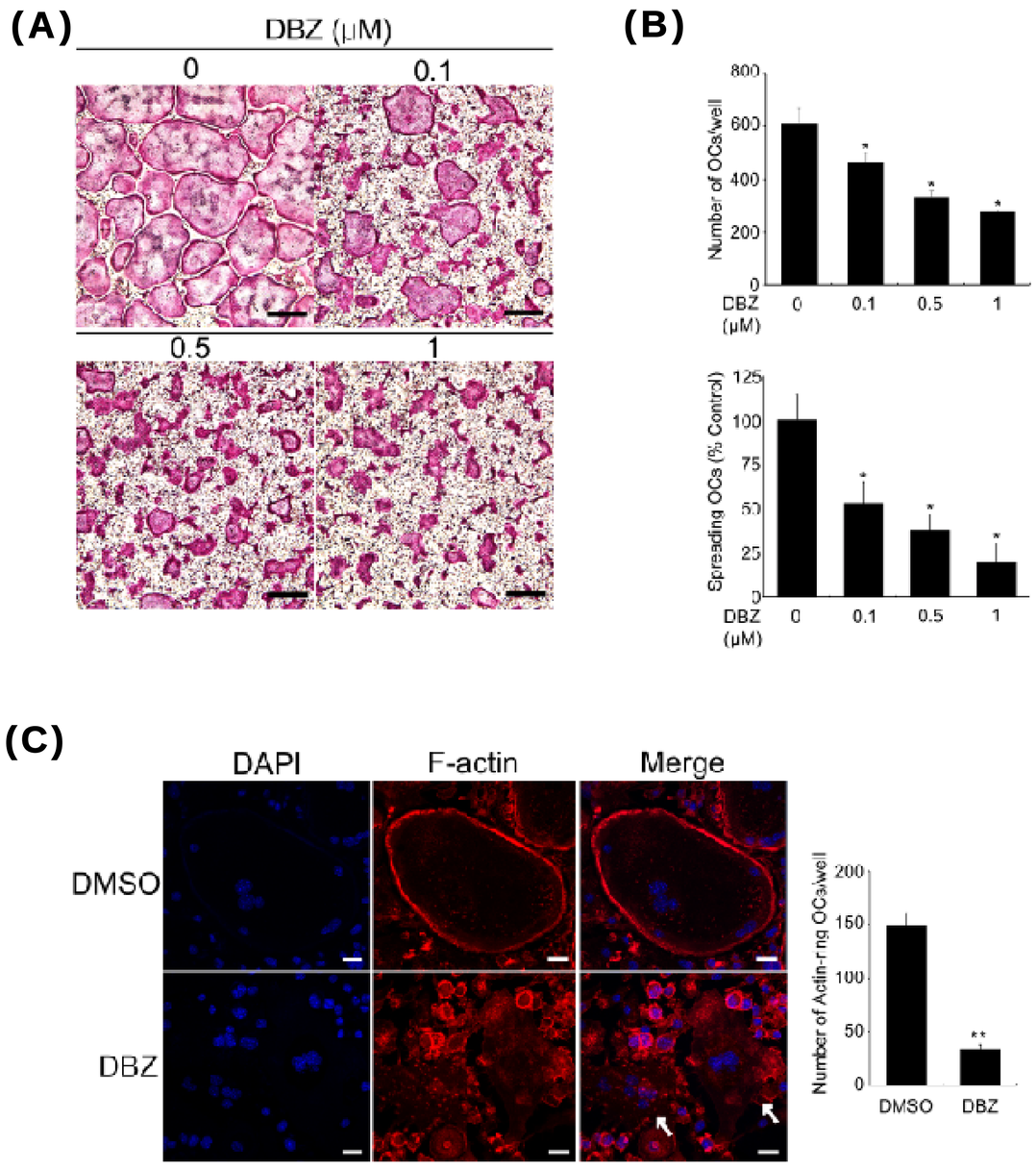

Fig. 1. DBZ suppresses osteoclast differentiation and spreading. (A) BMMs were cultured with DMSO or the indicated dose of DBZ in the presence of M-CSF (60 ng/ml) and RANKL (100 ng/ml) for 4 days. After culturing, the cells were stained for TRAP. Scale bar is 100 μm. (B) DMSO- or DBZ-treated osteoclasts that contained three or more nuclei were counted, and the number of spreading osteoclasts was measured ($^{*}p < 0.05$). (C) BMMs were cultured on cover glasses with DMSO or DBZ (1 μM) for 6 days in the presence of M-CSF (60 ng/ml) and RANKL (100 ng/ml). Distributions of F-actin (red) and nuclei (blue) are shown by using confocal microscopy. Scale bar is 20 μm. Arrows show defects in F-actin assembly of multi-nucleated osteoclasts. Cells with normal actin rings were counted (right, $^{**}p < 0.01$).

Figure 2

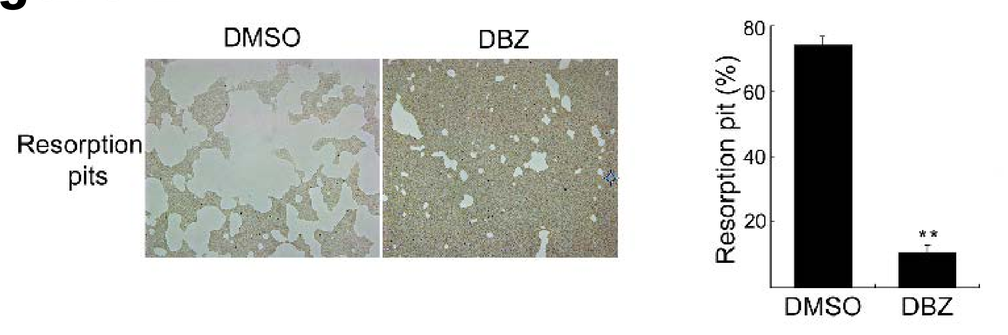

Fig. 2. DBZ inhibits resorption activity of mature osteoclasts. Mature osteoclasts were cultured on OAAS plates with DMSO or DBZ in the presence of M-CSF (60 ng/ml) and RANKL (100 ng/ml) for 24 h. The cells were removed, and pit areas were measured ($^{**}p < 0.01$).

Figure 3

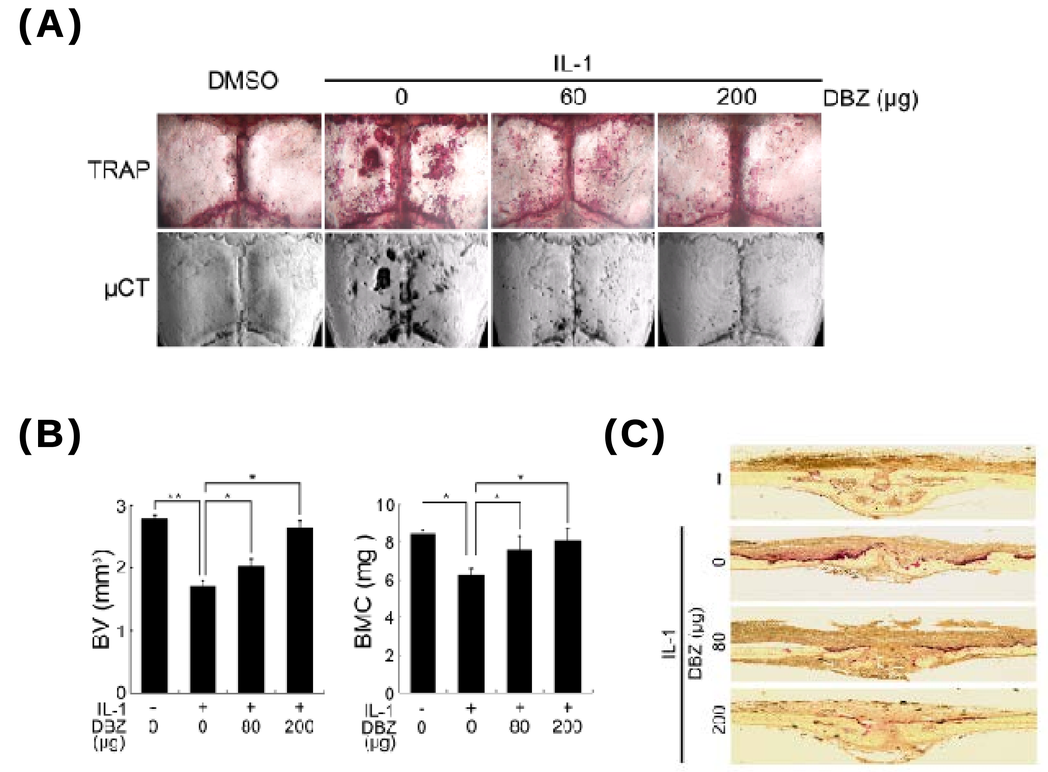

Fig. 3. DBZ inhibits IL-1-induced bone loss. Collagen sponges soaked with PBS or IL-1 (2 μg) were implanted over mouse calvarial bones, and DMSO or DBZ (60 or 200 μg/kg) was injected intraperitoneally (n = 5). (A) TRAP staining (top) and μCT images (bottom) of whole calvariae are shown. (B) Bone volume and bone mineral content of the calvariae were measured by μCT analysis ($^{*}p < 0.05$, $^{**}p < 0.01$). (C) Histological sections of calvarial bones were stained for TRAP.

Figure 4

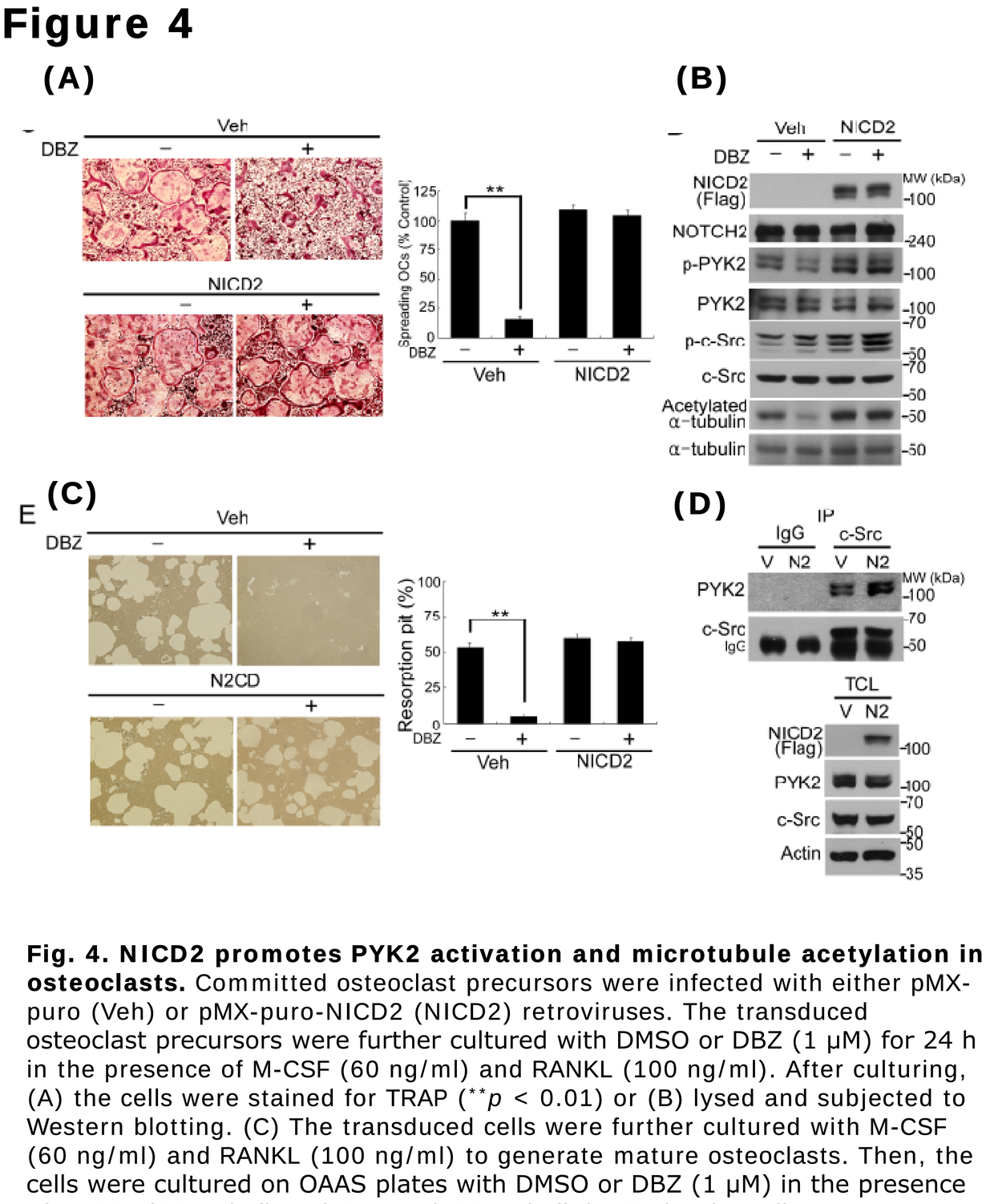

Fig. 4. NICD2 promotes PYK2 activation and microtubule acetylation in osteoclasts. Committed osteoclast precursors were infected with either pMX-puro (Veh) or pMX-puro-NICD2 (NICD2) retroviruses. The transduced osteoclast precursors were further cultured with DMSO or DBZ (1 μM) for 24 h in the presence of M-CSF (60 ng/ml) and RANKL (100 ng/ml). After culturing, (A) the cells were stained for TRAP ($^{**}p < 0.01$) or (B) lysed and subjected to Western blotting. (C) The transduced cells were further cultured with M-CSF (60 ng/ml) and RANKL (100 ng/ml) to generate mature osteoclasts. Then, the cells were cultured on OAAS plates with DMSO or DBZ (1 μM) in the presence of M-CSF (60 ng/ml) and RANKL (100 ng/ml) for 24 h. The cells were removed, and pit areas were measured ($^{**}p < 0.01$) (D) The transduced cells (V: vehicle or N2: NICD2) were lysed and immunoprecipitated with anti-c-Src antibody or irrelevant IgG. Immunoprecipitates (IP) and total cell lysates (TCL) were subjected to Western blotting. MW, molecular weight.

Conclusion

This study provides evidence that Notch2 plays a key role in osteoclast function. Our *in vivo* data suggest that DBZ may be clinically useful for preventing bone destruction with excessive osteoclast activation. However, given the fact that DBZ was developed as a gamma-secretase inhibitor, careful evaluation is needed before its clinical application. We propose that RANKL-induced activation of the Notch2/PYK2/c-Src/microtubule axis is necessary for osteoclast resorption function.