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Fibroblast growth factor-2 signaling in neurogenesis and neurodegeneration

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Abstract

Fibroblast growth factor-2 (FGF2), also known as basic FGF, is a multi-functional growth factor. One of the 22-member FGF family, it signals through receptor tyrosine kinases encoding FGFR1-4. FGF2 activates FGFRs in cooperation with heparin or heparin sulfate proteoglycan to induce its pleiotropic effects in different tissues and organs, which include potent angiogenic effects and important roles in the differentiation and function of the central nervous system (CNS). FGF2 is crucial to development of the CNS, which explains its importance in adult neurogenesis. During development, high levels of FGF2 are detected from neurulation onwards. Moreover, developmental expression of FGF2 and its receptors is temporally and spatially regulated, concurring with development of specific brain regions including the hippocampus and substantia nigra pars compacta. In adult neurogenesis, FGF2 has been implicated based on its expression and regulation of neural stem and progenitor cells in the neurogenic niches, the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampal dentate gyrus. FGFR1 signaling also modulates inflammatory signaling through the surface glycoprotein CD200, which regulates microglial activation. Because of its importance in adult neurogenesis and neuroinflammation, manipulation of FGF2/FGFR1 signaling has been a focus of therapeutic development for neurodegenerative disorders, such as Alzheimer's disease, multiple sclerosis, Parkinson's disease and traumatic brain injury. Novel strategies include intranasal administration of FGF2, administration of an NCAM-derived FGFR1 agonist, and chitosan-based nanoparticles for the delivery of FGF2 in pre-clinical animal models. In this review, we highlight current research towards therapeutic interventions targeting FGF2/FGFR1 in neurodegenerative disorders.

I. Introduction: Background on FGF2

Fibroblast growth factors (FGFs) are a superfamily of proteins, most of which bind heparin and extracellular heparin sulfate proteoglycans (HSPGs) and have a homologous central core of 140 amino acids [1]. FGF2 has pleiotropic effects in different tissues and organs, including potent angiogenic effects and an important role in the differentiation and function

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of the central nervous system (CNS). In human, five different polypeptides can be formed from the same *FGF2* gene via five different mRNA translation initiation sites [2, 3]. In mouse, *FGF2* mRNA generates two 22/21 kDa high molecular weight (HMW) isoforms and one 18 kDa low molecular weight (LMW) isoform. Signaling of FGF2 occurs through the high-affinity tyrosine kinase receptors FGFR1-4 [4], however for the purposes of this review we will focus on its main receptor in the CNS, FGFR1. FGF2 is an established neurogenic factor for proliferation and differentiation of multipotent neural stem cells both during development and in the adult mouse brain[5–7].

II. FGF2 and Neurogenesis

FGF2/FGFR Expression in Development and in the Adult

FGF2 is central to development of the CNS, which explains its importance in adult neurogenesis. During development, high levels of FGF2 are detected from neurulation onwards[8]. Moreover, expression of FGF2 and its receptors is temporally and spatially regulated during development, concurring with neurogenesis in specific brain regions [9]. For example, in the inferior colliculus and occipital cortex of the postnatal rat brain, expression of FGF2 and FGFR2 increased over the first month, with no increase in FGFR1 mRNA. In contrast, in the cerebellum, FGF2 and FGFR1 mRNA expression was highest at postnatal day 1, but FGFR2 expression showed little change with age [10]. This regional and temporal specificity of FGF2 and FGFR expression may contribute to its regulation in the development of specific neuronal populations within different regions, such as dopaminergic neurons in the nigrostriatal pathway in mice [11].

In the adult CNS, FGF2 is expressed in the neurogenic niches (the subventricular zone of the lateral ventricles- SVZ; and the subgranular zone of the hippocampal dentate gyrus- SGZ) and has been implicated in the control of adult neurogenesis based on changes in proliferation and differentiation of adult neural stem and progenitor cells [6, 7]. In the SVZ, FGF2 was found to be highly expressed by glial fibrillary acidic protein (GFAP)- positive cells, but not by nestin-positive immature neurons, suggesting a pro-proliferative role of astrocytes and GFAP-positive radial glia or neural stem cells in this area [12]. Indeed, astrocytic FGF2 has been implicated in enhanced neurogenesis in the SGZ following acute stress [13]. One of the FGF2 receptors, FGFR1, which is also highly expressed in neural stem cells (NSCs) in the SVZ and SGZ, is essential for hippocampal NSC proliferation. Ohkubo *et al* (2004) showed that genetic deletion of *FGFR1* in cells of the radial glial lineage, which are the progenitors of the majority of pyramidal neurons of the mouse dentate gyrus, resulted in dramatically reduced proliferation during embryonic development, accompanied by reduced hippocampal volume and impaired growth during postnatal development [14]. In the adult rat CNS, FGFR1 and FGFR4 are predominantly expressed in neurons, whereas FGFR2 and FGFR3 are more highly expressed in oligodendrocytes and astrocytes, respectively [15] [16]. It was found more than a decade ago that FGF2 administration to neuronal progenitor cells isolated from the septum and striatum of adult rats induces their proliferation *in vitro*, evidence that in the brain, neurogenic capacity is due not only to intrinsic properties of NPCs, but to regional differences in regulatory signals and receptor expression with FGF2/FGFR signaling being central to this capacity [17].

FGF2 and Proliferation

FGF2 regulates NSC propagation both *in vitro* and *in vivo*. *In vitro*, it has been found to stimulate efficient neural stem cell proliferation and can also induce proliferation of adult mouse stem cells by maintaining these progenitor cells in the cell cycle and preventing them from further differentiation [18].

Several studies have shown the importance of FGF2 in NSC proliferation *in vivo*. After subcutaneous injection of FGF2, there was a 30% increase in proliferating granule cell precursors in the external granule layer of the newborn rat cerebellum, and a significant increase in DNA synthesis in the SVZ and hippocampus, but not in the basal pons or cerebral cortex, confirming the regional specificity of FGF2 signaling in cell proliferation [5]. Furthermore, treatment with neutralizing antibodies to FGF2 reduced proliferation of cerebellar and hippocampal precursor cells in postnatal day 1 (P1) rats [19], and chronic infusion of FGF2 into the lateral ventricle of middle-aged rats leads to increased DCX-positive neuronal precursors [6]. However, although these studies show that pharmacological addition or blockade of FGF2 alters proliferation, several studies of FGF2^{-/-} mice show no deficit in overall NSC proliferation [20] [7], suggesting that FGF2 is a critical neurogenic factor, but that its neurogenic role can be compensated.

FGF2 and Differentiation

In contrast to studies describing FGF2 as a proliferative factor, multiple groups have shown that FGF2 deficiency does not cause defects in neuronal precursor proliferation during development and in adult neurogenesis. Dono *et al* reported that FGF2-deficient mice are viable and show normal neuronal progenitor proliferation during development, but that a fraction of these progenitors fail to colonize their target layers in the cerebral cortex, implying that FGF2 controls fate, migration and differentiation rather than proliferation of neuronal progenitors during development [20]. The authors concluded that although FGF2 had been shown as an essential proliferative factor *in vitro*, FGF2 in fact functions as a migratory signal for neural progenitors, or alternatively its deficiency is functionally compensated by other FGFs *in vivo*. Werner *et al* (2011) demonstrated that FGF2 (^{-/-}) mice had no deficits in overall proliferation of stem cells in the adult dentate gyrus as measured by total BrdU incorporation, but did show impairments in differentiation of progenitors assigned to the neuronal lineage, being double-positive for BrdU and doublecortin (DCX; a marker for immature neurons) or BrdU and neuronal nuclei (NeuN; a marker for mature neurons)[7]. In this study, exogenous FGF2 was unable to rescue the impairment, suggesting that LMW FGF2 is ineffective and HMW FGF2 isoforms may function as differentiation factors. Further study is needed since this was only tested in *ex vivo* hippocampal slices and not *in vivo*.

Role of Isoform-Specific FGF2 Signaling in Neurogenesis

FGF2 mRNA generates several isoforms, which have been suggested to mediate differential signaling in neurogenesis. In human, five different polypeptides can be formed from the same *FGF2* gene via five different mRNA translation initiation sites [2] [3]. In mouse, *FGF2* mRNA generates two 22/21 kDa high molecular weight (HMW) isoforms and one 18 kDa low molecular weight (LMW) isoform. Translation of the 18 kDa LMW isoform is

initiated at an internal AUG codon, while translation of the HMW isoforms are initiated at CUG codons (86, 319, 346, 361) that are 5' to the AUG site and thus contain the entire 18kDa amino acid sequence plus N-terminal extensions of different lengths. All isoforms contain a carboxyl-terminal bipartite nuclear localization signal (NLS). The HMW isoforms (34, 24, 22.5 and 22 kDa) also contain an amino-terminal Glutamic acid - Arginine (GR) repeat domain that acts as an NLS [21]. The CUG-initiated HMW isoforms are predominantly localized in the nucleus and function in an autocrine or intracrine manner [21], while the 18 kDa AUG-initiated isoform is predominantly localized in the cytoplasm but can be secreted, and binds to plasma membrane receptors including FGFR [22]. Intriguingly, FGF2 does not have a secretion signal peptide, but is secreted by an atypical ER/golgi-independent protein secretion pathway[23]. Although the pathway or pathways by which it is secreted remain unclear, studies have shown that FGF2 membrane translocation is unidirectional and requires integral and peripheral membrane proteins [23], including the Na⁺/K⁺-ATPase[24] and extracellular membrane-proximal HSPGs [25].

Differential isoform expression is highly regulated in both human and mouse, and LMW and HMW isoforms are thought to play unique roles in the cell. *In vitro*, differential isoform expression is translationally regulated by stress and cell density in non-transformed cells, but is constitutively active via the IRES in transformed cells [22]. HMW FGF2 is known to interact with nuclear Fibroblast Growth Factor Receptor-1 (nFGFR1), which acts as a developmental gene regulator via interactions with HMW FGF2, CREB-binding protein (CBP), and Ribosomal S6 Kinase-1 (RSK1), forming a transcription regulator complex which has been shown to regulate cell differentiation[26]. CBP induces gene expression associated with neuronal development and functions [27]. Indeed, CBP is critical in both short-term and long-term memory formation and regulates the gene expression of calcium-calmodulin-dependent kinases (CaMKs) and excitatory glutamate receptors: N-methyl-D-aspartate (NMDA) and 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA) receptors [28, 29]. Our preliminary study indicates that several neurogenic genes, such as *PROX1* and *SEMA5A*, are highly expressed in the hippocampus of AAV2/1-FGF2 infected vs. AAV2/1-GFP infected APP+PS1 mice, which provides a potential mechanism of enhanced neurogenesis (Fig. 1).

Furthermore, we have found that AAV-FGF2- infected neural stem cells mainly express HMW FGF2 in the nucleus and initiate neuronal differentiation *in vitro*, which is apparently a different mode of action from the mitogenic effect of LMW FGF2. In support of this idea, our laboratory recently found that silencing of endogenous CBP suppresses neuronal differentiation of neural stem cells expressing either wildtype (WT) or HMW FGF2. Interestingly, HMW FGF2 also binds to anti-apoptotic molecules Api5 and SMN and potentiates cell survival, suggesting further functional diversity as a neurotrophic factor (See Fig. 1B)[30, 31].

LMW FGF2, on the other hand, is a classic mitogenic factor, and induces transformation of normal or non-tumorigenic cells into tumor cells [32]. The differential functions of HMW and LMW isoforms are cell-type specific. In one study, HMW FGF2 was found to inhibit rat C6 glioma cell proliferation. Expression of HMW FGF2 in C6 glioma cells showed a cell-cycle arrest at G2M, suggesting a regulatory role of HMW FGF2 in entry into mitosis [33].

However, in another study by the same group, HMW or LMW FGF2 stimulated cell proliferation in the human U251MG glioma cell line, an effect believed to be mediated by the nuclear forms of FGFR1 [34]. On the other hand, some cell types such as rat pheochromocytoma (PC12) and Schwann cell precursors show differentiation after over-expression of HMW FGF2 [35]. These studies suggest that dependent on the cell type, HMW FGF2 can function as either a proliferation or differentiation factor, which may reflect the fine balance of different HMW FGF2-interacting molecules and their effect on signal transduction and gene expression.

FGF2 and Synaptic Plasticity

FGF2 has also been shown to modulate synaptic plasticity and axonal branching *in vitro* and *in vivo*. FGF2 applied to rat cerebral cortical neurons enhances neurite outgrowth [36] and axonal branching in hippocampal neurons [37]. Application of FGF2 enhanced LTP in rat hippocampal slices [38], and intracerebroventricular (ICV) injection of EGF and FGF2 promoted LTP generation in synapses between perforant path and dentate granule cells of anesthetized rats [39]. Although the exact mechanism of FGF2's synaptic effects is not fully understood, the dual action of FGF2 as a neurotrophic factor and inducer of synaptic plasticity is highly significant for development of therapies, as it has the potential to aid in the incorporation of new cells into a strengthened network.

III. FGF2 and Neurodegeneration: Therapeutic Innovations

FGF2 in Animal Models of Alzheimer's Disease

In line with its role in neurogenesis and synaptic enhancement, FGF2 has proven to be highly efficient at the regeneration of neurons in multiple experimental animal models, including those of optic nerve injury and excitotoxic cell death [40]. Several groups have accordingly shown the potential use of FGF2 as a therapeutic for neurodegenerative conditions such as Alzheimer's disease (AD) and Parkinson's disease (PD). Our lab has found that adeno-associated virus serotype 2/1 hybrid (AAV2/1)-mediated gene expression of FGF2 in AD transgenic mouse models (APP+PS1 and J20) significantly restores spatial learning, hippocampal CA1 long-term potentiation (LTP), and neurogenesis in the SGZ when administered both pre- and post-symptomatically [41]. Interestingly, in addition to its neurogenic properties, FGF2 appears to have an anti-inflammatory and amyloidosis-decreasing effect: AAV2/1-FGF2-injected APP+PS1 mice showed a reduction in total A β and plaque load accompanied by enhanced microgliosis around plaque regions. Furthermore, FGF2 treatment of primary cultured microglia enhanced A β phagocytosis, and AAV2/1-FGF2 infection of primary cultured neurons reduced A β production, suggesting that FGF2 has potent effects not only on neurons but on microglial phagocytosis.

FGF2 as a Therapeutic in Other Neurodegenerative Conditions

Parkinson's disease (PD) is a common neurodegenerative movement disorder involving degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to progressive motor dysfunction and cognitive impairment. FGF2 regulates dopaminergic neuron and nigrostriatal pathway development *in vivo*, the main pathway affected in humans in PD [11]. It has been shown that reactive astroglia display increased FGF2

immunoreactivity following dopaminergic cell degeneration induced by 6-hydroxydopamine (6-OHDA) lesions in the rat striatum, implying a possible mechanism of DAergic neuron repair [42]. Further evidence of the importance of FGF2 in dopaminergic neuron viability was shown by the finding that FGF2 enhances neuronal survivability and protects from 6-OHDA-induced cell death in the substantia nigra of PD mouse models [43]. In this study, both FGF2(−/−) mice and FGF2-overexpressing mice showed enlarged substantia nigra volumes, suggesting a critical developmental role of FGF2 in the establishment of the correct size and neuronal number in the substantia nigra. On the other hand, FGF2(−/−) mice showed significantly decreased survivability of dopaminergic neurons following 6-OHDA lesions to the substantia nigra as compared to FGF2-overexpressing mice and wildtype controls, suggesting an overcompensation of the absence of FGF2 during development, but a lack of these compensation mechanisms after lesion [43].

FGF2 has also been studied as a therapeutic for brain repair after traumatic brain injury (TBI). Rats treated with ICV FGF2 immediately following TBI showed enhanced neurogenesis in the SVZ and SGZ at 1 and 4-weeks post TBI, increased numbers of surviving neurons, and improved cognitive function as compared to controls [44]. In another study, after mice were given TBI according to the CHI model, which recapitulates focal TBI resulting in isolated frontoparietal cortical injury, mice treated with continuous infusion of FGF2 to the lateral ventricles showed improved motor function, increased astrocytic proliferation and number of blood vessels in the perilesional cortex vs. control, and increased neuronal proliferation when FGF2 was given in combination with vascular endothelial growth factor (VEGF)[45].

In Experimental Autoimmune Encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), FGF2 has been identified as a neuroprotective factor in preventing disease and in treating EAE. In one study, intrathecal injection of a recombinant herpes simplex virus (HSV) type-1 vector carrying the human *FGF2* gene significantly reverted pathological features of EAE in mice, including a decrease in the number of myelinotoxic cells (T-cells and macrophages) in the CNS parenchyma and leptomeningeal space and an increase in the number of oligodendrocyte precursors and myelin-forming oligodendrocytes in areas of demyelination and axonal loss [46].

In a recent study, more severe EAE was induced in FGF2(−/−) mice vs. FGF2(+/+) mice, specifically measured by increased infiltration of macrophages/microglia and CD8+ T-cells, increased nerve fiber degeneration, and decreased remyelination of axons, suggesting a protective role of FGF2 [47]. Additionally, spinal cord FGF2 mRNA levels were found to peak at the initial stage of remyelination in EAE, implying that FGF2 is involved in positive regulation of oligodendrocyte myelin production [48]. FGF2 levels are increased in the cerebrospinal fluid of MS patients, especially those in clinical relapse, possibly signifying a protective function of FGF2 [49]. Moreover, in postmortem MS human brain tissue, FGF2 is expressed in microglia and macrophages in active lesions (lesions exhibiting signs of remyelination), and oligodendrocyte precursor cells recruited to these lesions express FGFR1[50]. Consistent with this positive role of FGF signaling in myelination, transgenic mice lacking FGFR1 and FGF2 expression in oligodendrocyte lineage cells show no difference in proliferation or differentiation, but severe CNS hypomyelination [51]. These

studies are in contrast to findings suggesting that FGF2 is inhibitory to oligodendrocyte myelin production. In an animal study, increased levels of FGF2 led to brain demyelination in adult rats, while another study showed that FGF2 is inhibitory to oligodendrocyte progenitor differentiation [52]. Altogether, these opposing findings may suggest that an optimal level of FGF2 is necessary for remyelination in EAE and MS.

Novel Methods for FGF2 Delivery to the CNS

Intranasal Administration—Because growth factors cannot readily cross the blood-brain barrier (BBB), investigation has been done into alternative methods of delivering FGF2 to the brain. Intranasal administration is one of the methods investigated, particularly attractive because it delivers substances directly to the brain through the nasal epithelium, while eliminating the need for and potential risks of peripheral intravenous or intramuscular administration. Accordingly, intranasal administration of FGF2 led to increased neurogenesis in the SVZ of mouse brain [53], as well as improved spatial memory impairment and decreased hippocampal degeneration in AD rat models, although neurogenesis was not assessed in this study [54].

Chitosan-based Microspheres for FGF2 delivery—Another method being used for growth factor delivery across the BBB is microspheres, small spheres containing pharmacological agents which can be enzymatically degraded upon entry into the CNS. Skop *et al* (2013) recently developed microspheres made of chitosan, derived by alkaline deacetylation of chitin, a highly abundant natural polysaccharide [55]. Because chitosan is anionic, it can interact with cationic GAGs such as heparin, which binds FGF2. Heparin is often used in combination with FGF2 in stem cell culturing protocols, as it is essential for its binding to FGFR1 and prevents thermal degradation of the growth factor [56]. The group also used Genipin, a plant-derived crosslinking agent with potential anti-inflammatory properties, to crosslink heparin to chitosan [57].

Although the microspheres weren't tested *in vivo*, they have many properties that may be beneficial for *in vivo* use. For example, the degradation rate of chitosan can be reduced by crosslinking the polymer, which may be useful for *in vivo* applications. Interestingly, Nam *et al* (2010) found that in cultured rat brain microglial cells, Genipin inhibited lipopolysaccharide (LPS)-induced nitric oxide (NO) release, reduced the LPS-stimulated production of inflammatory molecules, and reduced NO release from microglia stimulated with interferon-gamma and amyloid-beta [57]. This suggests that Genipin-containing microspheres may be especially promising for neurodegenerative diseases involving neuroinflammation. *In vitro*, the FGF2 chitosan microspheres were more efficient than standard culture conditions in sustaining growth and survival of a neural stem cell line (GFP + RG3.6), suggesting their potential for *in vivo* testing. The *in vivo* neurogenic effect of other types of microspheres has been demonstrated, including striatal and cortical transplantation of FGF2-releasing gelatin microspheres in combination with neural progenitor cells in a rat stroke model [58] as well as the angiogenic effect in phase I/II trials of hydrogel gelatin FGF2 microspheres implanted into human patients with limb ischemia [59].

NCAM mimetic peptide, Fibroblast growth loop (FGL), as an alternative to FGF2

An interesting alternative to FGF2 therapies is the neural cell adhesion molecule (NCAM) mimetic peptide Fibroblast Growth Loop (FGL). In the CNS, NCAM shows ubiquitous expression and is involved in neuronal processes including synaptic outgrowth and plasticity [60]. The NCAM extracellular domain participates in homophilic interactions as well as heterophilic interactions with other molecules such as FGFR1. The NCAM FGFR1 binding site is in the extracellular second fibronectin type III (F3) region of NCAM. The FGL peptide is a synthetic 15-amino acid peptide derived from and encompassing this NCAM FGFR binding site [61]. Homophilic NCAM-NCAM binding to FGFR leads to FGFR phosphorylation and subsequent activation of intracellular signal transduction pathways, including Ras-mitogen activated protein kinase (MAPK) and Phosphatidylinositol-3-kinase (PI3K)-Akt pathways [62], which are known to control several fundamental cellular processes including proliferation, differentiation, cytoskeleton reorganization, death, and survival [63, 64]. FGL crosses the BBB and exerts its effects by acting on FGFR1 on neurons, simulating FGFR-NCAM heterophilic interactions [65].

FGL has been found to induce many neuroprotective effects similar to those induced by NCAM activation of FGFR, including in memory, synapse formation and anti-inflammatory effects in multiple disease models. In FGFR1-expressing rat dopaminergic, hippocampal and cerebellar granule neurons, FGL treatment induced neuronal differentiation and enhanced neurite outgrowth through direct activation of FGFR1, and this was dependent on MEK and PI3K activation similar to that induced by NCAM activation [66]. Rats showed memory improvement in water maze testing and fear conditioning paradigms immediately following ICV FGL administration, and in primary cultured rat hippocampal neurons, FGL promoted synapse formation and enhanced presynaptic function via FGFR1 activation [67]. Dallérac *et al* (2011) then demonstrated facilitation of LTP induction and maintenance in awake, behaving rats for up to 24 hours following ICV infusion of FGL, as well as altered neurogenesis associated with LTP [68].

It was recently shown that FGL mediates this enhanced synaptic transmission via delivery of excitatory glutamatergic AMPA receptors (AMPA) to synapses, along with activity-dependent enhancement of NMDA-dependent LTP. It was also found that the synaptic delivery of AMPARs is dependent on activation of PKC, a downstream effector of FGFR1 and molecule involved in synaptic plasticity, leading to persistent CaMKII activation [69]. It is especially significant that FGL enhances LTP in an activity-dependent manner, because this confers specificity of enhancement to active synapses, rather than global enhancement, which could lead to epileptic seizures. Furthermore, PKC activation persisted for at least 24 hours after FGL treatment, supporting the *in vivo* findings of long-term LTP enhancement.

The neuroprotective effects of FGL are beneficial in AD and PD animal models as well as in the aging brain. FGL administration has been shown to protect not only against cell death induced by 6-hydroxydopamine and A β in primary cultures of dopaminergic, hippocampal and cerebellar granule neurons [66], but also against A β -induced inflammation and cognitive deficits *in vivo* [70]. Altered synaptic morphology, progressive synapse loss and astro/microglial cell activation are hallmarks of the aging brain and are also key aspects of AD

pathology [71]. FGL has shown anti-inflammatory effects in the aging brain, specifically by restoring imbalances in levels of insulin-like growth factor-1 (IGF1) and pro-inflammatory interferon-gamma (IFN- γ) in hippocampal neurons, as well as reducing age-related glial activation *in vitro* [72].

Neuroimmunomodulation of CD200 signaling by FGF2 signaling

Interestingly, FGL's anti-inflammatory effects are associated with increased expression of the neuronal glycoprotein CD200 by astrocytes [72] and neurons [73] and have subsequently been found to be dependent on CD200 expression [73]. CD200 is known to play a role in maintaining microglia in a non-inflammatory ("quiescent") state, which has been suggested to protect synaptic function in the aging brain [74]. Its cognate receptor, CD200R, is expressed on cells of the myeloid lineage, which are mainly microglia in the brain [75]. Downer *et al* (2010) showed that *in vivo* administration of FGL to aged rats for 3 weeks attenuated age-related increases in microglial activation and corresponding deficits in LTP, effects postulated to be due at least in part to the actions of CD200 [76]. Importantly, CD200 expression is reduced in affected AD brain areas, and CD200(−/−) or CD200R(−/−) mice show enhanced inflammation in the brain [77]. Thus, a pathway involving activation of FGFR leading to enhanced CD200 expression, which leads to subsequent activation of anti-inflammatory pathways via microglial CD200R activation, may be disturbed in AD patients due to CD200 decrease by A β , pathogen-associated molecular patterns (PAMPs), or damage-associated molecular patterns (DAMPs) recognized by microglia (See Fig. 2). FGF2, NCAM mimetic peptide, or other molecules activating the FGFR1 receptor could potentially be used pharmacologically to reverse this disturbance.

One caution with interpretation of the results of Downer *et al* is that the authors did not systematically investigate microglial activation phenotype and any changes in phenotype with FGL treatment. They described observations of higher intracellular vacuole content, phagolysosomal and electron-dense material, which are attributed to greater phagocytic activity of microglia and an increased general 'activation state.' However, microglia may be protective to neurons when they are in an anti-inflammatory activation state, and continue to phagocytose apoptotic cells [78]. The effects of microglia on neural stem cells are highly dependent on the type of microglial activation state, which is known to be inhibitory to hippocampal neurogenesis when microglia are classically activated (M1 skewing; pro-inflammatory) by LPS [79], but supportive to neuronal differentiation and survival when microglia are activated by anti-inflammatory cytokines such as IL-4 (M2a skewing; see Fig. 2) [80]. Interestingly, Cox *et al* (2013) recently demonstrated FGL-induced, CD200-dependent attenuation of LPS-induced changes in microglial pro-inflammatory activation [73], suggesting that the earlier findings of Downer *et al* (2010) may correspond to attenuation of a pro-inflammatory phenotype of microglial activation.

FGL's beneficial effects on age-related synaptic changes have been suggested to be associated with changes in CD200 expression. Treatment with FGL in rats attenuated age-related loss of synaptophysin immunoreactivity (ir) (a synaptic vesicle protein expressed mainly at synapses) within the hippocampal CA3 and hilus regions, and of CD200-ir in the CA3 region. FGL treatment also prevented age-related loss of astrocyte-synapse contacts

and reduced the number of microglia-synapse contacts in the CA3 stratum radiatum, but had no effect on the average number of synapses in that region. This suggests that FGL may mediate its neuroprotective effects not only through neuron-neuron synaptic contacts but also by regulating altered glial-synaptic interactions associated with synaptic damage [81].

In contrast to its effects in the aging brain, subcutaneous injections of FGL to young (4-month old) rats resulted in reduced total volume of the dorsal hippocampus and an associated decrease in total pyramidal neuron numbers in CA1 and CA3. This suggests that the neuroprotective effects of FGL in aged animals may be dependent on glial activation status and its effects on synaptic viability, which are altered in aged animals [82]. In line with this idea, subcutaneous FGL injection reduced microglial CD11b and MHCII immunoreactivity, as well as MHC-II-positive microglial cell density in the hippocampi of aged (22 months) but not young rats [83]. Of note, FGL treatment actually enhanced MHC-II-ir in the dentate gyrus/hilus region of young rats, although the explanation for this opposite effect is unclear. Interestingly, although FGL was found to decrease pyramidal cell numbers in young rats, in these animals it was also associated with increased DCX-ir neurons in the neurogenic SGZ of the dentate gyrus, suggesting that FGL enhances SGZ neurogenesis in young rats possibly via its actions on FGFR1 [82].

Because of its observed effects on inflammation as well as synapse viability and hippocampal neuron growth, FGL is an attractive potential therapy for AD and other age-related neurodegenerative diseases. Gene therapy using AAV-FGF2 is similar in these regards, however FGL can be administered systemically and crosses the BBB, whereas AAV must be injected directly into the CNS, excluding AAV9 [84].

However, caution should be taken when assessing FGL's potential for treating disease in humans. Although FGL did show benefits in A β -injected rats, to our knowledge it has not been tested in published AD mouse models. FGL showed no toxicity in rats, dogs or monkeys in preclinical studies, and an 8-day safety trial in 24 males of one intranasal FGL dose showed no serious adverse effects [85]. In Europe, FGL is currently in clinical trials in AD patients, scheduled to end in 2014 [86]. However, the human safety study was only 8 days long, which may not reveal any long-term adverse effects of the drug. Additionally, since FGL was shown to potentially enhance inflammation in the dentate gyrus [83] and to reduce hippocampal cell volume and pyramidal cell number in young, healthy mice [82], in contrast to its effects on aged mice, FGL may differentially affect patients depending on age and/or disease severity.

IV. Summary

A plethora of studies have demonstrated the crucial role of FGF2 in neurogenesis, both in proliferation and differentiation of stem cells during development and in the adult brain. FGF2/FGFR1 signaling holds great promise for therapeutic interventions for CNS diseases, due to its established effects not only on neurogenesis, but on synaptic formation, neuron-glia interactions, inflammation, and amyloidosis. Much progress has been made on the role of FGF2 in the CNS and on development of therapeutic interventions, and although these interventions have not yet been applied for treatment of human patients, the research

devoted to advanced techniques such as gene therapy, microspheres, and novel BBB-permeable molecules are rapidly moving the field forward.

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Figure 1. Potential mechanism of FGF2-induced neurogenesis
A) Above: Heat map of differentially expressed genes derived from Affymetrix Genechip analysis of APP+PS1 mice infected with AAV2/1-FGF2 or AAV2/1-GFP vs. wildtype (WT) uninfected control. Several neurogenesis-related functions, such as neuronal differentiation, apoptosis, synaptic plasticity and nervous system development are among the most highly differentially regulated gene clusters. Below: 5 most significantly differentially regulated genes, as measured by one-way ANOVA and Tukey *post hoc*. PKP2, plakophilin 2; CPNE7, copine VII; PROX1, prospero-related homeobox 1; RAS1, RAS, dexamethasone-induced 1; SEMA5A, semaphorin-5A. B) Scheme of potential mechanism of FGF2-induced neurogenesis: binding of high-molecular weight (HMW) FGF2 to nuclear FGFR1 leads to induction of neurogenic genes, such as *PROX1* and *SEMA5A*, which are highly expressed in hippocampus of AAV2/1-FGF2 infected vs. AAV2/1-GFP infected APP+PS1 mice (see A). HMW FGF2 also binds to anti-apoptotic molecules apoptosis inhibitor 5 (Api5) and survival motor neuron 1 (SMN) to potentiate cell survival.

Figure 2. Scheme of neuroimmunomodulation of CD200 signaling by FGF2 signaling
 Activation of FGFR leads to enhanced CD200 expression, which leads to subsequent activation of anti-inflammatory pathways via microglial CD200 receptor (CD200R) activation. This pathway may be disturbed in AD patients due to CD200 decrease by amyloid- β (A β), pathogen-associated molecular patterns (PAMPs), or damage-associated molecular patterns (DAMPs) recognized by microglia, leading to neuroinflammation-induced suppression of LTP, synaptic transmission and neuronal viability in AD brain.