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iFGF14-Na_vs: A monogamous partnership?

Sulayman D. Dib-Hajj

Center for Neuroscience & Regeneration Research, Yale Medical School and Veterans Affairs Hospital, West Haven, CT, USA

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Fibroblast growth factors iFGF11-14, also known as fibroblast homologous factors (FHF1-4), are distinct from other members of the FGF family because they lack a signal peptide for secretion and do not bind to or activate FGF receptors [for review,[1]]. Members of the iFGFs subfamily, for example iFGF14, have identical core but divergent N-termini, which are encoded by alternatively spliced exons. The mystery of the iFGFs function began to unravel when they were shown to interact directly with the pore-forming α-subunits of several voltage-gated sodium channels (Na_vs) and regulated their current density, gating properties, and localization at the axon initial segment, with alternatively spliced isoforms of iFGF13 shown to localize at different neuronal compartments.[2-5] A role in regulating neuronal excitability *in vivo* was supported by identifying iFGF14 mutations in patients with spinocerebellar ataxia 27 (SCA27) as well as studies of mice carrying knockouts of these factors.[1] Similar to their interaction with Na_vs, iFGF13 and iFGF14 increase density of voltage-gated calcium channels, $Ca_v2.1$ and $Ca_v2.2$, at the plasma membrane enhancing synaptic transmission, however, this effect does not require a direct interaction of the iFGFs with these Ca_vs.[6] Although iFGFs interact with other proteins, the best understood role of these factors to date is that they modulate ion channels and regulate physiological processes of excitable cells.

The functional studies mentioned above were hypothesis-driven investigations of the effect of one iFGF regulator and one ion channel. This approach is necessary for determining the impact of a regulator interacting with an effector molecule. However, functional ion channel complexes are networks of channel subunits and partners that may enter into transient or long-term interactions with the pore-forming subunit and influence its trafficking, localization and gating properties. Thus, an unbiased proteomic approach is best suited to identify protein networks and gain insights into biologically-relevant interactions. For example, a comprehensive study has shown that different Ca_v2 channel subtypes could form complexes with roughly 200 proteins of distinct abundance and strength of interaction with the α-subunit.[7] In this volume of Channels, a similar approach was employed by Bosch et al.,[8] to identify a network of interactions with iFGF14 in mouse cerebellum. There are several strengths in this report including the use of a well-characterized antibody to immunoprecipitate protein complexes which form around iFGF14 or Na_vs, the use of wild-type and iFGF14-null mice to confirm the specificity of the captured complex, and the use of 2D-LC-MS/MS for protein identification. Confidence in the results is enhanced by the well-controlled and quantitative assays that optimized the concentrations of the antibodies and starting cerebellar lysates for the purification of the iFGF and Na_vs networks.

The main conclusions of this study are that the majority of the identified proteins are Na_vs or proteins that are known to interact with them, for example auxiliary β subunits, and that there were no major observed differences in the composition of the purified

CONTACT Sulayman D. Dib-Hajj sulayman.dib-hajj@yale.edu Center for Neuroscience & Regeneration Research, Yale Medical School and Veterans Affairs Administration Medical Center, 950 Campbell Ave, Bldg 34, West Haven, CT 06516, USA.
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complexes of Na_vs from wildtype or fgf14$^{-/-}$ mice, suggesting that iFGF14 does not recruit or substitute for another channel partner. This data is consistent with the role of iFGF14 in regulating cerebellar function. Notably, however, no Ca_v channels or their accessory subunits were identified in the iFGF14 complexes. This finding is consistent with the absence of the iFGF14 from the Ca_v protein networks that were reported earlier.[7] As the authors posit, the interaction with Ca_vs could be transient or weaker than necessary to be retained under the experimental conditions used in this study. Future work is needed to distinguish between these possibilities. The fact that synaptotagmin and calmodulin are identified in iFGF14 complexes from wildtype and fgf14$^{-/-}$ mice, albeit in reduced quantities, suggests some cross reactivity of the iFGF14 antibody with other proteins, including other members of the iFGF subfamily, or a non-specific interaction with synaptotagmin and calmodulin. Repeating these assays while varying conditions for lysate preparation, binding and elution conditions, and the use of multiple target specific antibodies will be needed to paint a comprehensive picture of the iFGF-ion channel network. Thus, it remains to be seen whether iFGF has a monogamous relationship with Na_vs *in vivo*.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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