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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Summary

DLK1/FA1 (delta-like 1/fetal antigen-1) is a negative regulator of bone mass that acts to inhibit osteoblast differentiation and stimulate osteoclast differentiation. However, the molecular mechanisms underlying these effects are not known. Thus, we studied the effect of DLK1/FA1 on different osteogenic factors-induced osteoblast differentiation. We identified DLK1/FA1 as an inhibitor of BMP2-induced osteogenesis in mouse myoblast C2C12 cells.

Methods

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.

Results

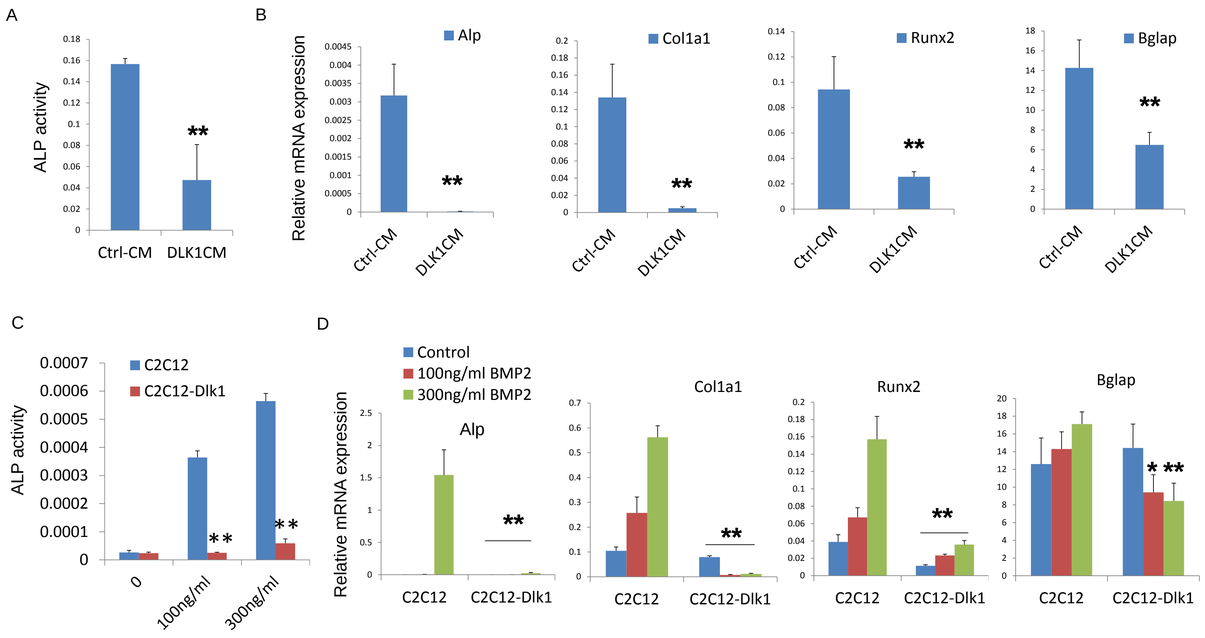

Fig. 1. DLK1 inhibits BMP2 induced osteoblast differentiation in C2C12 cells. (A,B) C2C12 cells were treated with control conditioned medium (Ctrl-CM) or human DLK1 conditioned medium (DLK1CM) in the presence of 100ng/ml BMP2 for 5 days. ALP activity was quantitated and normalized against cell viability (n=8) (A) and the expression of osteogenenic markers was analyzed by qRT-PCR and normalized against B2m (n=3) (B). (C,D) 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).

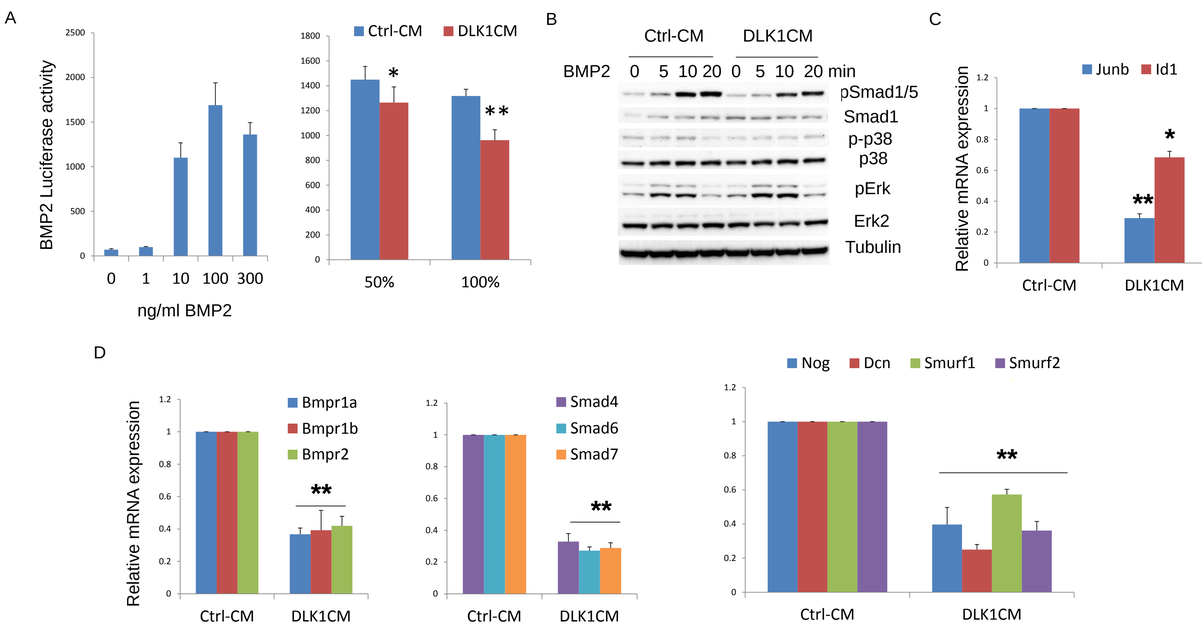

Fig. 2. DLK1 inhibits BMP signaling. (A) BMP luciferease reporter cells (C2C12BRA) were treated with 0-300ng/ml BMP2 (Left) or by 100ng/ml BMP2 with either control conditioned medium (Ctrl-CM) or human DLK1 conditioned medium (DLK1CM) for 6 hours (Right). The BMP activity was measured by luciferase assay 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 phosphor- and total Smad, p38, Erk were analyzed by western blot. (C, D) 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 Junb and Id1 (C) as well as BMP receptors, Smad and some other BMP components (D) were quantitated by qRT-PCR and normalized against B2m (n=3). *P<0.05, **P<0.005 comparing to Ctrl-CM.

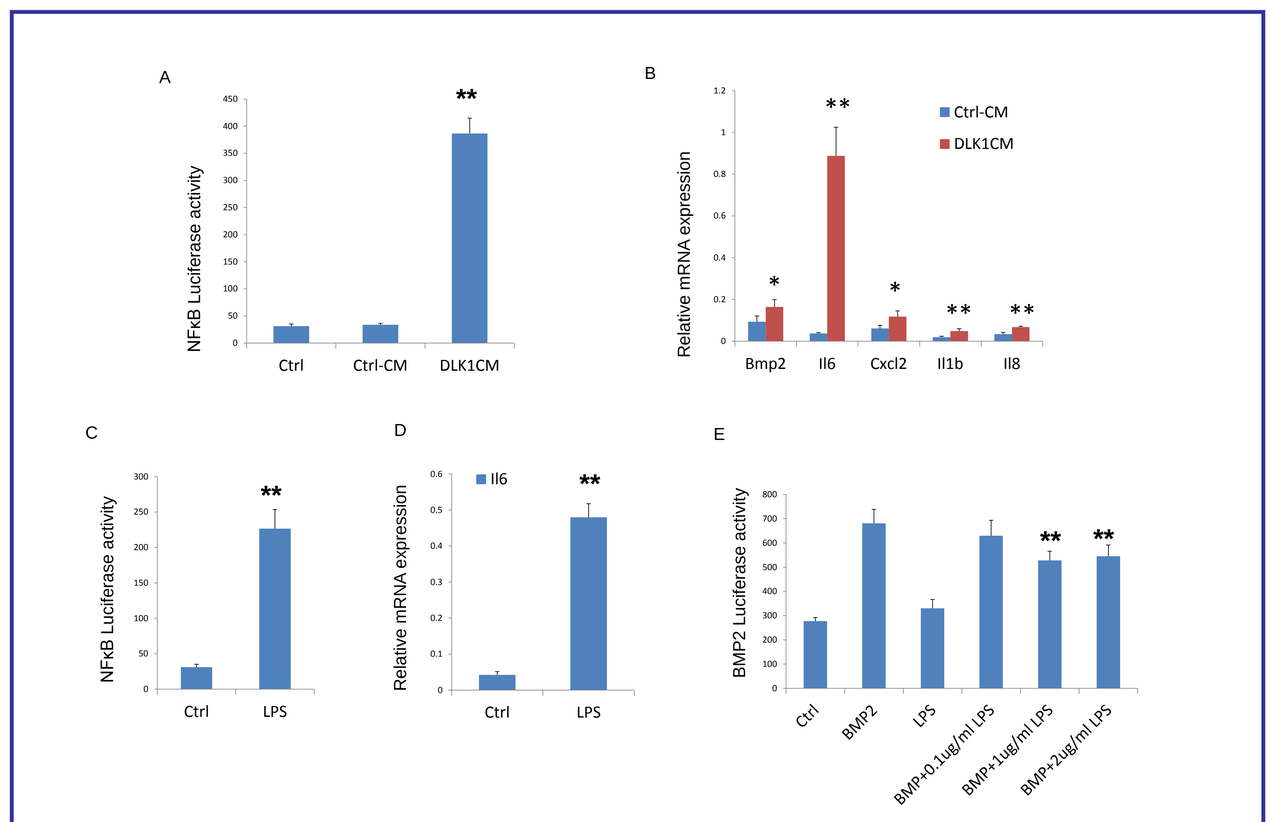

Fig. 3. DLk1 inhibits BMP signaling by activating NFκB pathway. (A) C2C12 cells were infected by NFκB luciferase reporter lentivirus (MOI=10) and then treated with normal culture medium as control (Ctrl), control conditioned medium (Ctrl-CM) or DLK1 conditioned medium (DLK1CM) for 4 hours. The NFκB activity 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 were 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 luciferase 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 20 hours. The expression of Il6 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.

Discussion

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 enery 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 lineage by BMP2. Thus, C2C12 cells are a good in vitro cell model for studying the interaction between DLK1 and BMP signaling during osteogenesis. Two possible mechanisms have been proposed. First, DLK1 affectes directly the expression of BMP receptors, Smads as well as some BMP inhibitors. Second, DLK1 inhibits BMP signaling indirectly through NFκB pathway. However, blocking canonical NFκB signaling could not rescue the osteoblast differentiation of C2C12 suggesting that non-canonical NFκB pathway or other signaling pathways may be involved in regulating DLK1 and BMP interaction. The inhibitory effect of DLK1 on BMP signaling was also observed in mouse calvaria cell suggesting such interaction may be a common mechanism regulating osteoblast differentiation.

Conclusions

Taken together, we revealed a novel mechanism by which DLK1 regulates in vitro osteoblast differetiation. 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 DLK1 on osteoblast differentiation and possibly on bone formation.

Acknowledgement

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