Phosphate, PTH, and FGF23 as mediators of the rachitic growth plate

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Dr. Demay’s CORT-supported study is focused on identifying the molecular basis for susceptibility of chondrocytes to phosphate-mediated apoptosis. This work characterizes hormone and growth factor (PTH, FGF23 and 1,25-dihydroxyvitamin D) effects on phosphate-mediated apoptosis using in vitro and in vivo models.

Interim Findings

Treatment of growing mice with the ERK inhibitor U0126 (panel A below) resulted in expansion of hypertrophic chondrocytes and impaired apoptosis of these cells, compared to Vehicle treated mice (V). We examined effects of low phosphate on Erk1/2 phosphorylation, and observed decreases in pErk1/2 immunoreactivity in hypertrophic chondrocytes of metatarsals cultured in low phosphate conditions, and in Hyp mice (panel B). pC-Raf was also decreased in Hyp growth plate (panel C).

This work demonstrates that Erk1/2 phosphorylation is required for phosphate-mediated apoptosis of hypertrophic chondrocytes and implicates the PTHrP signaling pathway in the impaired apoptosis of these cells. See: Miedlich SU, Zalutskaya A, Zhu ED, Demay MB; Phosphate-induced Apoptosis of Hypertrophic Chondrocytes is associated with a Decrease in Mitochondrial Membrane Potential and is Dependent upon Erk1/2 Phosphorylation; J Biol Chem; 285:18270-5, 2010.

Based on these responses, we examined whether C-Raf was upstream of pErk1/2 in the cascade that leads to phosphate-induced hypertrophic chondrocyte apoptosis. Inhibition of C-Raf (with GW5074) impaired phosphate-mediated pErk1/2 and C-Raf phosphorylation in these cells. Furthermore, PTH/PTHrP signaling blocked C-Raf and Erk1/2 phosphorylation. Thus we have established that C-Raf is in the signaling cascade between phosphate and hypertrophic
chondrocyte apoptosis and the cascade is modulated by PTH/PTHrP signaling and 1,25-dihydroxyvitamin D.

To examine if blocking vitamin D activity in NPT2a null mice results in progressive rickets, we generated double knockout (DKO) mice—lacking both Npt2a and the VDR—and employed a diet that prevents abnormal mineral ion levels in VDR null mice. Npt2a null mice have an expanded hypertrophic chondrocyte layer at 16 and 21 days of age (see below), regardless of whether they have a functional VDR. However, only DKO mice have expanded hypertrophic chondrocytes and abnormal primary spongiosa at 35 days of age.

VDR null mice fed this diet have a normal growth plate throughout all time points. To demonstrate that these abnormalities directly reflect receptor-dependent actions of 1,25-dihydroxyvitamin D, DKO mice were rendered vitamin D deficient and fed the diet that maintains normal mineral ion homeostasis. These mice also developed an expanded hypertrophic chondrocyte layer. Thus this study confirms that receptor-dependent actions of 1,25-dihydroxyvitamin D are required for a normal growth plate in the setting of persistent hypophosphatemia in Npt2a null mice. Moreover, the data suggest that 1,25-dihydroxyvitamin D may play a direct role at the growth plate in healing of the rickets in XLH. See: Miedlich SU, Zhu ED, Sabbagh Y, Demay MB; The receptor-dependent actions of 1,25-dihydroxyvitamin D are required for normal growth plate maturation in Npt2a knockout mice; Endocrinology; 151:4607-12, 2010.

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