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MEETING REPORT

Meeting Report from the 29th Annual Meeting of the American Society for Bone and Mineral Research

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PHOSPHATE METABOLISM

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Our understanding of phosphate metabolism has moved fast for the past few years. The big step was finding that FGF23 is a phosphatonin, a secreted messenger that directs the kidney to spill phosphate and inhibit synthesis of $1,25(OH)_2D$. Two years ago we learned that the klotho protein is a coreceptor for FGF23. Klotho binding converts certain FGF receptors to high-affinity FGF23 receptors (FGFRs), but which ones? Ablation of FGFR3 or FGFR4 does not impair FGF23 action (1), supporting the view that FGFR1c is the principal FGF23 receptor, but more work on this point will be necessary.

Klotho was described as an aging gene but in the mouse, loss of FGF23 and loss of *Klotho* produce similar phenotypes, which seem to be attributable to impaired FGF23 signaling, as the phenotype is greatly ameliorated by blocking the resultant hyperphosphatemia or the increase in $1,25(OH)_2D$. We now learn that the human disorder tumoral calcinosis, which was previously associated with mutations in either FGF23 itself or in the galacosyltransferase GALNT3 that processes FGF23, can also be caused by mutations that disable *Klotho* (2;3). The affected individual had all the manifestations of tumoral calcinosis but did not have evidence of premature aging. The role of *Klotho* in aging remains muddy; although many of the "aging" phenotypes seem to be attributable to disordered mineral metabolism, a recent paper suggests that *Klotho* causes cellular senescence through effects on Wnt signaling (4).

Klotho is expressed at only a few locations, making them prime candidates as sites for FGF23 action. One of these is the parathyroid. FGF23 inhibits the secretion of PTH from isolated bovine parathyroid cells, as subsequently reported by two groups (5-7). Unfortunately we don't know much about the relation of this effect to calcium signaling for PTH release. It's also curious that Klotho itself is reported to stimulate PTH secretion in the absence of FGF23 (8).

The principal renal phosphate transporter is NaPi2a. Removal of NaPi2a prevents phosphate reabsorption, and hypophosphatemia results. Crosses of hypophosphatemic NaPi2a null mice and hyperphosphatemic FGF23 nulls have hypophosphatemia (9), hence NaPi2a is the main renal target of FGF23 and therefore the NaPi2a null phenotype is dominant. In bone, rib nodules, increased mineralization of the primary spongiosa and increased osteoid characterize the FGF23 null mouse; these phenotypes are also observed in double knockout mice, which are hypophosphatemic, thus making the important point that some actions of FGF23 in bone are independent of serum phosphate.

Mutations in the NaPi2c transporter gene cause human hypophosphatemic rickets with hypercalciuria (HHRH) (5). Expression of both alleles from a compound heterozygotic HHRH patient showed that one is nonfunctional but the other is hypomorphic, apparently because of an inward-directed sodium leak (10). In the mouse, however, removal of the NaPi2c

gene does not produce rickets, but rather hypercalcemia and an increase in $1,25(OH)_2D$ levels, with no change in serum phosphate or TmP/GFR (11). FGF23 levels are reduced in NaPi2c null mice, which develop marked hypophosphatemia when treated with FGF23 (12). Relationships between renal phosphate handling, vitamin D activation and FGF23 levels are complex: loss of NaPi2c resets the level of vitamin D synthesis and FGF23 without any net change in phosphate excretion.

Extracellular phosphate levels in cartilage rise as chondrocytes mineralize their matrix, and eventually phosphate induces apoptosis of chondrocytes, ending their life cycle. Phosphate induces several pro-apoptotic molecules in ATDC5 cells (13). One of these is Bnip3. Silencing of Bnip3 by RNAi suppressed phosphate-induced apoptosis, and conversely expression of Bnip3 attenuated the antiapoptotic effect of Bcl-xL. Removal of Bcl-xL from chondrocytes with Cre-loxP produced dwarfism due to massive chondrocyte apoptosis. These abnormalities were largely rescued by a low-phosphate diet, which upregulated Bnip3 in chondrocytes. Another presentation reported that Hyp chondrocytes have reduced phosphate uptake via the Type III transporter Pit-1 (14). Overexpression of Pit-1 increases phosphate uptake, ATP levels, caspase-9 and -3 activation and apoptosis. Phosphate can also signal in renal tubule cells, possibly via NaPi2 (15) and overexpression of Pit-1 produces hyperphosphatemia without abnormalities in vitamin D activation (16).

One of the biggest pieces of news at last year's ASBMR meeting was that mutations in the SIBLING protein dentin matrix protein 1 (DMP-1) cause renal phosphate wasting and osteomalacia. The business end of DMP-1 is the carboxyl terminal domain (17). Mutations in DMP-1 that cause hypophosphatemic rickets impair processing of the molecule (18). DMP-1 expression patterns in bone are correlated with the pattern of strain (19). The sister SIBLING protein MEPE has a tantalizing but poorly understood relation to phosphate disorders. MEPE levels are high in tumors from patients with tumor-induced osteomalacia but MEPE null mice do not have a demonstrable phosphate disorder, establishing that MEPE is not a potent phosphatonin. MEPE overexpression, in fact, produces hyperphosphatemia and increased levels of $1,25(OH)_2D$ (20). Bones from these animals have reduced mass, mildly impaired mineralization, and low turnover (21).

Conflict of Interest: None reported.

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