Paget’s Disease Of Bone-like Disorder Is Caused By Chmp5 Deletion And Reversed By The Treatment Of OPG-Fc

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Introduction: Paget’s disease of bone (PDB) is a prevalent metabolic disorder characterized by exuberant bone turnover with increased osteoclastic bone resorption followed by an increase in osteoblastic bone formation. Despite major advances in understanding the pathophysiology of PDB, the mechanistic contributions of genetic components to PDB development remain unclear. Previously, we have co-purified CHMP5, a component of the ESCRT machinery, with the cytosolic NF-κB/IκBα complex from rabbit lung tissue and demonstrate that CHMP5 down-regulates NF-κB signaling. Given the importance of NF-κB signaling in osteoclast biology, we investigated the role of CHMP5 in osteoclast differentiation.

Methods: We generated mice lacking Chmp5 in osteoclasts using Cathepsin K (Ctsk)-Cre mice (referred as Chmp5Ctsk or CKO) to investigate in vivo function of CHMP5 in osteoclasts. To characterize skeletal phenotypes in Chmp5Ctsk mice, we performed skeletal preparation, μCT, bone histomorphometry, histological analysis and measurements of serum CTX, P1NP and ALP levels. Additionally, we conducted two experiments to reverse the skeletal phenotypes observed in Chmp5Ctsk mice. As a genetic approach, we generated Chmp5Ctsk/Rank-het mice by intercrossing Chmp5^floxfloxfloxflox;Rank^floxfloxflox;Cathepsin K-Cre mice with Chmp5^floxfloxfloxflox;Rank^floxfloxflox breeders and examined whether haploinsufficiency of Rank can reverse the skeletal phenotypes in Chmp5Ctsk mice. We also used a pharmacologic approach with antiresorptive agents including bisphosphonates (alendronate, zolendronate) and OPG-Fc, and examined whether antiresorptive treatment can reverse the skeletal phenotypes in Chmp5Ctsk mice.

Results: Loss of Chmp5 gene in osteoclasts leads to PDB-like skeletal disorder in mice. In Chmp5Ctsk mice, ectopic bone growth have observed at postnatal day 15. 12-week-old Chmp5Ctsk mice develop the progressive deposition of osteoclast and osteoblast-rich periosteal bone with a typical “Pagetoid” appearance in both the axial and appendicular skeleton, as observed by focal lytic and sclerotic lesions and gradual bone expansions in the skull, long bones, vertebrae and pelvis. μCT and bone histomorphometry showed that Chmp5Ctsk mice had a low bone mass phenotype in both their long bones and vertebrae due to an increase in osteoclast numbers and activity. Histological analysis of bone lesions revealed increased bone turnover with disorganized architecture of trabecular and cortical bones, increased bone resorption surface, increased bone formation as detected by calcein labeling, woven bone as detected by von Kossa staining, increased numbers of osteoclasts and osteoblasts as detected by TRAP staining and in situ hybridization of Collagen 1 (Col1), Osteopontin (Opn), and Osteocalcin (Ocn). Additionally, immunohistochemistry with PECAM and VEGF showed that blood vessel formation was significantly increased in bone lesions alongside a high expression of VEGF, which recapitulates a phenotype observed in PDB patients.
Next, we examined the genetic interaction between *Chmp5* and *Rank* by comparing WT, *Rank*-Het, CKO, and CKO;*Rank*-Het mice. CKO;*Rank*-Het mice displayed a partial reversal of the PDB-like bone phenotypes including bone lesions and expansion, low bone mass, elevated osteoclast numbers, and high serum levels of CTX, P1NP, and ALP. Additionally, we conducted antiresorptive treatments to inhibit RANK-induced signaling pathway and/or osteoclast differentiation. As expected, trabecular bone mass was slightly increased by treatments of alendronate and zolendronate and significantly increased by OPG-Fc treatment. In this regard, *Chmp5*<sup>Ctsk</sup> mice treated with alendronate and zolendronate displayed only a mild reduction in bone expansion of femur whereas OPG-Fc treatment almost completely reversed the bone expansion phenotype. In addition, serum CTX levels were decreased by treatments of alendronate and zolendronate and OPG-Fc treatment further reduced the CTX levels. Serum P1NP and ALP levels were dramatically decreased by both OPG-Fc and bisphosphonates. Taken together, treatments of the antiresorptive agents at least partially reversed the PDB-like bone phenotypes in *Chmp5*<sup>Ctsk</sup> mice. And our results demonstrate that OPG-Fc has a higher efficacy than bisphosphonates to reverse the entire spectrum of PBD-like bone phenotypes in *Chmp5*<sup>Ctsk</sup> mice.

**Discussion:** Understanding the pathogenesis of PDB has a broad significance for normal bone remodeling and osteoclast regulation of new bone formation. The dramatic increase in osteoblast activity secondary to alterations in osteoclast activity imply that potent endogenous stimulators of bone formation are produced by osteoclasts during the course of disease. Harnessing this endogenous mechanism to promote bone formation would be an attractive approach to identify potential new anabolic agents for resorptive bone disease. Here, our studies provide *Chmp5*<sup>Ctsk</sup> mice as an excellent model to understand the pathogenesis of PDB.

Given that PDB results from an increase in metabolic activity of osteoclasts, antiresorptive therapy with bisphosphonates has been used as the first-choice treatment for PDB so far. As an additional antiresorptive agent in clinical use, the RANKL inhibitor Denosumab can be considered as a good candidate for PDB treatment although this indication has been rarely reported. Our data provides a proof of principle that Denosumab can also be used as therapeutics for PDB treatment.

**Significance:** Our study provides a new mouse model of PDB, recapitulating the entire spectrum of clinical features. Despite substantial advances in understanding the pathophysiology of PDB over the past few years, no firm evidence as yet exists to show that antiresorptive therapy using anti-RANKL antibody (Denosumab) can prevent the progress of PDB. In this regard, Our *Chmp5*<sup>Ctsk</sup> mice will be an ideal in vivo tool to address these questions.
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