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Use of the transgenic approach to determine the role of DMP1 in phosphate regulation

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Dentin Matrix Protein 1 (DMP1) is a non-collagenous matrix protein expressed in all mineralized tissues with high expression in osteocytes, and low levels in soft tissues such as brain and kidney. Our previous studies showed that DMP1-KO mice appear normal at birth, but postnatally they develop profound abnormalities in mineralization of the skeleton resulting in an osteomalacia-like phenotype as well as abnormalities in the osteocyte/lacuno-canalicular system, which become worse with age[1]. Importantly, observations using this animal model prompted investigation and the discovery of DMP1 mutations in patients with Autosomal Recessive Hypophosphatemic Rickets (ARHR)[2]. From these studies we proposed that DMP-1 has both local and systemic functions with regards to controlling mineralization and bone remodeling. Multiple mouse models were used to determine the function of DMP1 including rescue of DMP1-null mice with a high phosphate or high calcium diet, and targeted re-expression of DMP1 in mice without endogenous DMP1 using an osteoblast specific promoter. Comprehensive approaches such as micro-CT, X-ray, resin cast SEM (a technique developed for better visualization of osteocyte morphology), procion red injection, calcein/alizarin red/dapi labeling (a technique developed for the visualization of the relationship between osteocytes and mineralization deposition rate), *in situ* hybridization, and immunostaining, as well as serum biochemistry.

No rescue of the DMP1 null bone phenotype was observed using a high calcium diet. However, high phosphate diet returned the defective, disorganized growth plate to normal in the DMP1-null mouse even in the presence of greater circulating levels of FGF23. However, the high phosphate diet failed to restore normal mineralization in DMP1-null bone specifically surrounding osteocyte lacunae, therefore, the osteomalacia was only partially corrected.

Next, we re-expressed DMP1 in mice lacking the DMP1 gene by targeted re-expression of *DMP1* in osteoblasts and therefore osteocytes by using the 3.6 kb rat type 1a1 collagen promoter. The DMP1-null phenotype was completely rescued with restored phosphate and FGF23 levels to control. Interestingly, no apparent effect was observed with targeted overexpression of DMP1 using this promoter on the wild-type background including no changes in FGF23 levels.

We have also generated a mouse model using the 10kb DMP1 promoter to drive Cre expression in order to obtain targeted deletion of genes in osteocytes[3]. We have examined this promoter and found that it is up-regulated by phosphate using a 10 kb DMP1 promoter-luciferase construct. This promoter responds to phosphate in a dose-dependent manner. In summary, we have used the approach of various transgenic mouse models that we have created to determine the function of DMP1, its role in phosphate homeostasis through FGF23, its localization in osteocytes, and its regulation by phosphate.

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The authors have no conflict of interest.

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