Roles of intracellular fibroblast growth factors in neural development and functions

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Fibroblast growth factors (FGFs) can be classified as secretory (FGF1–10 and FGF15–23) or intracellular non-secretory forms (FGF11–14). Secretory forms of FGF and their receptors are best known for their regulatory roles in cell growth, differentiation and morphogenesis in the early stages of neural development. However, the functions of intracellular FGFs remain to be explored. FGF12 and FGF14 are found to interact with voltage-gated sodium channels, and regulate the channel activity in neurons. FGF13 is expressed in primary sensory neurons, and is colocalized with sodium channels at the nodes of Ranvier along the myelinated afferent fibers. FGF13 is also expressed in cerebral cortical neurons during the late developmental stage. A recent study showed that FGF13 is a microtubule-stabilizing protein required for regulating the neuronal development in the cerebral cortex. Thus, non-secretory forms of FGF appear to have important roles in the brain, and it would be interesting to further investigate the functions of intracellular FGFs in the nervous system and in neural diseases.

fibroblast growth factors, nervous system, development, microtubule, ion channel, X-linked mental retardation

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their receptors play essential roles in the early stages of neural development [4]. Some secretory FGFs are also involved in affective disorders and modulate emotionality in animal models [8]. Conversely, knowledge about the functions and related mechanisms of intracellular FGFs in the nervous system is still limited when compared with what is known about secretory FGFs and their receptors. It is known that intracellular FGFs can interact with voltage-gated Na$^+$ channels, and are required for normal motor function [5]. Recently, we also found that FGF13B is required for the development of the cerebral cortex [9].

Although the expression and functions of FGFs have been summarized and discussed recently [4,5], we feel that it is necessary to further summarize and discuss the research progress of intracellular FGFs in particular. Therefore, the present review will focus on recent studies of the expression and functional roles of intracellular FGFs in the nervous system (Table 1). The potential roles of intracellular FGFs in neural diseases will be also discussed.

1 Expression of intracellular FGFs in the nervous system

Members of the FGF11 family are expressed in cerebral cortical neurons during development [5,7,9,21]. FGF13 is also widely distributed in the developing brain with an expression level ~5-fold higher than FGF11, FGF12 and FGF14 [7]. Two alternatively spliced isoforms of FGF13 have been identified: FGF13A, which contains a NLS, and cytoplasmic FGF13B which does not contain a NLS [7,22]. FGF13B expression in the cerebral cortex of mice and rats gradually increases from embryonic day 14 (E14) until postnatal day 7 (P7), after which its expression level begins to decrease [9]. In the developing brain of mice, FGF13 is present in the subplate (SP), ganglionic eminences (GE) and proliferative zones of the cortical wall [the ventricular zone (VZ) and the subventricular zone (SVZ)] at E14. It is present in the cortical plate (CP) of the cerebral cortex, hippocampus and striatum from E17 to P14. FGF13 expression is largely absent in the adult mouse brain, except for low levels in the hippocampus.

In contrast to brain, the dorsal root ganglia (DRG) show expression of FGF13 and FGF14 in adult animals [23,24]. FGF13 is expressed in 60% of DRG neurons under normal circumstances [23]. It has been shown that the gene expression profiles of DRG and the dorsal spinal cord are modified by peripheral nerve injury, leading to gene-regulation of many molecules, such as neurotransmitter receptors, ion channels and the Na$^+$,K$^+$-ATPase activator [25–30]. Indeed, after sciatic nerve transection, the expression of FGF2 and FGF7 increased in the DRG, whereas FGF13 expression decreased [23,24]. Seven days after nerve injury, FGF13-containing neurons decreased to 18%, and partially recovered to 40% at 28 days after injury. Despite these observed changes in FGF expression, their functional role in DRG neurons under normal circumstances and after nerve injury remain to be explored.

It is interesting to note that the expression patterns of non-secretory FGFs in the central nervous system are different from that in the peripheral nervous system during development and adulthood. A particular example is the...
differential FGF13 expression pattern, where FGF13 is expressed in the brain during development but not in adulthood, whereas it is present in adult DRG neurons [23,24]. Such distinct expression patterns suggest differential roles of these FGFs in the nervous system throughout life.

2 Role of intracellular FGFs in modulation of Na+ channel activity

With regard to the function of intracellular FGFs, accumulated evidence shows that they are important for regulating the activity of Na+ channels (Table 1). Intracellular FGFs are often colocalized with Na+ channels at the initial segments of axons and nodes of Ranvier [10,31,32]. Therefore, these FGFs might modulate the initiation and the propagation of action potentials [11,33–35]. FGF12B was found to bind to Na+,1.5 and Na+,1.9 channels, but not to Na+,1.7 or Na+,1.8 [36,37]. The interaction of FGF12B with Na+,1.5 channel caused a hyperpolarizing shift in steady-state inactivation [37]. FGF13B was found to associate with Na+,1.6, which is present at the nodes of Ranvier along central and peripheral axons and unmyelinated afferent fibers in the peripheral nervous system. FGF13B increased the amplitude of Na+,1.6 sodium currents in a transfected DRG-derived cell line ND7/23 [10]. Two alternatively spliced N-terminal FGF14 variants, FGF14-1a and FGF14-1b, differentially regulate currents produced by Na+,1.2 and Na+,1.6 channels [38]. Furthermore, intracellular FGF12 may be a component of tissue-specific protein kinase signaling modules, since FGF12 was found to interact with the MAP kinase (MAPK) scaffold protein Islet-Brain-2 (IB2) in the brain [22]. It remains unclear whether these intracellular FGFs increase the open probability of channels upon membrane depolarization by binding to the C-terminus of Na+, channels, by inserting more Na+ channels into the plasma membrane from the cytosolic pool, or by recruiting a kinase to the channel complex and the subsequent phosphorylation of the channel. It would be interesting to study the correlation between the regulation of Na+, channel activity and the signaling modules of intracellular FGFs in neurons.

Analysis of the crystal structure of FGF13A reveals a conserved surface required for binding to the C-terminal domain of the pore-forming (α) subunits of Na+, channels and for modulating the inactivation of Na+, channels by partially occluding the inner face of the pore [17]. The 152-residue-long homologous region of intracellular FGFs is sufficient for channel binding, and the 18-residue-long, C-terminal extension of the homologous region plays an essential role in channel binding [17]. Alternative splicing at the N-terminus of intracellular FGFs does not contribute to their interaction with the C-terminal domain of Na+, channels.

Table 1 Function of FGF11 family members in the nervous system

|                | Knockout | Knockdown | Overexpression | Mutation |
|----------------|----------|-----------|----------------|----------|
| **FGF12**      | Fg12+/−/Fg14+/− mice show severe ataxia behavior [10]. | Knockdown of FGF13 impairs neuronal polarization and increases the branching of axons and leading processes [9]. | Overexpression of FGF13B in ND7/23 cells increases the peak current amplitude and causes depolarizing shift of voltage-dependent inactivation of Na+ channels [11]. | Interruption of Fg13 by a duplication breakpoint in human is associated with XLMR [12]. |
| **FGF13**      | FGF13-deficient mice show neuronal migration defects in both neocortex and hippocampus and weakened learning and memory [9]. | In utero electroporation with FGF13 shRNA leads to neuronal migration defect in the somatosensory cortex [9]. | Overexpressing FGF13B induces stabilization of microtubule bundles in cultured cortical neurons [9]. | FGF14F145S mutation is associated with autosomal dominant cerebral ataxia [18]. |
| **FGF14**      | Fg14−/− mice show abnormalities in synaptic plasticity in hippocampus. [14]. | Fg14−/− mice exhibit impaired spatial learning and defective theta burst-induced LTP [15]. | Overexpression of FGF14-1a/b alters Na+, channel currents, and shifts the voltage dependence of Na+, channel activation and inactivation [17]. | Frame-shift mutation in the exon 4 of FGF14 gene and DNA polymorphisms is found in patients with inherited ataxias [19]. |
|                | Fg14−/− mice show impaired spontaneous and repetitive firing in Purkinje neurons [16]. | Fg14−/− mice show impaired spontaneous and repetitive firing in Purkinje neurons [16]. | The expression of FGF14F145S reduces the α subunit Na+, channels at the initial segment of axons, the currents of Na+, channels, and the excitability of hippocampal neurons [20]. | |
channels. FGF13A induces a greater shift in the voltage-dependence of inactivation when compared with FGF13B [10, 17, 39]. This suggests that the alternatively spliced N-terminus of FGF13A is more effective in occluding the channel pore.

Animal models of FGF-deficiency correlate with human diseases and give insight into the functions of intracellular FGFs. Studies show that Fgfl2 knockout mice display muscle weakness [5] (Table 1). FGF14-deficient mice develop ataxia, paroxysmal hyperkinetic movement disorder and cognitive impairment [13, 32, 40]. This may be attributed to the loss of FGF14, which impairs the intrinsic excitability of cerebellar Purkinje neurons [15] and the long-term potentiation of synapses in the hippocampal CA1 region [16, 31, 40]. Likewise in humans, an autosomal dominant missense mutation, FGF14P145S, results in progressive spinocerebellar ataxia and cognitive impairment [14, 41]. This is because the stability of FGF14P145S is reduced, which leads to a loss of FGF14 function [18, 41]. Furthermore, FGF14P145S may disrupt the interaction of FGF14 with Nav1.2 channel and impair neuronal excitability [42]. Frameshift mutation and polymorphisms of Fgf14 are also found in patients with inherited ataxias [20]. Therefore, Fgf12 and Fgf14 are important for regulating the neurotransmission of motor-functions. The functional roles of FGF13 expressed in adult hippocampal neurons and DRG neurons remain to be further investigated.

3 FGF13 functions in neural development

The extensive expression of FGF13 in the developing brain has prompted studies to investigate its functions. Early studies showed that FGF13 could induce tyrosine phosphorylation of mitogen-activated protein kinase (MAPK) and phospholipase C-gamma (PLC-γ) in hippocampal astrocytes [19]. Treatment of neuronal cultures from rat embryonic cortex with FGF13 increased the number of neurons containing gamma-aminobutyric acid (GABA) and choline acetyltransferase enzymes [19]. FGF13 is also involved in Xenopus neural differentiation [43]. In addition, FGF13 may be involved in limb development [44]. Pretreatment with intravenous FGF13 reduces infarct volume and ameliorates neurological deficits following focal cerebral ischemia in rats [45].

During brain development, cortical neurons migrate from the site of their last mitotic division towards their final destination and generate the proper neural circuits [46–48]. Proper regulation of microtubule dynamics during axon/leading process formation is crucial for the transition of neurons from the multipolar stage to a bipolar morphology, and is a prerequisite for initiating radial migration [49–51]. Tubulins, a number of microtubule-regulating proteins that promote microtubule assembly and proteins that protect microtubules from depolymerization (such as microtubule-stabilizing proteins), are all involved in this regulatory process [52]. Our recent study shows that FGF13B is a microtubule-stabilizing protein that is enriched in the growth cones of cortical neurons [9] (Table 1). FGF13 was found to promote microtubule polymerization and stabilization, enabling the polarization of developing cortical neurons. Knockdown of FGF13 expression impairs the polarization and migration of cortical neurons in developing brains of mice and rats [9] (Figure 2). Neuronal migration was delayed in both the neocortex and the CA1 region of hippocampus of Fgf13 knockout mice. Learning and memory were also impaired in these FGF13-deficient mice, although the positioning of neurons in the neocortex and hippocampus of adult mutant mice was similar to that in control mice [9]. These data indicate an essential role of FGF13 in establishing neural circuits in the cerebral cortex and enabling cognitive functions.

The core function of FGF13B in brain development is its direct interaction with tubulin via a tubulin-binding domain, FGF13B [50, 51]. Several members of the microtubule-stabilizing protein family, such as microtubule-associated proteins and doublecortin (DCX), are essential for the early phase of neuronal migration [53–57]. Reduction in DCX also attenuates the transition of multipolar neurons to bipolar ones in the developing cortex [54, 58]. Interestingly, FGF13 is enriched in the axons of cortical neurons while its level in the dendrites is lower than DCX [9]. FGF13 and DCX may function independently during neural development, because the inhibition of neuronal migration caused by loss of DCX could be rescued by overexpressing FGF13 and vice versa. Therefore, it would be interesting to investigate the differential functions and correlations among FGF13, DCX and other microtubule-stabilizing proteins.

4 FGF13 in X-linked mental retardation

In humans, FGF11, FGF12, FGF13 and FGF14 genes are located on chromosome 17, 3, X and 13, respectively [7]. X-linked mental retardation (XLMR) is an inherited intellectual disability with disordered neural development arising from many mutations along the X chromosome. Although mutations resulting in XLMR have been reported in 102 genes [59–61], only a few of them have been proven to directly cause intellectual disability via defined mechanisms that are supported by experimental approaches, such as in animal models. Interestingly, FGF13 is a candidate gene for the syndromal and non-specific forms of XLMR mapped to the q26 region of the X chromosome [5, 12, 61]. Direct evidence for an important role of FGF13 gene in XLMR is the impairment of learning and memory in FGF13-deficient mice [9]. We found that FGF13 loss delayed neuronal migration in both the neocortex and the hippocampus, and impaired learning and memory [9], indicating that the FGF13 function is essential for the development of cortical
structures and cognitive functions.

Duplication in Xq26 may contribute to XLMR [61,63,65]. Duplication may disrupt the coding or regulatory region of the gene located at its boundaries, or induce a dosage effect of proteins encoded by genes located within the duplicated region. In a Börjeson-Forssman-Lehmann syndrome-like patient, FGF13 may be interrupted by a duplication breakpoint [63]. However, it has to be pointed out that the phenotypes induced by FGF13 mutations can be different because these various mutations can generate diverse isoforms [44,63]. Different FGF13 isoforms or mutants may have distinct expression patterns, subcellular distribution and functions in development. Therefore, the phenotypes of FGF13 gene mutations may be determined largely by the mutation sites and the mutated products.

Although FGF13 is transiently expressed in the cerebral cortex only from E14 until P14 with a peak level at P7, FGF13-deficient mice exhibit marked defects in learning and memory. Therefore, the delayed neuronal migration and increased collateral branching of axons could disrupt the formation of the neural circuits and networks required for generating cognitive functions. At the moment, it remains unclear how synaptogenesis, action potential firing and synaptic transmission might be affected by loss of FGF13 function in adulthood, but some effects are expected because FGF13 may regulate neuronal excitability through modulating Na channels [10,17,32]. Therefore, FGF13-deficient mice may be a useful animal model for studying XLMR-related mechanisms.

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