Bone and Mineral Metabolism

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Global Wnt Inhibition With a Porcupine Inhibitor Decreases Established Trabecular Bone in a Mouse Model of Fibrous Dysplasia

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Background: G protein-coupled receptors (GPCRs) mediate a wide spectrum of physiological functions, including bone development and remodeling. Fibrous dysplasia (FD) is a common skeletal dysplasia caused by increased G_s-GPCR signaling and characterized by fibrotic expansile bone lesions. FD has no effective medical treatments. Our prior studies used the ColI(2.3)$^+$/Rs1$^+$ mouse model where osteoblastic-cell G_s signaling induced a dramatic FD-like phenotype and increased Wnt signaling, which we hypothesized is a major driver of the phenotype. Furthermore, we previously showed that blocking Rs1 expression could reverse the abnormal bone phenotype, providing proof-of-concept for finding therapies for FD.
Methods: Long bone stromal cells from wildtype and ColI(2.3)$^+$/Rs1$^+$ 9-week-old male mice were analyzed by single-cell RNAseq to identify potential cellular sources of Wnt ligands. We compared these findings with global inhibition of Wnt activity by oral administration of the porcupine inhibitor LGK974 to ColI(2.3)$^+$/Rs1$^+$ mice. These mice were analyzed by histology and micro-computed tomography (micro-CT).
Results: Control and ColI(2.3)$^+$/Rs1$^+$ bones showed similar scRNAseq results, except for a large expansion of osteoblastic lineage cells and increased differential expression (DE) genes in this cluster. Expression of G_i-GPCRs was increased, potentially as compensation for the strong G_s-GPCR pathway activation induced by Rs1 expression. We also found increased GH/IGF1 pathway activation in the osteoblastic cluster, and expression of multiple Wnt ligands within multiple cell clusters. We also identified a cell population unique to the ColI(2.3)$^+$/Rs1$^+$ FD-like bone lesions. Broad Wnt production inhibition of porcupine by LGK974 induced dose-dependent resorption of the abnormal FD bone shown by decreased bone volume and trabecular thickness; however, the fibrocellular infiltrate in the ColI(2.3)$^+$/Rs1$^+$ mice was still present.
Conclusions: FD-like bones of ColI(2.3)$^+$/Rs1$^+$ mice showed broad activation of Wnt signaling in multiple cell types, suggesting both cell autonomous and non-cell autonomous activity. Broad Wnt inhibition decreased established FD-like trabecular bone, but the fibrocellular infiltrate did not fully reverse. These results suggest distinct roles of G_s-GPCR and Wnt signaling in FD pathogenesis.

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Importance of Extra-Renal CYP24A1 Expression for Maintaining Mineral Homeostasis

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Calcium homeostasis involves a complex interplay between kidneys, parathyroid glands, intestine and bone. Specifically, $1,25(OH)_2D_3$ is a key calciotropic hormone which stimulates intestinal calcium absorption. A growing body of evidence suggests that circulating levels of $1,25(OH)_2D_3$ depend not only on its synthesis under the action of PTH in the kidneys, but also its catabolism by 24-hydroxylase, herein referred to as CYP24A1. The clinical importance of CYP24A1 has been demonstrated by human loss-of-function mutations, which lead to severe hypercalcemia due to exaggerated levels of $1,25(OH)_2D_3$. Despite its growing importance, little is known about its tissue-specific contributions to normal vitamin D metabolism. To explore the physiology of CYP24A1 and delineate renal-specific effects of CYP24A1 in calcium metabolism, we generated a mouse with constitutive kidney-specific deletion of *Cyp24a1* ($Six2^{Cre}$-$Cyp24^{flox}$). Six2 marks the nephron progenitor population throughout nephrogenesis. We hypothesized that hypercalcemia as seen in CYP24A1 inactivating mutations is related to lack of both renal and extrarenal expression, and that renal deletion does not lead to severe hypercalcemia. To confirm *Cyp24a1* deletion, we measured mRNA expression in the kidney using qPCR and RNA *in situ hybridization.* All mice were fed a standard commercial rodent diet and followed longitudinally for five months with interval calcium measurements. At time of termination, serum PTH levels were measured along with vitamin D-dependent calcium transporters as a functional measure of $1,25(OH)_2D_3$ action. *Cyp24a1* expression was significantly knocked down in total kidneys from $Six2^{Cre}$-$Cyp24^{flox}$ mice as compared to intestinal expression suggesting successful gene deletion. Compared to age-matched wildtype controls, $Six2^{Cre}$-$Cyp24^{flox}$ mice were mildly but persistently hypercalcemic (diff between means= 0.46 mg/dL, p-value: 0.03, n=8 per group). As expected, 1,25D-dependent calcium transporters in the kidney (*Calb1, Trpv5, Slc8a1, Atp2b1)* and intestine *(Trpv6, s100g)* were all increased, consistent with increased systemic $1,25(OH)_2D_3$ activity. PTH levels were appropriately suppressed in the $Six2^{Cre}$-$Cyp24^{flox}$ mice (diff between means=83 pg/mL, p-value 0.2, n=9 control, n=3 exp) as were renal *cyp27b1* mRNA expression. These data suggest that renal CYP24A1 is important for systemic $1,25(OH)_2D_3$ regulation, but the lack of severe hypercalcemia supports critical contributions of extra-renal CYP24A1.

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In Vitro Effect of CCL11 on Myogenic Differentiation and Its Relevance With Sarcopenia Parameters in Older Adults

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Background: The C-C motif chemokine ligand 11 (CCL11) has been receiving attention as a potential pro-aging factor