Targeted Disruption of BMP Signaling Through Type IA Receptor (BMPRIA) in Osteocyte Leads to Dramatic Increase in Bone Density and Mechanical Strength

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Introduction: Bone morphogenetic proteins (BMPs) are known as ectopic bone inducers. The FDA approved BMPs (BMP2 and BMP7) for clinical use. However, our recent studies demonstrated challenging evidence that BMP signaling in osteoblasts can negatively regulate endogenous bone mass because loss of BMP receptor IA (BMPR1A) in osteoblasts dramatically increased bone mass in mice (ref 1-3). Although more than 90% of bone cells are osteocytes, roles of BMP signaling in osteocytes are largely unknown. The purpose of this study was to investigate BMP function in osteocytes and prove our concept.

Methods: We generated conditional knockout mice (cKO) with osteocyte-specific deletion of BMPR1A under DMP1 promoter (Dmp1Cre+:Bmpr1a fx/fx) and compared them with controls (Dmp1Cre-:Bmpr1a fx/fx). Bone phenotype was characterized by X-ray, micro CT, histology (i.e. H&E, TRAP staining), bone histomorphometry (static and dynamic), and serum biochemistry (i.e. Ca, P, RANKL, SOST). Bone strength was analyzed by biomechanical test (i.e. 3-bending test) using femurs. In addition, osteocyte phenotype was further investigated by osteocyte-morphometry and electron microscopy (i.e. acid-etched resin cast SEM, backscattered SEM).

Results: The cKO mice grew normally, however, cKO bones (i.e. femur, tibia, spine, tail, rib) were dramatically sclerotic at 3M as assessed by X-ray. Micro CT revealed significantly increased bone parameters in cKO femur and spine (i.e. more than 100% increase in trabecular bone volume, trabecular thickness, trabecular number, and bone mineral density). H&E staining demonstrated increased trabecular bone in metaphysis, epiphysis, and diaphysis and thickened cortical bone in cKO femur. Osteoclast number assessed by TRAP staining was reduced in cKO bones. These findings were consistent with results from bone histomorphometry (i.e. significantly increased bone volume and reduced osteoclast number in cKO bones). In addition, osteoid volume was increased and bone formation rate was reduced in cKO bones. Interestingly, serum protein levels of SOST and RANKL were significantly reduced in cKO mice. Mechanical strength was better in cKO mice (i.e. max force and stiffness were increased in cKO femurs). For osteocyte-morphometry, both total and empty lacuna numbers were significantly increased in cKO bones; however, empty lacuna ratio was higher in cKO with disorganized osteocyte shape assessed by SEM.

Discussion: These results suggest that loss of BMP signaling specifically in osteocytes dramatically increases bone mass presumably through simultaneous inhibition of RANKL and SOST (i.e. osteoclast inhibition and Wnt activation together). It is reported that RANKL and SOST are more expressed by osteocytes than osteoblasts. Thus, BMP signaling through BMPR1A can play more important roles in osteocytes than osteoblasts by controlling RANKL and SOST. This study proves the concept that BMP signaling can negatively regulate endogenous bone mass in vivo.
**Significance:** 1. Many complications have been reported in BMP therapy in these days. It is true that exogenous BMPs can induce ectopic bones; however, endogenous roles of BMP signal could be opposite. This interpretation may help understand such complication and could lead to a better direction of BMP therapy.

2. Both RANKL and SOST have latest drug molecular targets of osteoporosis therapy. This study provides another line of evidence that BMPR1A can be a new drug target which controls RANKL and SOST together.

![Image showing bone mineral density](image-url)