

The structure, function, and pharmacologic inhibition of FGF23

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FGFs mediate their responses by binding to and activating a family of four receptor tyrosine kinases (FGFRs 1 – 4). FGFRs 1 – 3 have two isoforms produced by alternate splicing which differ in their ligand-binding specificities and tissue expression patterns.

FGF23 is the largest of the 22 known FGFs and differs from others by a unique C-terminal domain. XLH patients have elevated circulating levels of FGF23, as do patients with autosomal dominant hypophosphatemic rickets (ADHR). In ADHR, FGF23 is resistant to proteolytic cleavage leading to delayed clearance and accumulation in the circulation. Furthermore, tumor-induced osteomalacia (TIO), another disorder with a similar phenotype to XLH, is caused by tumor overproduction of FGF23. Recessive mutations resulting in low circulating levels of intact FGF23 cause tumoral calcinosis (TC), in which serum P levels are elevated. These clinical observations have revealed a novel role for FGF23 although little is known about its mode of action. Our studies aim to discover new information about this pathway and apply it to human disease.

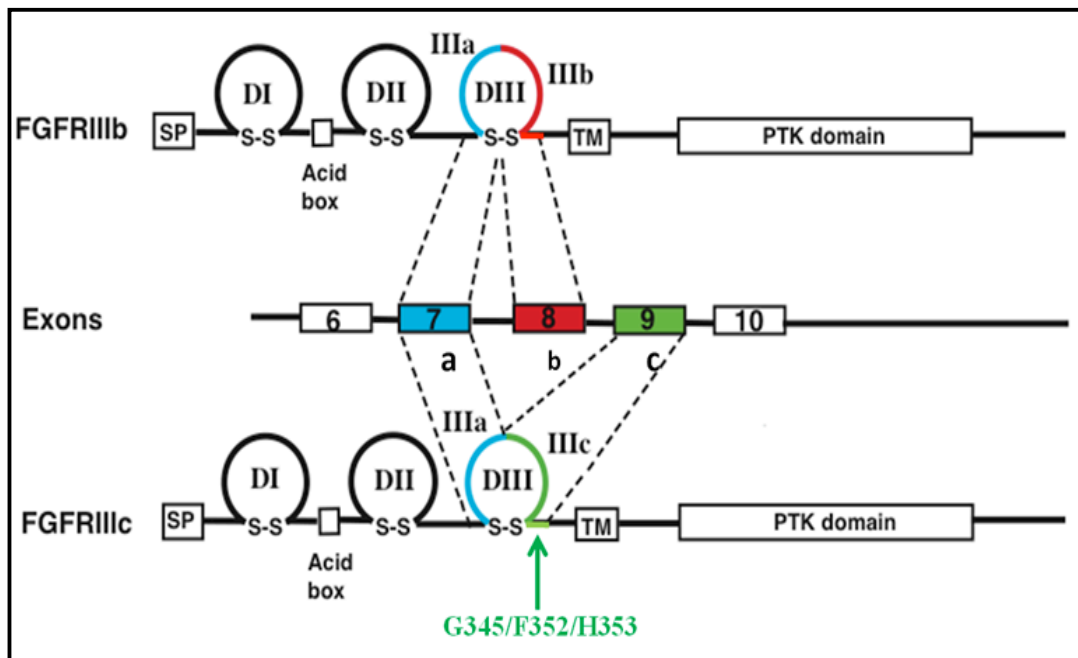
We are focused on developing pharmacological inhibitors of FGF23 signaling in kidney, thereby correcting renal phosphate wasting, which is central to the pathophysiology of XLH. The kinase domains of FGFRs are highly conserved, and consequently small molecule kinase inhibitors of FGFRs block all the FGFR isoforms, precluding the use of this class of inhibitors as specific therapeutic agents. To identify inhibitors with greater selectivity for FGF23 actions, alternative therapeutic targets such as the interaction between Klotho and FGF receptors are being examined.

Interim Findings

Recent studies have shown that Klotho functions as an accessory molecule in transducing FGF23 signaling via FGF receptors. We have successfully generated expression vectors for FGF23, Klotho, FGFR1c, FGFR1b, FGFR2c, FGFR2b, and FGFR3c, and have purified Klotho for antibody production.

Based on the observation that Klotho binds primarily to the c-isoforms of FGFRs, we hypothesized that the 'c' type isoforms of FGFRs may have a unique binding site that interacts with Klotho. We then determined the minimal interaction site of Klotho on the FGFR surface by creating FGFR2 chimeric constructs where b-isoform-specific sequences in the third Ig-like domain were replaced by corresponding c-isoform sequences. We examined the ability of the chimeric proteins to associate with Klotho as compared to both the wild-type c- and b-isoforms. The results show that the c-isoform-specific sequences at the F and G strands of the Ig-like domain 3 converted the b-isoform to associate with Klotho.

We performed alanine scan mutational analyses, in which residues in the F and G strands of the c-isoform were replaced by alanine to determine the key residues that mediate the interaction of FGFR with Klotho. We have narrowed down the Klotho interaction region of FGFR2 to three critical residues of the second half of the third Ig-like domain of the c-isoform, shown below. These results will direct us toward production of antibodies that block the interaction of Klotho with FGFR.



Identification of Klotho interacting site of c-type FGFRs.

Two major isoforms of FGFRs, the b and c, are produced by alternate splicing of exons 8 and 9, respectively. The location of putative Klotho interacting residues, Glycine 345, Phenylalanine 352 and Histidine 353, on the c-isoforms is marked by the arrow.