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Fibroblast growth factor homologous factors (FHF): new roles in neuronal health and disease

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Abstract

FHFs (fibroblast growth factor homologous factors) are a noncanonical subset of intracellular FGFs (fibroblast growth factors) that have been implicated in a variety of neurobiological processes and in disease. They are most prominently regulators of voltage-gated Na⁺ channels (Navs). In this review we discuss new insights into how FHFs modulate Navs. This is followed by a summary of a growing body of evidence that FHFs operate in much broader fashion. Finally we highlight unknown aspects of FHF function as areas of future interest.

I. Background

The discovery and early history of FHFs has been reviewed elsewhere (Goldfarb 2005). Briefly, FHFs were first identified as FGF-like cDNAs during a search for novel retinal-specific genes. The FHFs are composed of four members of the FGF superfamily: FGF11, FGF12, FGF13, and FGF14 (aka FHF3, FHF1, FHF2, and FHF4, respectively). Each member has at least two distinct isoforms generated by alternative splicing of different first exons. FGF13 displays the most extensive isoform diversity with at least five different isoforms (Figure 1) (Munoz-Sanjuan and others 2000; Smallwood and others 1996). Several of the alternatively spliced isoforms have a restricted nuclear localization (Smallwood and others 1996). Their roles within the nucleus have not yet been determined.

Though similar in overall structure to the FGFs (see **Section II**), important differences render FHFs incapable of activating FGF receptors. First, lacking a signal sequence in their N-termini, they are not secreted extracellularly (Smallwood and others 1996) and thus are unavailable to bind to FGF receptors. Additionally, specific structural differences appear to prevent FHFs from being able to activate FGF receptors (Olsen and others 2003).

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Declaration of Conflicting Interests

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The first breakthrough in understanding roles for FHF as non-growth factors came when Waxman and colleagues identified FGF12B (aka FHF1B) while screening for proteins that interact with the intracellular C-terminal tail of the voltage-gated sodium channel Nav1.9 (Liu and others 2001). Since then it has become clear that FHFs are critical cytoplasmic Nav regulators that determine the *in vivo* kinetic parameters of channel function and at least in some neurons may also control Nav subcellular localization (discussed in detail in **Section III**).

Interestingly, FHFs have been implicated in neurobiological disease. The best defined link is for spinocerebellar ataxia 27 (SCA27). SCA27 was mapped to *FGF14* in a Dutch kindred in whom all affected individuals harbored a heterozygous phenylalanine to serine point mutation (corresponding to F145S in FGF14A or F150S in FGF14B) (van Swieten and others 2003). Additional *FGF14* mutations have been described in other patients with spinocerebellar ataxia (Dalski and others 2005). Consistent with this disease association, mice that lack *Fgf14* are ataxic and perform poorly in motor behavioral tasks, recapitulating major phenotypes observed in SCA27 patients (Wang and others 2002). Because heterozygous *Fgf14*^{+/-} mice displayed no obvious phenotype, it appears that the F150S mutation in the Dutch kindred must act as a dominant-negative loss-of-function mutation. Beyond SCA27 associated with *FGF14*, there are suggestions that *FGF13* is a locus for neuropsychiatric diseases. *FGF13* is located on the chromosomal segment that maps to an X-linked spinocerebellar ataxia (SCAX5) (Zanni and others 2008). Linkage analysis has also implicated *FGF13* in X-linked mental disorders such as Borjesson-Forssman-Lehmann syndrome (Gecz and others 1999). Possible mechanisms for FHF dysfunction in neural disease are discussed further in **Section VI**.

II. The structure of FHFs

Crystal structures of FGF12B, FGF13S and FGF13U show that the FHFs do not only share sequence homology with the FGFs but are structurally similar to FGFs, with the minor, but important differences for engaging FGFRs, as noted above (Goetz and others 2009; Olsen and others 2003; Wang and others 2012; Wang and others 2014). FHFs and canonical FGFs contain 12 antiparallel β strands arranged in a structure with pseudo threefold-symmetry called a β -trefoil (Olsen and others 2003; Zhu and others 1991). Non-homologous substitutions in FHFs of a few amino acids known to be critical for FGF interaction with FGF receptors may introduce steric clashes or remove hydrogen bonds thus rendering FHFs incapable of binding FGF receptors (Olsen and others 2003). There is no structural information on the extended FHF N- and C-termini, which are not found on the canonical FGFs (Figure 1).

The interaction sites between FHFs and Nav channels have recently been defined at the atomic level, extending our understanding of how FHFs bind to the Nav intracellular C-terminus, a rich site of protein interactions and Nav modulation (Liu and others 2001; Liu and others 2003; Olsen and others 2003). Crystal structures of ternary complexes containing an FHF, a Nav C-terminus and calmodulin reveal how specific amino acids conserved among all four FHFs form protrusions that fit into Nav CTD depressions that are comprised of amino acids conserved across the Nav channel family (Wang and others 2012; Wang and

others 2014; Yan and others 2014). Critical among these protrusions on the FHF are Leu⁵⁶ and Arg⁵⁷ (numbered according to FGF13B), which interact with Nav His¹⁸⁴⁹ and Asp¹⁸⁵² (numbered according to Nav1.5). Indeed, mutation of Arg⁵⁷ is sufficient to completely abrogate interaction with the Nav channel (Figure 2) (Yan and others 2014). These new structures also provide insight into how FHF differentially modulate specific Nav channels. For example, a negatively charged Lys¹⁴ in FGF13U (Lys⁹ in FGF12B) forms a salt bridge with a positively charged Glu¹⁸⁹⁰ in Nav1.5. In Nav1.1 however the corresponding residue is a neutral glutamine (Gln¹⁹⁰⁴). Disruption of this salt bridge explains why FGF12B regulates Nav1.5 but has no effect on Nav1.1 (Wang and others 2012; Wang and others 2011) and provides a basis for why the nearby Q7R mutation in FGF12 affects cardiac Nav1.5 channel function and leads to a cardiac arrhythmia syndrome (Hennessey and others 2013).

III. FHFs regulate voltage-gated sodium channels

The best characterized role for FHFs is the regulation of voltage-gated sodium channels. Besides directly binding to Navs (see **Section II**), FHFs are enriched in neuronal subdomains where Navs concentrate. FGF14 in both the hippocampus and the cerebellum localizes to the axon initial segment (AIS), the proximal region of the axon where action potentials initiate and Navs are highly concentrated (Goldfarb and others 2007; Lou and others 2005). FGF13U localizes to nodes of Ranvier, another location at which Navs concentrate in order to propagate action potentials down the length of an axon (Wittmack and others 2004).

FHFs have profound effects on neuronal Navs in heterologous expression systems, but the specific effects depend upon the individual FHF and Nav pair studied. Moreover, the same FHF isoform may have different effects on different Nav channels. For example, both S and U (aka A and B) isoforms of FGF13 increase Nav1.6 currents in the DRG-derived cell line ND7/23 and shift steady-state inactivation to more depolarized voltages yet the two FGF13 isoforms impart different effects on recovery from inactivation. While FGF13S greatly slows Nav1.6 recovery from inactivation, FGF13U does not show any appreciable effect (Rush and others 2006; Wittmack and others 2004). The fact that different isoforms of the same FHF can have contrasting effects on particular kinetic parameters has been a recurring theme for FHFs. For example, in heterologous expression systems current density and steady-state inactivation of neuronal Nav1.2 and Nav1.6 sodium channels are differentially regulated by FGF14A and FGF14B. While FGF14A does not affect current density through either coexpressed Nav1.2 or Nav1.6, FGF14B reduces current densities for both Navs. Further, FGF14A shifts steady-state inactivation of Nav1.2 to more depolarized voltages while FGF14B exhibits no significant effect. In contrast, both isoforms shift the steady-state state inactivation of Nav1.6 to more depolarized voltages (Laezza and others 2009).

An additional layer of complexity is that these effects differ depending on the system in which they are studied. While FGF14b overexpression leads to a *reduction* in current density for any Nav isoform coexpressed in HEK cells, the same isoform when transfected into hippocampal neurons actually *increases* current density (Lou, Laezza et al. 2005, Laezza, Lampert et al. 2009). Further, cultured cerebellar granule cells from *Fgf12*^{-/-}; *Fgf14*^{-/-} knock-out mice do not display any differences in Nav current density

(compared to wild-type controls), while *Fgf12*^{-/-};*Fgf14*^{-/-} neurons in slices have significantly smaller transient Nav peaks (Goldfarb and others 2007).

Although dissecting the individual roles of specific FHF s may be challenging in the face of such observations, it remains clear that FHF s are critical in setting Nav kinetic parameters important for normal neuronal physiology. Both *Fgf14*^{-/-} Purkinje neurons and *Fgf12*^{-/-};*Fgf14*^{-/-} granule cells are much less excitable and fire fewer spontaneous or evoked action potentials when compared to control cells. In *Fgf12*^{-/-};*Fgf14*^{-/-} granule cells this is because Nav s inactivate at more negative voltages, are quicker to inactivate, and slower to recover from inactivation (Goldfarb and others 2007). Another consequence of FGF14 loss-of-function is a decrease in Purkinje cell “resurgent” current, a component of Nav current that manifests when Nav s are protected from inactivation by a putative “blocking particle”. This normally allows Nav s to re-open quickly and facilitates Purkinje cells’ ability to fire subsequent closely-timed action potentials (Yan and others 2014). FGF14 knockdown biases the Nav channels towards an inactivated state that decreases resurgent current and consequently the rate of firing. While this regulation is imparted by the B isoform of FGF14, the A isoforms of the FHF s also compete with intrinsic Nav fast inactivation for a different purpose: to decrease action potential firing during a steady excitatory current (Dover and others 2010; Venkatesan and others 2014).

FHF s have been suggested to regulate the localization of Nav s, particularly to the axon initial segment, although the results have also been mixed. A modest decrease in Nav1.1 and Nav1.6 immunofluorescence within axon initial segments was detected in *Fgf14*^{-/-} Purkinje neurons (Shakkottai and others 2009; Xiao and others 2013). In contrast, no such decrease has been reported in *Fgf12*^{-/-};*Fgf14*^{-/-} cerebellar granule cells (Goldfarb and others 2007). Interestingly, expression of the SCA27 mutant FGF14^{F150S} in hippocampal cells caused reduction of both Nav immunofluorescence and current density (Laezza and others 2007). Whether these contrasting results derive from cell-type differences between the cerebellum and the hippocampus, or from how closely FGF14^{F150S} recapitulates FGF14 knockdown remains to be elucidated.

Kinase signaling cascades that affect Nav regulation may do so through FHF s. The FGF14-Nav complex was identified as a target for the kinase glycogen synthase 3 (GSK3). GSK3 inhibition reduces the localization of both Nav and FGF14 at the AIS, indicating that a kinase pathway is important for the assembly of this protein complex in neurons (Shavkunov and others 2013).

IV. FHF s regulate voltage-gated calcium channels and synaptic transmission

Recent reports have shown that FHF s also influence voltage-gated Ca²⁺ channels (Cav s). shRNA-mediated knockdown of *Fgf14* in cerebellar Purkinje neurons leads to a pronounced decrease in the currents flowing through Cav s. In a heterologous expression system FGF14 increases current density through the presynaptic Cav2.1 and Cav2.2 but not through the somatodendritic L-type Cav1.2 or R-type Cav2.3. Similar to the observations with Nav channels, these data demonstrate that FHF s exert specific effects on different Cav channel

targets. Examining synaptic transmission at the granule cell to Purkinje cell synapse showed the consequences of Cav regulation by FHF. FGF14 knockdown in the presynaptic granule cells alters short-term plasticity (as measured by post-synaptic responses to paired pulse stimuli) in the manner that would be expected from a decrease in presynaptic calcium influx (Yan and others 2013). Consistent with this observation of impaired synaptic transmission at the granule cell to Purkinje cell synapse is a report that *Fgf14*^{-/-} mice display impaired hippocampal synaptic transmission and plasticity due to reduced presynaptic function (Wozniak and others 2007; Xiao and others 2007).

The mechanism by which FGF14 increases current density through presynaptic Cav_s appears to be through increasing the number of Cav_s present at the cell membrane rather than by affecting single channel function. How FGF14 regulates the number of Cav_s at the synaptic membrane remains unclear, however, as FHF_s and Cav_s do not display a direct interaction analogous to FHF_s and Nav_s.

V. FHF_s have been implicated in novel roles outside channel regulation

Because of the similarity between FHF_s and canonical FGF_s, several investigations have focused on whether FHF_s show functions different from voltage gated ion channel modulation. A yeast two-hybrid screen for FGF12 interacting proteins yielded the MAPK scaffold protein islet brain-2 (IB2). Co-immunoprecipitation experiments confirmed an interaction in cerebellar lysates. The consequences of this interaction are not clear, but in HEK293T cells FGF12-IB2 recruits MAPK p38 δ and thus activates signal transduction down the MAPK pathway (Schoorlemmer and Goldfarb 2001; Schoorlemmer and Goldfarb 2002). Whether this holds true in the brain and what this signaling module may do is not known. FGF12B was also identified as a novel interactor of NEMO, which is a critical subunit of I κ B kinase (IKK) whose activity eventually leads to activation and nuclear translocation of the transcription factor NF- κ B. Overexpression and knockdown experiments showed that FGF12B is a negative regulator of NEMO and thus of NF- κ B. FGF12B knockdown increased neuronal arborisation, neurite lengths and dendritic spine numbers in a NEMO-dependent manner (Konig and others 2012). Together with the report that the Nav-FGF14 complex is a GSK3 target (see **Section III**), these results implicate FHF_s as participants in kinase signaling cascades.

FGF12B's regulation of neuronal morphology is not the only evidence that FHF_s are participants in shaping neuronal architecture. Knockdown of FGF13 in cultured neurons leads to nonpolarized neurons wherein all neurites maintained dendritic characteristics. The number of dendritic branches increases and the cells that do have axons often have axons that are abnormally branched. The failure to form proper axons leads to deficits in cortical migration, as seen in *Fgf13*^{-Y} hemizygous mice, which display disordered cortical layers during development. The underlying mechanism is hypothesized to involve FGF13 regulation of microtubule stabilization through a direct interaction with β -tubulin (Wu and others 2012). Intriguingly, the FHF interacting protein IB2 (discussed above) also associates with microtubules (Schoorlemmer and Goldfarb 2001; Schoorlemmer and Goldfarb 2002). Thus, FHF_s may play several roles in regulating the neuronal cytoskeleton, whether through

signaling pathways that culminate in cytoskeletal changes or by directly interacting with components of the cytoskeleton.

VI. Unexplored questions and future directions

As discussed in **Section III**, a challenging problem in the study of FHF is that their modulatory effects on ion channels in heterologous expression systems are not always consistent with observations from *in vivo* models. As a case in point, manipulation of FHFs affects channel current density in heterologous systems and *in vivo* (Laezza and others 2007; Laezza and others 2009; Lou and others 2005; Rush and others 2006; Wittmack and others 2004; Yan and others 2013), but not always in a consistent direction. Reasons underlying these differences are not clear, but may relate to the effects FHFs appear to exert on trafficking and targeting ion channels to the plasma membrane and inherent differences in how this occurs in neurons *versus* heterologous expression systems. Ion channels travel to the plasma membrane through the ER-Golgi pathway and via vesicular transport along the microtubule cytoskeleton (Barry and others 2014; Kaplan and others 2001; Xu and others 2010; Zhang and others 2012). They are removed from the membrane via endocytosis (Fache and others 2004). For reviews of neuronal voltage-gated ion channel trafficking see (Lai and Jan 2006; Leterrier and others 2011; Vacher and Trimmer 2012). Thus, FHFs may be involved in anterograde or retrograde trafficking, or both, and the aspects of the machinery necessary for these processes may differ between neurons and heterologous expression systems. FHFs could conceivably act as adaptors anchoring channel containing vesicles to motor proteins or as cytoskeleton bound regulators orienting traffic towards specific domains within the cell. For example, a report that the Nav scaffolding molecule ankyrin G interacts with kinesin-1 to regulate Nav trafficking raises the possibility that FHFs may play a similar role (Barry and others 2014). The direct interaction between FHFs and Nav channels provides a mechanistic suggestion for Nav channel trafficking, yet how FHFs participate in the regulation of surface quantities of Cav channels, which do not bind FHFs directly, would seem to be different.

The specific mechanisms by which FGF14 mutations cause disease phenotypes such as spinocerebellar ataxia also remain unclear, thus preventing a complete understanding of the underlying pathology and potential therapeutic strategies. Loss-of-function FGF14 mutations that affect neuronal excitability could do so by loss-of-function effects upon Nav channels and resultant decreased cellular firing; by reducing Ca²⁺ influx through presynaptic Cav1 channels; or through both mechanisms. Other ataxias have been attributed to effects on either Nav or on Cav channels (Shakkottai and Paulson 2009) but none have yet been shown to be caused by effects on both.

Beyond spinocerebellar ataxia, deficiencies in FHF function have also been associated with cognitive impairment in mouse behavioral models as well as in humans (Brusse and others 2006), but the underlying molecular mechanisms are not known. *Fgf14*^{-/-} mice displayed impaired acquisition performance in a Morris water maze test (Wozniak and others 2007). Interestingly, *Fgf13*^{-Y} hemizygous mice exhibit the same acquisition deficit as well as a decreased time in the target quadrant. No motor deficits were observed for these mice (Wu and others 2012). These observations and those discussed previously in this review imply

that FHF s play important roles in establishing normal central nervous system circuitry probably through several distinct yet possibly related mechanisms: by setting electrophysiological parameters determining excitability, by contributing to normal synaptic function and plasticity, or through morphological changes in neurite patterning and cortical cell migration. It is likely that different FHF family members have varying levels of contribution to these processes but the degree to which any two FHF s are redundant remains incompletely understood.

Finally, certain FHF splice variants are localized within the nucleus, but the functions of this subset of FHF s remain unknown (Smallwood and others 1996). It is unlikely that these nuclear-localized splice variants affect Nav or Cav channels, thus suggesting other functions such as transcription or gene expression, but this has not yet been established.

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References

- Barry J, Gu Y, Jukkola P, O'Neill B, Gu H, Mohler PJ, et al. Ankyrin-G directly binds to kinesin-1 to transport voltage-gated Na⁺ channels into axons. *Dev Cell*. 2014; 28(2):117–131. [PubMed: 24412576]
- Brusse E, de Koning I, Maat-Kievit A, Oostra BA, Heutink P, van Swieten JC. Spinocerebellar ataxia associated with a mutation in the fibroblast growth factor 14 gene (SCA27): A new phenotype. *Mov Disord*. 2006; 21(3):396–401. [PubMed: 16211615]
- Dalski A, Atici J, Kreuz FR, Hellenbroich Y, Schwinger E, Zuhlke C. Mutation analysis in the fibroblast growth factor 14 gene: frameshift mutation and polymorphisms in patients with inherited ataxias. *Eur J Hum Genet*. 2005; 13(1):118–120. [PubMed: 15470364]
- Dover K, Solinas S, D'Angelo E, Goldfarb M. Long-term inactivation particle for voltage-gated sodium channels. *J Physiol*. 2010; 588(Pt 19):3695–3711. [PubMed: 20679355]
- Fache MP, Moussif A, Fernandes F, Giraud P, Garrido JJ, Dargent B. Endocytotic elimination and domain-selective tethering constitute a potential mechanism of protein segregation at the axonal initial segment. *J Cell Biol*. 2004; 166(4):571–578. [PubMed: 15302857]
- Gecz J, Baker E, Donnelly A, Ming JE, McDonald-McGinn DM, Spinner NB, et al. Fibroblast growth factor homologous factor 2 (FHF2): gene structure, expression and mapping to the Borjeson-Forssman-Lehmann syndrome region in Xq26 delineated by a duplication breakpoint in a BFLS-like patient. *Hum Genet*. 1999; 104(1):56–63. [PubMed: 10071193]
- Goetz R, Dover K, Laezza F, Shtraizent N, Huang X, Tchetchik D, et al. Crystal structure of a fibroblast growth factor homologous factor (FHF) defines a conserved surface on FHF s for binding and modulation of voltage-gated sodium channels. *J Biol Chem*. 2009; 284(26):17883–17896. [PubMed: 19406745]
- Goldfarb M. Fibroblast growth factor homologous factors: evolution, structure, and function. *Cytokine Growth Factor Rev*. 2005; 16(2):215–220. [PubMed: 15863036]
- Goldfarb M, Schoorlemmer J, Williams A, Diwakar S, Wang Q, Huang X, et al. Fibroblast growth factor homologous factors control neuronal excitability through modulation of voltage-gated sodium channels. *Neuron*. 2007; 55(3):449–463. [PubMed: 17678857]
- Hennessey JA, Marcou CA, Wang C, Wei EQ, Wang C, Tester DJ, et al. FGF12 is a candidate Brugada syndrome locus. *Heart Rhythm*. 2013; 10(12):1886–1894. [PubMed: 24096171]

- Kaplan MR, Cho MH, Ullian EM, Isom LL, Levinson SR, Barres BA. Differential control of clustering of the sodium channels Na(v)1.2 and Na(v)1.6 at developing CNS nodes of Ranvier. *Neuron*. 2001; 30(1):105–119. [PubMed: 11343648]
- Konig HG, Fenner BJ, Byrne JC, Schwamborn RF, Bernas T, Jefferies CA, et al. Fibroblast growth factor homologous factor 1 interacts with NEMO to regulate NF-kappaB signaling in neurons. *J Cell Sci*. 2012; 125(Pt 24):6058–6070. [PubMed: 23097049]
- Laezza F, Gerber BR, Lou JY, Kozel MA, Hartman H, Craig AM, et al. The FGF14(F145S) mutation disrupts the interaction of FGF14 with voltage-gated Na⁺ channels and impairs neuronal excitability. *J Neurosci*. 2007; 27(44):12033–12044. [PubMed: 17978045]
- Laezza F, Lampert A, Kozel MA, Gerber BR, Rush AM, Nerbonne JM, et al. FGF14 N-terminal splice variants differentially modulate Nav1.2 and Nav1.6-encoded sodium channels. *Mol Cell Neurosci*. 2009; 42(2):90–101. [PubMed: 19465131]
- Lai HC, Jan LY. The distribution and targeting of neuronal voltage-gated ion channels. *Nat Rev Neurosci*. 2006; 7(7):548–562. [PubMed: 16791144]
- Leterrier C, Brachet A, Dargent B, Vacher H. Determinants of voltage-gated sodium channel clustering in neurons. *Semin Cell Dev Biol*. 2011; 22(2):171–177. [PubMed: 20934527]
- Liu C, Dib-Hajj SD, Waxman SG. Fibroblast growth factor homologous factor 1B binds to the C terminus of the tetrodotoxin-resistant sodium channel rNav1.9a (NaN). *J Biol Chem*. 2001; 276(22):18925–18933. [PubMed: 11376006]
- Liu CJ, Dib-Hajj SD, Renganathan M, Cummins TR, Waxman SG. Modulation of the cardiac sodium channel Nav1.5 by fibroblast growth factor homologous factor 1B. *J Biol Chem*. 2003; 278(2):1029–1036. [PubMed: 12401812]
- Lou JY, Laezza F, Gerber BR, Xiao M, Yamada KA, Hartmann H, et al. Fibroblast growth factor 14 is an intracellular modulator of voltage-gated sodium channels. *J Physiol*. 2005; 569(Pt 1):179–193. [PubMed: 16166153]
- Munoz-Sanjuan I, Smallwood PM, Nathans J. Isoform diversity among fibroblast growth factor homologous factors is generated by alternative promoter usage and differential splicing. *Journal of Biological Chemistry*. 2000; 275(4):2589–2597. [PubMed: 10644718]
- Olsen SK, Garbi M, Zampieri N, Eliseenkova AV, Ornitz DM, Goldfarb M, et al. Fibroblast growth factor (FGF) homologous factors share structural but not functional homology with FGFs. *J Biol Chem*. 2003; 278(36):34226–34236. [PubMed: 12815063]
- Rush AM, Wittmack EK, Tyrrell L, Black JA, Dib-Hajj SD, Waxman SG. Differential modulation of sodium channel Na(v)1.6 by two members of the fibroblast growth factor homologous factor 2 subfamily. *Eur J Neurosci*. 2006; 23(10):2551–2562. [PubMed: 16817858]
- Schoorlemmer J, Goldfarb M. Fibroblast growth factor homologous factors are intracellular signaling proteins. *Curr Biol*. 2001; 11(10):793–797. [PubMed: 11378392]
- Schoorlemmer J, Goldfarb M. Fibroblast growth factor homologous factors and the islet brain-2 scaffold protein regulate activation of a stress-activated protein kinase. *J Biol Chem*. 2002; 277(51):49111–4919. [PubMed: 12244047]
- Shakkottai VG, Paulson HL. Physiologic alterations in ataxia: channeling changes into novel therapies. *Arch Neurol*. 2009; 66(10):1196–1201. [PubMed: 19822774]
- Shakkottai VG, Xiao M, Xu L, Wong M, Nerbonne JM, Ornitz DM, et al. FGF14 regulates the intrinsic excitability of cerebellar Purkinje neurons. *Neurobiol Dis*. 2009; 33(1):81–88. [PubMed: 18930825]
- Shavkunov AS, Wildburger NC, Nenov MN, James TF, Buzhdygan TP, Panova-Elektronova NI, et al. The fibroblast growth factor 14.voltage-gated sodium channel complex is a new target of glycogen synthase kinase 3 (GSK3). *J Biol Chem*. 2013; 288(27):19370–19385. [PubMed: 23640885]
- Smallwood PM, Munoz-Sanjuan I, Tong P, Macke JP, Hendry SH, Gilbert DJ, et al. Fibroblast growth factor (FGF) homologous factors: new members of the FGF family implicated in nervous system development. *Proc Natl Acad Sci U S A*. 1996; 93(18):9850–9857. [PubMed: 8790420]
- Vacher H, Trimmer JS. Trafficking mechanisms underlying neuronal voltage-gated ion channel localization at the axon initial segment. *Epilepsia*. 2012; 53(Suppl 9):21–31. [PubMed: 23216576]

- van Swieten JC, Brusse E, de Graaf BM, Krieger E, van de Graaf R, de Koning I, et al. A Mutation in the Fibroblast Growth Factor 14 Gene Is Associated with Autosomal Dominant Cerebral Ataxia. *The American Journal of Human Genetics*. 2003; 72(1):191–199. [PubMed: 12489043]
- Venkatesan K, Liu Y, Goldfarb M. Fast-Onset Long-Term Open-State Block of Sodium Channels by A-type FHF_s Mediates Classical Spike Accommodation in Hippocampal Pyramidal Neurons. *J Neurosci*. 2014; 34(48):16126–16139. [PubMed: 25429153]
- Wang C, Chung BC, Yan H, Lee SY, Pitt GS. Crystal structure of the ternary complex of a NaV C-terminal domain, a fibroblast growth factor homologous factor, and calmodulin. *Structure*. 2012; 20(7):1167–1176. [PubMed: 22705208]
- Wang C, Chung BC, Yan H, Wang HG, Lee SY, Pitt GS. Structural analyses of Ca²⁺/CaM interaction with NaV channel C-termini reveal mechanisms of calcium-dependent regulation. *Nat Commun*. 2014; 5:4896. [PubMed: 25232683]
- Wang C, Wang C, Hoch EG, Pitt GS. Identification of novel interaction sites that determine specificity between fibroblast growth factor homologous factors and voltage-gated sodium channels. *J Biol Chem*. 2011; 286(27):24253–24263. [PubMed: 21566136]
- Wang Q, Bardgett ME, Wong M, Wozniak DF, Lou J, McNeil BD, et al. Ataxia and paroxysmal dyskinesia in mice lacking axonally transported FGF14. *Neuron*. 2002; 35(1):25–38. [PubMed: 12123606]
- Wittmack EK, Rush AM, Craner MJ, Goldfarb M, Waxman SG, Dib-Hajj SD. Fibroblast growth factor homologous factor 2B: association with Nav1.6 and selective colocalization at nodes of Ranvier of dorsal root axons. *J Neurosci*. 2004; 24(30):6765–6775. [PubMed: 15282281]
- Wozniak DF, Xiao M, Xu L, Yamada KA, Ornitz DM. Impaired spatial learning and defective theta burst induced LTP in mice lacking fibroblast growth factor 14. *Neurobiol Dis*. 2007; 26(1):14–26. [PubMed: 17236779]
- Wu QF, Yang L, Li S, Wang Q, Yuan XB, Gao X, et al. Fibroblast growth factor 13 is a microtubule-stabilizing protein regulating neuronal polarization and migration. *Cell*. 2012; 149(7):1549–1564. [PubMed: 22726441]
- Xiao M, Bosch MK, Nerbonne JM, Ornitz DM. FGF14 localization and organization of the axon initial segment. *Mol Cell Neurosci*. 2013; 56:393–403. [PubMed: 23891806]
- Xiao M, Xu L, Laezza F, Yamada K, Feng S, Ornitz DM. Impaired hippocampal synaptic transmission and plasticity in mice lacking fibroblast growth factor 14. *Mol Cell Neurosci*. 2007; 34(3):366–377. [PubMed: 17208450]
- Xu M, Gu Y, Barry J, Gu C. Kinesin I transports tetramerized Kv3 channels through the axon initial segment via direct binding. *J Neurosci*. 2010; 30(47):15987–16001. [PubMed: 21106837]
- Yan H, Pablo JL, Pitt GS. FGF14 regulates presynaptic Ca²⁺ channels and synaptic transmission. *Cell Rep*. 2013; 4(1):66–75. [PubMed: 23831029]
- Yan H, Pablo JL, Wang C, Pitt GS. FGF14 modulates resurgent sodium current in mouse cerebellar Purkinje neurons. *Elife*. 2014:3.
- Zanni G, Bertini E, Bellcross C, Nedelec B, Froyen G, Neuhauser G, et al. X-linked congenital ataxia: a new locus maps to Xq25-q27.1. *Am J Med Genet A*. 2008; 146A(5):593–600. [PubMed: 18241076]
- Zhang Y, Bekku Y, Dzhashiashvili Y, Armenti S, Meng X, Sasaki Y, et al. Assembly and maintenance of nodes of ranvier rely on distinct sources of proteins and targeting mechanisms. *Neuron*. 2012; 73(1):92–107. [PubMed: 22243749]
- Zhu X, Komiya H, Chirino A, Faham S, Fox GM, Arakawa T, et al. Three-dimensional structures of acidic and basic fibroblast growth factors. *Science*. 1991; 251(4989):90–93. [PubMed: 1702556]

Summary

FHFs are emerging as an intriguing family of molecules that have broad effects on neuronal function, playing critical roles in the development, physiology and disease of the mammalian nervous system (Figure 3). Though FHFs have been established as intracellular modulators of voltage gated sodium-channels, much is still unknown about their specific modes of action and their roles in other neurobiological processes. Answering these questions will not only enlighten specific roles for FHFs, but will also reveal novel aspects of neuronal cell biology and yield insight into disease mechanisms.

Figure 1. The organization of FHF domains
All four members of the FHF family share a highly similar core region homologous to the canonical FGF β -trefoil core. In addition, FHF's have extended N- and C-termini. Each member of the FHF family has isoforms that are generated by the alternative splicing of different first exons thus leading to proteins that are identical except for their N-termini. FGF11, 12 and 14 have two isoforms each while FGF13 has five.

Figure 2. The Nav binding site of FHF

Crystal structures of FHF in complex with Navs show that this interaction depends on FHF “protrusions” that fit into Nav “depressions”. Shown here is a representative ribbon diagram of an FHF (FGF13) binding to a space-filling model of a Nav C-terminus. The Arg57 residue on FHF is highlighted in yellow as mutation of this residue is sufficient to abrogate FHF-Nav binding.

Figure 3. The diversity of FHF functions

Shown is a summary of the various FHF functions discussed in the text. Functions directly supported by evidence in the literature are in black text while functions that have only been speculated at are in violet.