

The molecular background to hypophosphataemic rickets

Bone mineral loss disorders are major, worldwide health concerns, and can be familial, idiopathic, oncogenic, dietary, or hormonal. The mechanisms controlling bone integrity are complex. Extensive research has been directed towards the characterisation of the key factors involved in bone mineral regulation and two specific bone diseases, X-linked hypophosphataemic rickets (HYP) and oncogenic hypophosphataemic osteomalacia (OHO). The primary defect in HYP is a defective zinc metalloendopeptidase (PHEX),¹⁻⁴ and a new candidate glycoprotein (MEPE) has been proposed as the phosphaturic factor released by OHO tumours.^{5,6} Both diseases cause severe changes in bone morphology and have an overlapping pathophysiology. This review will discuss the recent molecular advances in our understanding of the role of the kidney and other organs in bone mineral homeostasis, with emphasis on X-linked hypophosphataemic rickets and tumour osteomalacia.

Clinical features of HYP and tumour osteomalacia

Classic, vitamin D resistant HYP (MIM #307800), is characterised by:

- Hypophosphataemia
- Renal phosphate leak as expressed as a lowered transfer maximum of phosphate per unit volume of glomerular filtrate (TMPO4/GFR)
- Inappropriate vitamin D metabolism in the presence of low serum phosphate (low to normal serum 1,25 dihydroxy vitamin D3; calcitriol)
- High alkaline phosphatase
- Skeletal defects.⁷

OHO shares many clinical, biochemical, and physiological features with HYP.^{8,9} The tumours are mainly of mesenchymal origin, although a number of different tumour types have been reported.^{10,11} Evidence indicates that changes in renal phosphate handling, vitamin D metabolism, and skeletal mineralisation are caused by factor(s) secreted by OHO tumours.^{8-10,12-19} The resection of OHO tumours results in the disappearance of disease symptoms and bone healing and is a key observation that supports this.^{8,12,20}

Cloning of the rickets gene

The rickets gene PHEX (phosphate regulating gene with endopeptidase activity on the X chromosome),^{1,2} is a zinc metalloendopeptidase with close homologies to an M13 family of type II glycoproteins. Neprilysin (NEP) is a prototypic member of the M13 family. Human PHEX and its cDNA have been characterised fully,^{1,3,4} and extensive mutation analysis has confirmed that the defect in HYP can be attributed to primary mutations in the PHEX gene.^{3,21-24} Two murine homologues (HYP and Gy), for X-linked rickets have been characterised,^{25,26} and deletions in the murine Phex gene were discovered at the 3' and 5' ends respectively.^{27,28} Mutation analysis combined with molecular modelling and comparison of data for NEP and thermolysin has enabled a model for the catalytic site of human PHEX to be proposed.^{3,8,11,12}

PHEX/Phex tissue expression

A defined pattern of PHEX/Phex expression has emerged after screening a large range of tissue types. Mouse bone,^{28,29} mouse teeth,³⁰ human fetal bone,^{28,29} human lung,^{28,31} and adult human ovary³¹ all express PHEX/Phex mRNA. The highest relative levels of expression were

measured in bones²⁸ and teeth.³⁰ No PHEX expression has yet been recorded in kidney,^{28,30-32} while PHEX expression occurs in OHO tumours.²⁹ Recombinant expression of PHEX and assessment of its subcellular localisation supports the molecular predictions that PHEX is a type II integral membrane glycoprotein.²⁹

Physiological and molecular changes in HYP and tumour osteomalacia

Extensive research has been carried out on murine models of rickets, and a number of reviews have described this work.^{8,11,12,33-36} Several distinct molecular processes are defective and include:

- Renal phosphate handling (renal Na dependent phosphate co-transport)
- Aspects of vitamin D metabolism
- Phosphorylation of specific extracellular bone matrix proteins (EBMPs)
- Expression of specific EBMPs
- Protein kinase C and skeletal casein kinase II expression
- Osteoblast bone cell function and skeletal mineralisation.

All the abnormal changes described are now known to be due to a primary defect in the PHEX gene.^{1,3,4,21,23,24,27,28} Tumour osteomalacia has very similar clinical and biochemical features to HYP, and it is likely that the tumour may secrete a key factor regulated or processed by PHEX. The following section will review the above areas in relation to HYP, OHO and normal molecular physiology.

RENAL PHOSPHATE TRANSPORT

Inorganic phosphate (PO₄) is reabsorbed from the glomerular filtrate by a number of distinct Na⁺ dependent phosphate co-transporters (NaPi). Three classes of NaPi transporters have been characterised:

- NPT1 (type 1),
- NPT2 (type 2)
- Viral cellular receptors Glrv 1, and RAM 1.^{37,38}

Various isoforms of these transporters have also been discovered,³⁹ and a separate gene has been cloned that has close homologies to renal NPT2. The new transporter is classified as type 2b (NPT2b) and the human gene (NaPi 3b) is expressed in intestines and lung.^{40,42} Also, human NPT2 (NaPi 3) contains a vitamin D response element and is thus regulated by calcitriol⁴² at the nuclear level.

In familial X-linked rickets, early renal phosphate transport studies were carried out on the HYP mouse.³⁵ Renal defects were found in a NaPi transporter localised to the brush border membranes of the proximal convoluted tubules, and this was later confirmed to be due to down regulation of mRNA and protein of the NPT2 class of phosphate transporters.^{34,36,43,44} Mouse gene knockout experiments have shown that NPT2 has a major role in regulating phosphate homeostasis and skeletal development, but does not produce an X-linked rickets phenotype.⁴⁵ More recently, downregulation of murine NPT1 transporters in HYP has also been observed, although the decrease was 20% compared with 50% for NPT2.⁴⁴ The phosphate defect in rickets mice persists after parathyroidectomy and predominantly involves a high affinity, low capacity Na⁺ phosphate co-transport system.^{8,34,35} There is considerable and impressive experimental evidence that supports the role of a circulating factor regulating phosphate in the kidney. These data have been extensively reviewed.^{8,11,34,35}

VITAMIN D METABOLISM

In HYP and OHO, low serum phosphate does not initiate an increase in calcitriol and the levels are either inappropriately low to normal (HYP), or severely reduced (OHO). Also, in complete contrast to normal mice, HYP mice fed low phosphate diets have decreased 1, 25-(OH)₂D₃ levels and HYP mice on phosphate supplemented diets have increased concentrations of 1,25 (OH)₂D₃ in serum. The acute, maladaptive regulatory response of at least two key enzymes, renal 1 α hydroxylase and the 24 hydroxylase (upregulated), are responsible for the abnormal modulation of serum calcitriol.^{46–47}

PHEX expression is downregulated by calcitriol,^{2–48} and it is of interest to speculate how this might also contribute to aberrant vitamin D metabolism in HYP. It is possible that PHEX is required to process a molecule that directly or indirectly regulates aspects of vitamin D metabolism, and calcitriol modulates this by feedback suppression of PHEX synthesis. Loss of PHEX activity in HYP might therefore interfere with this regulatory mechanism, resulting in inappropriate 1 α hydroxylase and 24 hydroxylase activity. Also, Phex expression increases as osteoblasts mature and appears to be associated with matrix mineralisation.^{35–48} In contrast, calcitriol inhibits bone mineralisation and the interconnected expression of both Phex and calcitriol may therefore be required for appropriate bone mineral homeostasis.

BONE

Profound changes in bone morphology occur in both HYP and OHO.^{7–8} In HYP mice, intrinsic osteoblast defects are apparent that are not associated directly with the hypophosphataemia, renal phosphate leak, or altered vitamin D status. Intramuscular transplantation of normal and HYP mice osteoblasts or periosteal have provided experimental evidence for osteoblast defects.^{49–51} Histomorphometric analysis of the transplanted cells demonstrate that HYP osteoblast mineralisation function is intrinsically abnormal. Major differences in Hyp osteoblast cultures in response to alkaline phosphatase and calcitriol mediated proliferation regulated by phosphate also suggests that an intrinsic osteoblast factor plays a role.⁵² Other skeletal related defects have been reported and include increased osteoblast gluconeogenesis associated with decreased intracellular pH,⁵³ reduced casein kinase II activity and decreased osteopontin (OPN) phosphorylation.⁵⁴

MODELS

Key features concerning phosphate/calcium renal bone homeostasis are beginning to emerge and models can now be proposed that take into account the new findings. PHEX/Phex may cleave and process a factor or factors that are essential for phosphate handling, osteoblast/odontoblast function, mineralisation, and growth. It is also possible that PHEX/Phex as a homodimer and/or heterodimer plays a pivotal role in the processing of an important renal phosphate regulating factor that directly or indirectly impacts on bone mineralisation/remodelling. This factor is likely processed sequentially via specific proteolytic cleavage to generate a number of bioactive peptides that impact on phosphate regulation and/or bone mineralisation. Other modes of post-translational cleavage of these peptides cannot be excluded and phosphorylation may also play a role. The under-phosphorylation of osteopontin due to defective skeletal casein kinase II (CK-II) in Hyp⁵⁴ and the discovery of cell surface CK-II ectokinase activity in osteoblasts⁵⁵ underlines the importance of protein phosphorylation. The fact that Phex expression is suppressed by calcitriol suggests that one or more of these peptides may well impact on the regulation of vitamin D metabolism by

suppressing or activating key catabolic or anabolic enzymes such as 1- α -hydroxylase or 24-hydroxylase. This in turn may affect osteoblast and osteoclast function. Elegant experiments demonstrating the role of an intrinsic osteoblast defect in Hyp mice,^{49–50–56–57} and the finding that Phex expression is predominant in osteoblasts/bone/teeth,^{28–30–32} suggests that some of the processed peptides may have an autocrine effect on osteoblast/odontoblast function as well as a paracrine effect on renal phosphate handling.

Of direct relevance to a putative phosphaturic factor is the recent cloning from OHO tumours of an RGD containing glycoprotein MEPE (matrix extracellular-phosphoglycoprotein).^{5–6} MEPE has close similarities to bone and dentin extracellular matrix proteins (Dentin-matrix protein-I, osteopontin, dentin sialoprophosphoprotein, bone sialo protein-II). This protein is highly expressed in OHO tumours but not in non-phosphaturic tumours and is also highly expressed in bone marrow and brain. Although yet to be confirmed unequivocally, MEPE is a good candidate for the uncharacterised phosphaturic factor (phosphatonin), that has been proposed to be processed directly or indirectly by PHEX.

An increased understanding of the mechanisms controlling bone-renal biochemistry and physiology is prerequisite to improving the clinical management of bone loss disorders (inherited or acquired). Recent advances have provided researchers with new insights that will ultimately contribute to the achievement of this goal.

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