Delta-like 1/fetal antigen 1 (DLK1/FA1) inhibits BMP2 induced osteoblast differentiation through modulation of NFκB signaling pathway: a novel mechanism for effects on skeletal homeostasis

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Osteoblast differentiation of C2C12 cells were analyzed by real-time RT-PCR analysis of osteogenic markers and ALP quantitation.

BMP and NFκB signaling pathways were analyzed by luciferase reporter assay, western blot and qRT-PCR analysis of target genes.

In the previous studies, DLK1 has been shown to inhibit both adipogenesis and osteoblastogenesis but favors bone resorption. Increased level of DLK1 was observed in the postmenopausal women suggests a possible mechanism mediating the effects of estrogen deficiency on bone turnover. Recently, DLK1 is identified as a novel negative regulator of every metabolism through controlling osteocalcin bioavailability.

Although DLK1 plays important role in bone turnover, its interaction with signaling pathways which are crucial for bone homeostasis are poorly understood. C2C12 cells are mouse myoblast cells which could be differentiated into osteoblast suggesting that non-canonical signaling pathway: a novel mechanism for effects on skeletal homeostasis.

Taken together, we revealed a novel mechanism by which DLK1 regulates in vitro osteoblast differentiation. DLK1 affects both the expression of major BMP components and NFκB activity to inhibit BMP signaling. Our results provide new insight into molecular control of DLK1induced osteoblast differentiation and possibly on bone formation.

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Fig. 3. DLK1 inhibits BMP signaling by activating NFκB pathway. (A) C2C12 cells were infected by NFκB luciferase reporter lentivirus (MOI=10) and then treated with normal culture medium as control (Ctrl), control conditioned medium (Ctrl-CM) or DLK1 conditioned medium (DLK1CM) for 4 hours. The NFκB activity was measured by luciferase assay and normalized against protein concentration. (n=8). **P<0.005 comparing to Ctrl. (B) C2C12 cells were treated with Ctrl-CM or DLK1CM for 20 hours. The expression of NFκB target genes was analyzed by qRT-PCR and normalized against B2m. (n=3) *P<0.05, **P<0.005 comparing to Ctrl-CM(C) C2C12 cells were infected by NFκB reporter lentivirus (MOI=10) and then treated with normal culture medium (Ctrl) or 1μg/ml LPS for 4 hours. The NFκB activity was measured by luciferase assay and normalized against protein concentration. (n=8) **P<0.005. (D) C2C12 cells were treated with normal culture medium or 1μg/ml LPS for 2 hours. The expression of Rel was analyzed by qRT-PCR and normalized against B2m. (n=3) **P<0.005. (E) BMP luciferase reporter cells (C2C12BRA) was treated with normal culture medium (Ctrl), 100ng/ml BMP2, 1μg/ml LPS, or 100ng/ml BMP2 with 0.1-2μg/ml LPS for 6 hours. The BMP activity was measured by luciferase assay and normalized against protein concentration. (n=8) **P<0.005 comparing to BMP2.

Fig. 1. DLK1 inhibits BMP2 induced osteoblast differentiation in C2C12 cells. (A) C2C12 cells were treated with control conditioned medium (Ctrl-CM) or human DLK1 conditioned medium (DLK1CM) in the presence of 100ng/ml BMP2 for 5 days. ALP activity was quantified and normalized against cell viability (n=6). (A) and the expression of osteogenic markers was analyzed by qRT-PCR and normalized against B2m (n=3). (B) C2C12 or C2C12 overexpressing mouse Dlk1 (C2C12-Dlk1) cells were treated with 0-300ng/ml BMP2 for 6 days. The ALP activity and the expression of osteogenic markers were analyzed as above. *P<0.05, **P<0.005 comparing to Ctrl-CM (A,B) or C2C12 (C,D).

Fig. 2. DLK1 inhibits BMP signaling. (A) BMP luciferase reporter cells (C2C12BRA) were treated with 0-300ng/ml BMP2 (Left) or by 100ng/ml BMP2 with other control conditioned medium (Ctrl-CM) or human DLK1 conditioned medium (DLK1CM) for 6 hours (Right). The BMP activity was measured by luciferase assay and normalized against protein concentration (Left) (n=8). (B) C2C12 cells were treated with Ctrl-CM or DLK1CM in the presence of 100ng/ml BMP2 for 0 to 20 min and the expression of phospho- and total Smad3, p38, Erk were analyzed by western blot. (C) C2C12 cells were treated with Ctrl-CM or DLK1CM in the presence of 100ng/ml BMP2 for 5 days and the expression of BMP target genes Jund and Id1 (C) as well as BMP receptors, Smad5 and some other BMP components (D) were quantified by qRT-PCR and normalized against B2m (n=3). *P<0.05, **P<0.005 comparing to Ctrl-CM.