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Fibroblast growth factor homologous factors in the heart: a potential locus for cardiac arrhythmias

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Abstract

The four fibroblast growth factor homologous factors (FHF, FGF11–FGF14) are intracellular proteins that bind and modulate voltage-gated sodium channels (VGSCs). While FHF have been well studied in neurons and implicated in neurologic disease, their role in cardiomyocytes was unclear until recently. In this review, we discuss the expression profile and function of FHF in mouse and rat ventricular cardiomyocytes. Recent data show that FGF13 is the predominant FHF in the murine heart, directly binds the cardiac VGSC α subunit, and is essential for normal cardiac conduction. FHF loss-of-function mutations may be unrecognized causes of cardiac arrhythmias, such as Long QT and Brugada syndromes.

Introduction

Fibroblast growth factor homologous factors (FHF) are a subset of the fibroblast growth factor (FGF) family (Goldfarb 2005). Comprised of four genes (FGF11–14), FHF were cloned by homology after FHF1 (FGF12) was first identified in a screen for novel genes expressed in the retina (Smallwood et al. 1996). For each FHF, multiple isoforms with distinct N-terminal sequences are produced through alternative promoter usage and alternative splicing, yielding 10 FHF isoforms in humans (Munoz-Sanjuan et al., 2000). Unlike canonical FGFs, FHF lack signal sequences, are not secreted, and do not bind to or activate FGF receptors. Rather, these intracellular proteins bind to and modulate voltage-gated sodium channels (VGSCs) and are potential loci for cardiac conduction abnormalities (Wang et al. 2011a).

VGSCs, of which there are nine isoforms (Nav1.1–Nav1.9) (Catterall et al. 2005), are a family of proteins that mediate inward Na^+ current upon membrane depolarization. Nav1.5 is the principal VGSC found in heart, is responsible for the phase 0 upstroke of the cardiac action potential, and is essential for propagation of the electrical impulse through the heart. VGSCs are comprised of a main α , pore-forming subunit which contains four homologous domains (Figure 1). The α subunit is sufficient to form a functional channel on its own but is typically associated with one or more β subunits, which regulate channel gating kinetics and trafficking to the membrane (Meadows et al. 2005). Further, the three cytoplasmic

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domain interlinker regions and the N- and C- termini bind multiple interacting proteins, providing additional modulation (Abriel 2010).

The four mammalian FHF s bind directly to the C-termini of VGSC s (Wang et al. 2011b, Wang et al. 2011a, Liu et al. 2001, Goetz et al. 2009), and different FHF isoforms differentially modulate channel function (Lou et al. 2005, Rush et al. 2006). FHF dysfunction alters VGSC behavior in the nervous system and has been implicated in neurologic disease (Shakkottai et al. 2009, Laezza et al. 2007). Spinocerebellar ataxia 27 is caused by a missense mutation in FGF14 (van Swieten et al. 2003). Additionally, nonspecific forms of X-linked mental retardation have been mapped to the FGF13 locus, suggesting that the FGF13 gene may be a candidate for these disorders (Gecz et al. 1999).

While the role of FHF s in neurons has received increasing attention, their function in cardiomyocytes has only recently been investigated (Wang et al. 2011a). This review focuses on FHF regulation of Na_v1.5 in cardiomyocytes and explores the idea that FHF dysfunction may be an unrecognized cause of cardiac arrhythmias.

FGF13 is the predominant cardiac FHF in mice

While all FHF s are highly expressed in the nervous system and developing heart (Smallwood 1996), the expression profile of FHF s in adult cardiomyocytes remained unclear until recently (Wang et al. 2011a). Quantitative reverse-transcriptase polymerase chain reaction with isoform-specific primers showed that FGF13 is the most abundant FHF in adult mouse and rat cardiomyocytes and that other FHF genes were expressed at relatively low levels. Furthermore, only FGF13 could be detected by western blot of mouse cardiomyocyte lysate. These results are consistent with a recently published expression atlas of the FGF system in adult mouse showing high levels of FGF13 in the heart and low levels of other FHF s (Fon Tacer et al. 2010). The expression patterns of FHF s in human heart have not been reported and may be different than in mouse.

FGF13 binds Na_v1.5 directly in mouse cardiomyocytes

FGF13, in addition to being the predominant cardiac FHF, directly binds Na_v1.5, the principal cardiac VGSC α subunit (Wang et al. 2011a). FGF13 co-immunoprecipitated with Na_v1.5 from mouse cardiomyocyte lysates, suggesting that FGF13 and Na_v1.5 are in the same complex. An in vitro binding assay utilizing the Na_v1.5 C-terminus and lysates expressing specific FGF13 isoforms indicates that this interaction is likely direct and that the C-terminus of the Na_v1.5 binds to the core of FGF13. Data from SPR spectroscopy also support the hypothesis that the VGSC α subunit C-terminus and FGF13 core region interact directly (Goetz et al. 2009, Wang et al. 2011b, Wang et al. 2011a). A recently published crystal structure of a ternary complex of the Na_v1.5 C-terminus domain (CTD), FGF13, and calmodulin shows that FGF13 binds to the globular domain of the Na_v1.5 CTD, and residues distal to Ser1885 contribute significantly to the FHF interaction site on Na_v1.5 (Figure 2) (Wang et al. 2012). Furthermore, immunocytochemistry shows that a portion of FGF13 colocalizes with Na_v1.5 at the lateral membrane and intercalated discs in cardiomyocytes, two regions with distinct Na_v1.5 populations (Wang et al. 2011a, Petitprez et al. 2010).

FGF13 modulates sodium channel current density and kinetics in cardiomyocytes

The effect of FHF-VGSC α subunit binding on channel properties has been widely studied in heterologous expression systems and neurons. In these settings, FHF binding modulates Na⁺ current density, channel availability, and channel expression, the degree to which varies

depending on the FHF isoform and VGSC α subunit (Lou et al. 2005, Rush et al. 2006, Laezza et al. 2007).

The function of FHFs in cardiomyocytes and their role in the modulation of endogenous Na^+ currents has not been studied until recently (Wang et al. 2011a). Knockdown of FGF13 by infection of adult mouse ventricular cardiomyocytes with an adenovirus expressing a shRNA to all FGF13 splice variants reduced peak Na^+ current density by 49%. Knockdown of FGF13 did not alter $\text{Na}_V1.5$ total protein or mRNA levels. However, surface $\text{Na}_V1.5$, measured by surface biotinylation, was reduced by 45%. In addition, FGF13 knockdown hyperpolarized the steady-state inactivation curve and delayed channel recovery from inactivation, further reducing channel availability. Together, these data suggest that FGF13- $\text{Na}_V1.5$ binding is necessary for proper channel function in cardiomyocytes.

FGF13 is required for normal cardiac conduction

Cardiac conduction can be studied in ventricular monolayers using optical mapping (Sathaye et al. 2006). FGF13 knockdown in rat cardiomyocyte monolayers resulted in markedly slowed conduction velocity (Wang et al. 2011a). Maximum capture rate, a measure of monolayer response to pacing, was significantly reduced after FGF13 knockdown. These studies shed light on the physiologic role of FGF13 in modulating VGSC function. Loss of function of FGF13 is associated with decreased cardiac conduction properties, due to reduced Na^+ channel current density and delayed recovery from inactivation.

FHF mutations may be unidentified loci for cardiac arrhythmias

The alterations in $\text{Na}_V1.5$ sarcolemmal expression and gating kinetics induced by FGF13 knockdown suggest that FHF loss-of-function mutations may be unrecognized causes of cardiac arrhythmias associated with VGSC loss of function. In addition, mutations in the gene encoding $\text{Na}_V1.5$ (SCN5A) that disrupt FHF interaction may produce equivalent effects as FHF loss-of-function mutations. FGF13 knockdown in mouse provides a model for studying potential loss-of-function mutations in cardiac FHFs, which may promote arrhythmogenesis via a combination of several mechanisms: (1) reduced trafficking of $\text{Na}_V1.5$ to the plasma membrane; (2) reduced channel availability; and (3) delayed recovery from inactivation, leading to (4) decreased conduction velocity (Figure 3).

VGSC dysfunction is central to various inherited arrhythmias, including Long QT syndrome (LQTS) and Brugada syndrome. LQTS type 3 arises from mutations in SCN5A which disrupt fast inactivation of $\text{Na}_V1.5$, increasing inward sodium current and thereby prolonging the QT interval (Wang et al. 1995). Moreover, in recent years, mutations in genes encoding $\text{Na}_V1.5$ regulatory proteins have been discovered as new loci for cardiac arrhythmias. For instance, congenital LQTS types 9, 10, and 12 are linked to mutations in CAV3 (Caveolin-3), SCN4B (Beta subunit 4 of sodium channel), and SNTA1 (Alpha 1 syntrophin), respectively, all of which lead to an increase in $\text{Na}_V1.5$ persistent current (Ueda et al. 2008, Medeiros-Domingo et al. 2007, Vatta et al. 2006).

While no mutations in FHFs have yet been associated with inherited arrhythmias, it is possible that FHF dysfunction in cardiomyocytes may disrupt fast inactivation of cardiac VGSCs, serving as an additional, yet unidentified locus for LQTS. It has been speculated that overexpression of FHFs above their endogenous low levels in the heart may produce physiological changes similar to those of LQTS (Liu et al. 2003). The recent crystal structure of the FGF13 binding sites on $\text{Na}_V1.5$ suggests that FGF13 binds in a region of the C-terminus that contains a D1839G mutation associated with LQTS (Figure 2) (Wang et al. 2012, Benhorin et al. 1998). This mutation may alter interaction with a knob formed by a conserved asparagine on FHFs, possibly affecting the affinity of FHF binding. Thus, it is

possible that mutations at sites on either the FHF or Nav1.5 CTD binding surfaces may produce a LQTS phenotype.

Of note, Liu et al. previously reported that a D1790G LQTS mutation abolished binding of FGF12B to the Nav1.5 CTD (Liu et al. 2003, An et al. 1998). However, as shown in Figure 2, the aspartate at 1790 does not contribute to the critical residues that form the FHF binding surface. Given that the interaction motif is conserved among Nav1.x CTD-FHF pairs (Wang et al. 2012), it is unlikely that FGF12B binds at a site different from FGF13; the identification of aspartate 1790 as a contributor to FHF binding may have been due to the use of a sensitive but not specific yeast two-hybrid method that used indirect assessment of interaction to perform the binding site mapping experiment.

Brugada syndrome is often associated with a decrease in peak sodium current due primarily to Nav1.5 biophysical defects (Hedley et al. 2009). Given that only about 20% of Brugada cases to date have defined genetic defects (Hedley et al. 2009), FHF mutations may be unexplored loci for the loss of Nav1.5 channel function. Mechanisms for the decrease in current density include (1) reduced expression of the channel; (2) a shift in voltage or time dependence of activation, inactivation, or reactivation; and (3) accelerated or prolonged recovery from inactivation (Antzelevitch et al. 2005). FHF dysregulation may contribute to the pathogenesis of Brugada syndrome through many of these mechanisms. The FGF13 knockdown data suggest that FHF loss-of-function mutations may reduce current density by decreasing channel surface expression and causing a hyperpolarizing shift in inactivation. Furthermore, one of the hallmarks of Brugada syndrome – slowed conduction – could be explained by delayed Na⁺ channel recovery from inactivation. FHF loss-of-function mutations may lower the threshold of heart rate for which VGSCs can fully recover, decreasing conduction velocity and maximum capture rate, and providing a substrate for ventricular tachycardia/ventricular fibrillation and re-entrant arrhythmias.

In addition to modulating channel gating kinetics, FHFs play an important role in proper trafficking of sodium channels to the cell membrane. In neurons, FGF14 appears to participate in targeting VGSCs to the axon initial segment (AIS): expression of an FGF14 spinocerebellar ataxia 27-causing dominant negative mutant abolished binding of wild-type FGF14 to Nav1.2 and reduced targeting of VGSCs to the AIS (Laezza et al. 2007). In the heart, FGF13 appears to play an analogous role in targeting sodium channels to the sarcolemma, as knockdown of FGF13 is associated with reduced surface expression of Nav1.5. Just as FHF mutations in the CNS impair neuronal excitability, mutations of FHFs in the heart, in addition to altering channel biophysical properties, may reduce cardiac excitability by disrupting channel membrane trafficking. It is unclear whether FGF13-mediated trafficking requires direct binding between FGF13 and the Nav1.5 C-terminus, or whether FGF13 acts in an integrated fashion with other channel modulators. Because numerous modulatory proteins as well as the Nav β subunits also bind to the Nav1.x C-terminus and regulate channel trafficking (Abriel 2010), it is conceivable that FHFs may work in concert with those other trafficking proteins. Laezza et al. speculate, for instance, that FHFs may modulate Nav α -Nav β subunit-subunit interactions (Laezza et al. 2007).

FHFs appear to be similar in function to Ankyrins, adaptor proteins responsible for proper expression and localization of ion channels and transporters in excitable and non-excitable cells (Mohler 2006). Ankyrin-B (ANK2) and Ankyrin-G (ANK3) are the two predominantly expressed isoforms in the heart. Ankyrin-G binds to Nav1.5 at a motif in the linker loop between domains II and III (Figure 1) (Lemaitre et al. 2003); no direct interaction has been found between Ankyrin-B and Nav1.5. Genetic variants in ANK2 have been linked to LQTS type 4, also known as “Ankyrin-B Syndrome,” an atypical arrhythmia with risk of sudden cardiac death (Mohler 2006). Additionally, a mutation in the consensus sequence in

Nav1.5 for binding to Ankyrin-G has been associated with Brugada syndrome (Mohler et al. 2004). This mutation reduced surface expression of Nav1.5 in cardiomyocytes, and affected channel gating behavior in a heterologous expression system, suggesting that Ankyrin-G may modulate gating properties, in addition to its role in trafficking VGSCs to the cell membrane (Mohler et al. 2004). In a similar manner, mutations in the sequences encoding the C-terminus of Nav1.5 may abolish FHF binding, leading to reduced channel surface expression and abnormal gating properties. Whether FHFs and Ankyrins interact to regulate channel activity remains unclear and merits further investigation.

It is likely that FHFs play other important roles in cardiomyocytes that have yet to be explored. Immunocytochemistry shows that FGF13 exhibits a widespread expression pattern in ventricular cardiomyocytes, including a punctate distribution in the cytoplasm and prominent nuclear localization (Wang et al. 2011a). Of particular interest for future investigation are potential interactions between FHFs and other cardiac ion channels, as well as nuclear proteins.

Summary

FHFs are intracellular proteins that play a critical role in the membrane targeting and function of VGSCs in the heart. FGF13 is the predominant FHF in adult murine ventricular cardiomyocytes. It binds directly to Nav1.5, modulating channel trafficking to the sarcolemma, gating kinetics, current density, and conduction velocity through cardiac tissue. These modulatory effects on VGSCs suggest that loss-of-function mutations in FHFs may underlie cardiac arrhythmias, including LQTS and Brugada syndrome.

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Figure 1.

Illustration of the α subunit, two β subunits, and key modulatory proteins of Na_V1.5. The pore-forming α subunit contains four homologous domains (I–IV) – each made up of six transmembrane segments (S1–S6) – which assemble into a tetrameric structure. The N and C termini and three inter-domain linker loops are cytoplasmic. Channel activation is mediated by a charged S4 transmembrane segment; fast inactivation is mediated by hydrophobic residues in the III–IV linker. Ankyrin-G binds in the II–III linker and regulates channel membrane trafficking. The α subunit interacts with one or more β subunits, which regulate gating kinetics and membrane trafficking. FGF13 binds directly to the cytoplasmic C-terminus. The aspartate at 1790 has been reported to be associated with a LQTS mutation but lies outside the FGF13 binding site.

Figure 2.

Crystal structure of the complex of Na_v1.5 C-terminus domain (CTD) and FGF13 (Wang et al. 2012). This model contains the Na_v CTD amino acids 1776–1928 (blue) and FGF13U amino acids 11–158 (red). The Na_v CTD is comprised of a globular domain followed by an extended 29 amino acid helix. FGF13 binds directly to the globular domain. Highlighted in yellow are two aspartic acid residues that have been associated with LQTS mutations. One of these, D1839, is a critical residue that mediates binding to FGF13 whereas the other, D1790, is proximal to the FGF13 binding site.

Figure 3.

Illustration of FGF13 regulation of $\text{Na}_v1.5$ gating kinetics and membrane trafficking in wildtype (WT) cardiomyocyte (left panel) and FGF13 knockdown cardiomyocyte (right panel). Binding of FGF13 to the C-terminus of $\text{Na}_v1.5$ modulates the voltage-dependence of channel inactivation. FGF13 knockdown reduces the number of channels available to be activated – in the patch of sarcolemma illustrated, 1 of 3 channels is activated in the knockdown cardiomyocyte compared to 4 of 5 channels in WT cardiomyocyte. FGF13 is also required for proper trafficking of channels to the membrane – in the knockdown, only 3 of 5 channels are expressed at the membrane. Collectively, the reduced channel availability and surface expression lead to reduced current density. Furthermore, in the knockdown, delayed recovery from inactivation slows conduction velocity through cardiac tissue, providing a possible substrate for re-entrant arrhythmias.