Constitutively Expressed COX-2 Contributes to the Earlier Stage of Bone Growth in Cultured Calvaria

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INTRODUCTION
Cyclooxygenase-2 (COX-2) was thought to be an inducible enzyme when growth factor stimulation or inflammation, but increasing reports indicated COX-2 expresses constitutively in several organs1. Whether COX-2 constitutive expression and has its physiological role in bone remains undefined. Our previous results showed that a COX-2 selective inhibitor, celecoxib, significantly suppressed proliferation of human bone marrow mesenchymal stem cells (hBMSCs) and osteoblasts (hOBs)2–3, suggesting COX-2 may be highly related to the regulations of osteogenic cell functions. Furthermore, COX-2-deficient mice have decreased bone density and new bone formation compared with normal littermates4, while COX-1 deficiency slightly enhances bone formation5. Accordingly, we hypothesized that COX-2 may constitutively express in osteoblasts and contribute to regulating osteogenic cell functions and bone formation. In this study, the mechanism of COX-2 on bone formation was investigated.

METHODS
We examined the localization of COX-2 in normal mouse femurs by using immunohistochemistry (IHC). We further silenced COX-2 or COX-1 mRNA in mouse calvaria to test its influence on COX-1 and COX-2 protein levels, new bone formation, and osteogenic cell proliferation by using IHC, H&E stain and BrdU assay, respectively.

RESULTS
Our results showed that COX-2 was constitutively expressed in osteoblasts sitting adjacent to trabeculae, periostium and endostium (Fig. 1). In mouse calvaria, transfection of COX-1 siRNA at day 0 inhibited COX-1 protein level (Fig. 2A), but had no significant effect on new bone formation of mouse calvaria (Fig. 2B). Alternatively, transfection of COX-2 siRNA at day 0 suppressed new bone formation of mouse calvaria at day 3 and 7 (Fig. 3A&B), and inhibited proliferation of osteogenic cells in mouse calvaria at day 3 (Fig. 3C). However, transfection of COX-2 siRNA in the 3-day-cultured calvaria showed no suppressive effect on new bone formation (Fig. 3D).

DISCUSSION
Our results showed that COX-2 was constitutively expressed in osteoblasts sitting adjacent to trabeculae, periostium and endostium in mouse femurs. The ex-vivo study of cultured mouse calvaria demonstrated that silencing of COX-2 suppressed proliferation of osteogenic cells. Our previous study also revealed that COX-2, but not COX-1, suppressed hOB proliferation contributed by the PTEN/Akt signaling. We suggested that COX-2 may highly contribute to the regulation of proliferation of osteogenic cells. Moreover, our results showed that COX-2, but not COX-1, deficiency inhibited new bone formation of cultured mouse calvaria. However, COX-2 silencing at later stage of bone formation did not influence new bone formation of cultured mouse calvaria. Accordingly, we suggest that COX-2 may associate with the regulation of osteogenic cell proliferation and early stage of new bone formation. In this study, our finding provides new insights for bone physiology that COX-2 is constitutively expressed in osteoblasts in dynamic bone growth area, which contributes to the regulation of osteoblast proliferation associated new bone formation.

REFERENCES