The influence of GDF11 on brain fate and function

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Abstract Growth differentiation factor 11 (GDF11) is a transforming growth factor β (TGF β) protein that regulates aspects of central nervous system (CNS) formation and health throughout the lifespan. During development, GDF11 influences CNS patterning and the genesis, differentiation, maturation, and activity of new cells, which may be primarily dependent on local production and action. In the aged brain, exogenous, peripherally delivered GDF11 may enhance neurogenesis and angiogenesis, as well as improve neuropathological outcomes. This is in contrast to a predominantly negative influence on neurogenesis in the developing CNS. Seemingly antithetical effects may correspond to the cell types and mechanisms activated by local versus circulating concentrations of GDF11. Yet undefined, distinct mechanisms of action in young and aged brains may also play a role, which could include differential receptor and binding partner interactions. Exogenously increasing circulating GDF11 concentrations may be a viable approach for improving deleterious aspects of brain aging and neuropathology. Caution is warranted, however, since GDF11 appears to negatively influence muscle health and body composition. Nevertheless, an

expanding understanding of GDF11 biology suggests that it is an important regulator of CNS formation and fate, and its manipulation may improve aspects of brain health in older organisms.

Keywords Growth differentiation factor 11 · GDF11 · Brain aging · Brain development · Neurogenesis · Stroke · Alzheimer's disease

Introduction

GDF11, also known as bone morphogenic protein 11, is a member of the TGF β superfamily (Nakashima et al. 1999). It is highly homologous to myostatin (MSTN/GDF8), sharing 90% amino acid sequence identity in their active domains (Nakashima et al. 1999) (Fig. 1). GDF11, as well as MSTN/GDF8, activin type I (activin-like receptor kinase 4, 5, and 7 [ALK4, ALK5, ALK7]) and activin type II (activin receptor kinase IIA and IIB [ActRIIA/ACVR2A, ActRIIB/ACVR2B]) receptors, which canonically activate SMAD-targeted transcription but may also activate additional pathways, including mitogen-activated protein kinase (MAPK) signaling (Oh et al. 2002; Rebbapragada et al. 2003; Andersson et al. 2006; Egerman et al. 2015; Philip et al. 2005). Non-redundancy of GDF11 and MSTN/GDF8 function has been strongly attributed to distinct expression patterns. GDF11 is expressed broadly, but MSTN/GDF8 expression is predominantly restricted to skeletal and cardiac muscle (Nakashima et al. 1999; McPherron et al. 1997;

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McPherron et al. 1999). However, biochemical analyses suggest that, although highly similar, variability in critical structural domains (Padyana et al. 2016; Cash et al. 2012; Cash et al. 2009) (Fig. 1) may confer important differences in interactions with receptors (Khalil et al. 2016) and inhibitory binding partners, including follistatin (FST), follistatin-like 3 (FSTL3), and GDF-associated serum proteins (GASP1 and GASP2) (Lee and Lee 2013; Lee and McPherron 2001; Schneyer et al. 2008). Indeed, relative to GDF8, GDF11 appears to be a more potent signaling ligand in certain contexts, particularly regarding utilization of activin type I receptors (Walker et al. 2017). Thus, distinct functional roles for GDF11 and MSTN/GDF8 may also be driven by differential cell-type and context-specific expression and utilization of ALK4, ALK5, ALK7, ACTRIIA, and ACTRIIB (Hammers et al. 2017). Collectively, these distinguishing features of GDF11 appear to contribute to its unique influence on brain development and function throughout the lifespan.

Fig. 1 GDF11 and MSTN/GDF8 are highly homologous. The protein structures of the monomeric, active domains of human GDF11 (blue) and mouse MSTN/GDF8 (red) are superimposed. Differing residues are highlighted (GDF11: green; MSTN/GDF8: orange). Protein structures were obtained from: (Padyana et al. 2016; Cash et al. 2009) and compared using Molsoft ICM Browser version 3.8-7b

GDF11 expression in the CNS

Gdf11 is highly expressed in many embryonic murine tissues, including the developing CNS (Nakashima et al. 1999). In early embryonic development, *Gdf11* is expressed in both anterior and posterior neural epithelium, and in later stages, it is expressed in the thalamus, hippocampus, striatum, preoptic area, inferior colliculi, ventral midbrain, anterior hindbrain, cerebellum, and fornix (Nakashima et al. 1999; McPherron et al. 1999), as well as the developing retina and spinal cord (Kim et al. 2005; Shi and Liu 2011). Transcriptional analysis of young mouse brain cortex revealed that multiple cell types express *Gdf11*, including astrocytes, neurons, and oligodendrocytes (Fig. 2) (Zhang et al. 2014). In contrast, *Mstn/Gdf8* is very lowly or not expressed, highlighting potential specificity of GDF11 in brain development. *Gdf11* levels appear to decrease in myelinating oligodendrocytes, relative to newly formed or precursors of oligodendrocytes, suggesting that in some cell types, GDF11's developmental functions may be temporally regulated (Fig. 2). In adult mice, *Gdf11* can be detected in the thalamus, cerebellum, hippocampus, midbrain, and hindbrain (Nakashima et al. 1999), although although the corresponding cell types are yet unknown.

Due to high homology between GDF11 and MSTN/GDF8, most protein structure-based detection methods lack the specificity to accurately distinguish between these factors, so exploration of brain-specific production and action of GDF11 in development has predominantly relied on analysis of mRNA abundance and/or transgenic mouse models. Nevertheless, immunohistochemical analysis of GDF11 in the adult rat CNS indicated that GDF11 is widely expressed throughout the adult brain, including the olfactory bulb, cortex, nucleus accumbens, caudate putamen, hippocampus, thalamus, hypothalamus, midbrain, cerebellum, brainstem, and spinal cord, with observed expression in neurons, astrocytes, and ependymal cells (Hayashi et al. 2018a). Similar expression patterns were observed for MSTN/GDF8 in the adult rat brain also using antibody-based detection, raising questions of functional redundancy and/or detection cross-reactivity (Hayashi et al. 2018b).

GDF11 in brain development

Consistent with its expression pattern, GDF11 influences developmental patterning in multiple tissues,

Fig. 2 *Gdf11* is highly expressed, relative to *Mstm*/*Gdf8* in multiple cell types in the young mouse cortex. RNAseq expression data, indicated as Fragments Per Kilobase of transcript per Million mapped fragments (FPKM), were derived from purified populations of cells from pooled young mouse brain cortices. The data were obtained from: (Zhang et al. 2014)

including brain (Oh et al. 2002; Andersson et al. 2006; McPherron et al. 1999; Vanbekbergen et al. 2014). Experiments employing a mouse embryonic stem (ES) cell patterning culture system demonstrated that GDF11 may regulate brain organization at the earliest developmental states, specializing progenitors toward posterior forebrain, midbrain, and anterior hindbrain fates at the expense of anterior forebrain fate (Vanbekbergen et al. 2014). Furthermore, GDF11 appears to directly regulate *Hox* genes to influence the patterning of the developing spinal cord (McPherron et al. 1999; Liu 2006).

Experiments in model organisms and in vitro systems have implicated GDF11 as a negative regulator of developmental neurogenesis (Kim et al. 2005; Kawauchi et al. 2009; Wu et al. 2003; Gokoffski et al. 2011). This includes work demonstrating that GDF11 is a coordinator of olfactory epithelium neurogenesis. *Gdf11* and *Actr11b* are expressed in olfactory receptor neurons and neuronal progenitor cells within the olfactory epithelium, as is *Fst*, a GDF11 antagonist (Nakashima et al. 1999; Wu et al. 2003). In olfactory epithelium explants, GDF11 inhibits neurogenesis and promotes neuronal progenitor quiescence, likely through activation of p27^{Kip1} and/or p21^{Cip1} and inactivation of FOXG1, a positive regulator of olfactory epithelium neurogenesis (Kawauchi et al. 2009; Wu et al. 2003). Although *Gdf11*-null mice die within 24 h of birth, analysis of embryonic pups revealed significant increases in total number of proliferating olfactory epithelium neuron progenitors (Wu et al. 2003). As an endogenous GDF11 inhibitor, FST antagonizes the anti-neurogenic influence of GDF11 in olfactory epithelium (Wu et al. 2003). Furthermore, FOXG1 exerts its pro-neurogenic actions, in part, by

antagonizing GDF11 (Kawauchi et al. 2009). Coordinated actions between GDF11 and another FST-binding protein, activin β B, also instruct neuronal versus glial progenitor fate (Wu et al. 2003; Gokoffski et al. 2011). Cumulatively, these findings suggest that GDF11 and signaling partners dynamically function through negative and positive feedback loops to balance neurogenesis within the developing olfactory epithelium.

GDF11 also negatively regulates neuron number in the retina (Kim et al. 2005). *Gdf11*-null embryos possess as much as 50% more retinal ganglion cells (RGCs) as much as 50% more retinal ganglion cells (RGCs) than wild type controls, which corresponds to an expansion of optic nerve area, indicating that excess cells are capable of differentiating. *Fst*-null mice display a reduction in RGC number, suggesting that, like the olfactory epithelium, coordinated GDF11-FST function regulates RGC abundance. Examination of temporal patterning demonstrated that *Gdf11*-null mice have an extended duration of RGC production. GDF11 also appears to directly influence progenitor fate specialization, since RGC expansion is at the expense of other cell types, including amacrine and photoreceptor cells (Kim et al. 2005). A negative role for GDF11 in olfactory epithelium and retinal neuronal development is consistent with GDF8's negative regulation of muscle growth and development (McPherron et al. 1997). In contrast, in the developing spinal cord, newly born neurons transiently express *Gdf11*, which triggers adjacent progenitor cells to exit the cell cycle and differentiate into neurons (Shi and Liu 2011), indicating that GDF11 is also capable of facilitating developmental neurogenesis in distinct contexts.

Functional neuronal morphology and identity may also be influenced by GDF11 (Hocking et al. 2008; Augustin et al. 2017; Lo and Frasch 1999; Awasaki et al. 2011; Wang et al. 2018). Immature neurons may require extrinsic signals for dendritic initiation and maturation, and work by Hocking and colleagues suggests that GDF11, as well as other TGF β superfamily ligands, extrinsically influence neuronal differentiation. Studying *Xenopus* RGCs in vitro, they demonstrated that treatment with recombinant Gdf11 stimulates the formation of dendrites (Hocking et al. 2008). Furthermore, *gdf11* and its cognate receptor *actr11b* are expressed in the developing *Xenopus* retina during early stages of dendritic extension. Using transgenic *Xenopus* harboring dominant negative forms of Actr11b and/or Bmpr11, (the receptor for Bmp2, a Tgf β ligand that triggers dendritic initiation), they demonstrated that dendritic number was reduced only when dominant negative receptors were co-expressed but not when expressed alone, collectively suggesting that redundant or cooperative Tgf β superfamily signaling plays a role in *Xenopus* RGC dendritic formation (Hocking et al. 2008). This is consistent with coordinated but distinct TGF β superfamily signaling in the regulation of embryonic chick retinal development (Franke et al. 2006).

Mammalian cell culture experiments also support GDF11 as a regulator of functional neuronal morphology. A study of C17.2 neural stem cell identity and behavior indicated that concentration and temporal kinetics of GDF11 treatment highly influence its effects (Wang et al. 2018). Treatment of neural stem cells with GDF11 induced differentiation, including neurite outgrowth, and increased protein levels of neuronal and astrocytic lineage markers (Wang et al. 2018). A 24-h treatment slightly improved cell viability, but this effect was lost at 72-h assessments. Furthermore, GDF11 treatment induced apoptosis and suppressed migration in a dose-dependent manner. (Wang et al. 2018). However, treatment of primary rat cortical neuron cultures with recombinant human GDF11 or MSTN/GDF8 decreased neurite outgrowth area, while treatment with TGF β had the opposite effect (Augustin et al. 2017). Of note, GDF11 increased the formation of excitatory synapses, and MSTN/GDF8 reduced the formation inhibitory synapses, suggestive of distinct modulatory roles for these homologs in synaptogenesis in vitro (Augustin et al. 2017). Thus, GDF11 may dynamically influence brain cell differentiation, morphology, and

survival, with outcomes highly dependent on cell-type, target receptor, concentration, and stimulation duration.

In *Drosophila*, *myoglianin* (*myo*) encodes the protein MYO, which is the TGF β /activin signaling ligand with highest amino acid sequence homology to mammalian GDF11 and MSTN/GDF8 (Lo and Frasch 1999). Its developmental expression pattern includes muscle and brain (Lo and Frasch 1999; Awasaki et al. 2011), which is consistent with its evolutionarily conserved counterpart. MYO mediates glia-dependent neuronal remodeling during *Drosophila* development (Awasaki et al. 2011). Neuro-muscular synaptic physiology also appears to be influenced by MYO signaling (Augustin et al. 2017; Lo and Frasch 1999; Awasaki et al. 2011). In addition to having a negative regulatory role in *Drosophila* muscle growth, inhibition or upregulation of MYO potentiates and suppresses neuro-muscular junction (NMJ) synaptic activity, respectively, in part, through coordination of synaptic receptor density (Augustin et al. 2017). Disruption of MYO also influences motility, likely driven by NMJ dysfunction (Augustin et al. 2017). Thus, GDF11 is an evolutionarily conserved regulator of multiple aspects of brain development, including NMJ synaptic physiology.

GDF11 in brain aging and disease models

Although GDF11's developmental neurogenic role is largely suppressive, accumulating evidence indicates that it may function as a growth and trophic factor in adult neurogenesis (Katsimpardi et al. 2014; Ozek et al. 2018; Zhang et al. 2018a), suggestive of differential influence throughout the lifespan. Mammalian adult neurogenesis is believed to be restricted to the subventricular zone (SVZ) of the lateral ventricle and subgranular zone (SGZ) of the hippocampal dentate gyrus, wherein, putative progenitor cells may be influenced by vascular- and/or peripheral-derived signaling factors (Zhao et al. 2008; Alvarez-Buylla and Lim 2004; Villeda et al. 2011). Similarly, age-related changes in the cerebrovasculature may impair brain tissue function and reduce neurogenesis (Palmer et al. 2000), in part, through alterations in circulating factors (Villeda et al. 2011; Villeda et al. 2014). In line with this notion, daily intraperitoneal injection of recombinant GDF11 to 23-month old mice increases SVZ vascularization and the abundance of SOX2+ neural stem cells, relative to PBS treatment (Katsimpardi et al. 2014). Furthermore,

treatment of cultured endothelial cells with recombinant GDF11 increases SMAD activation and endothelial proliferation. It is, therefore, possible that in the aged SVZ, GDF11 may enhance neurogenic function by rejuvenating vascular networks in aging (Katsimpardi et al. 2014).

Building on these findings, the same group also demonstrated a role for GDF11 in SGZ hippocampal neurogenesis. Similar to observations in the SVZ, recombinant, peripheral administration of GDF11 to 22–23-month old mice increases the abundance of BrdU+/NEUN+ newborn neurons, SOX2+ neural stem cells, and DCX+ neural progenitors in the dentate gyrus of aged mice (Ozek et al. 2018). GDF11 treatment also enhances aged hippocampal vascular networks and increases levels of excitatory neuronal activity markers in both the dentate gyrus and cortex, suggesting angiogenic and neuronal-activation benefits are not limited to neurogenic regions. The pro-neurogenic and pro-angiogenic effects of GDF11 were not observed in young mice administered GDF11, which is consistent with the notion of distinct age-dependent roles for GDF11 in the brain. Interestingly, a single intraperitoneal dose of GDF11 does not lead to increased GDF11 signaling, as measured by SMAD2/3 phosphorylation in whole brain samples, despite the ability of GDF11 to activate SMADs in many peripheral tissues, as well as in neurons and astrocytes *in vitro*. The authors argued that peripherally delivered GDF11 may not be able to cross the blood-brain barrier, which they further supported by a pulse-chase experiment using biotinylated GDF11. Thus, the beneficial effects of GDF11 on neurogenesis in the aged brain may be heavily dependent on improvement of vascular homeodynamics. Of note, *in vivo* and *in vitro* treatment with GDF11 leads to increased vascular endothelial growth factor (VEGF) concentrations, a potent angiogenic factor, but treatment with MSTN/GDF8 does not alter VEGF levels in endothelial cell cultures, providing a potential mechanism for differing vascular effects of these homologs in the aged brain (Ozek et al. 2018).

In agreement with these findings, a separate study demonstrated that a single intraperitoneal injection of GDF11 to 9-month old “middle-aged” mice enhances spatial memory performance in the object location task and increases SOX2+ neural stem cell signal but does not increase SMAD2/3 phosphorylation in the dentate gyrus (Zhang et al. 2018a). Artificially increasing circulating concentrations of GDF11, therefore, appears to

provide potent trophic growth and support in the aged brain. Whether endogenous GDF11 influences neuronal activity, including neurogenesis and synaptic transmission, under normal conditions in the adult brain is less clear. One study that relied on antibody-based detection found that GDF11 expression does not correlate with synaptic plasticity, as measured by long-term potentiation physiology assessment (De Domenico et al. 2017). Surprisingly, hippocampal GDF11 levels decreased in aged mice following rotarod training, although the causes and implications of such a reduction in response to physical exercise are unknown (De Domenico et al. 2017).

Consistent with pro-neurogenic and pro-angiogenic influence in the adult brain, GDF11 treatment may improve stroke recovery (Lu et al. 2018; Ma et al. 2016; Ma et al. 2018), which is characterized by neuronal precursor cell (NPC) migration from SVZ to SGZ regions to infarct-damaged areas (Arvidsson et al. 2002; Teng et al. 2008; Osman et al. 2011; Walter et al. 2010) and is supported by angiogenesis (Krupinski et al. 1994; Hayashi et al. 2003). Revascularization and perfusion of the insult region is critical for tissue recovery after stroke, which may be influenced by infusion of peripheral growth and trophic factors (Tsai et al. 2006). Using the distal middle cerebral artery occlusion mouse model of stroke, Lu and colleagues demonstrated that daily intraperitoneal injection of human recombinant GDF11 from days 7–13 following occlusion enhances NPC proliferation in the SVZ, revascularization in peri-infarct zones, and trophic factor levels at day 14 post-occlusion (Lu et al. 2018). GDF11’s positive, proliferative effects are blocked by treatment with a TGF β /Activin signaling antagonist. Importantly, newly differentiated neuron abundance (marked by BrdU+ NEUN+ cells) in peri-infarct cortex increases at 42-day post-occlusion, and consistently, sensorimotor performance improves in mice treated with GDF11 at 28- and 42-day post-infarct, suggestive of long-term functional benefits afforded by delayed GDF11 treatment during stroke recovery (Lu et al. 2018). Similarly, in a rat model of right middle cerebral artery occlusion, daily intravenous delivery of GDF11 beginning 2 h after reperfusion and continuing for 7 days increases the number of CD34+ endothelial progenitor cells and ALK5 + CD31+ endothelial cells in peri-infarct cortex, while increasing SMAD2/3 phosphorylation (Ma et al. 2018). GDF11 treatment also improves behavioral outcomes, endothelial proliferation, and revascularization.

Importantly, treatment with a TGF β /Activin signaling antagonist prevents GDF11-dependent behavioral improvements, cortical SMAD activation, and increases in proliferating endothelial cells and vascular density (Ma et al. 2018).

A growth and trophic role for GDF11 following ischemia is further supported by experiments implementing transcutaneous electrical stimulation of the auricular branch of the vagus nerve (ta-VNS) as a means to stimulate angiogenesis and improve neurological outcomes following middle cerebral artery occlusion in rats (Ma et al. 2016). Occlusion followed by ta-VNS improves behavioral performance and reduces infarct volume, relative to occluded rats receiving sham-stimulation. Antibody-based assays were used to quantify GDF11 levels, which may not have been able to distinguish between MSTN/GDF8. Regardless, the authors reported increases in plasma and peri-infarct cortical GDF11 levels in all occlusion groups, peaking at 3 days post-stimulation, with highest levels in ta-VNS rats. GDF11 mRNA levels also increase in the peri-infarct cortex following ischemia/reperfusion, with ta-VNS stimulating the highest increase in association with increased Ki67+ and ALK5+ endothelial cell (CD31+) abundance, further implicating GDF11-stimulated vascular proliferation as a potential mechanism underlying improved recovery responses (Ma et al. 2016). These findings suggest that stimulating endogenous increases in both circulating and local production of GDF11 may be a means to promote the beneficial effects of GDF11 in the brain.

Increasing GDF11 levels may also improve aspects of Alzheimer's disease pathology (Zhang et al. 2018b; Wang et al. 2015). In the APP/PS1 mouse model of β -amyloid (A β) Alzheimer's disease, twice daily intravenous injection of recombinant GDF11 for 28 days increases cortical cerebral blood flow and correspondingly, increases the number of CD31+ endothelial cells, enhances VEGF expression, and improves vascular architecture, relative to PBS-treated mice (Zhang et al. 2018b). GDF11 treatment results in slightly reduced A β 40, but not A β 42 levels, and reduced markers of glial activation in the cortex. Morris water maze testing indicated improved spatial learning and memory in transgenic mice administered GDF11, but not PBS (Zhang et al. 2018b). Interestingly, in the distinct APPswe/PSEN1dE9 transgenic mouse model of A β Alzheimer's pathology, intraperitoneal delivery of splenocytes from young wild-type mice increases

plasma concentrations of GDF11, determined by an antibody-based method (Wang et al. 2015). This is associated with reduced cortical A β plaques and improved spatial learning and memory in the Morris water maze behavioral task. Although benefits cannot be mechanistically linked to increased GDF11 levels, this study implicates modulation of immune cell populations as a means to increase GDF11 levels, while improving aspects of A β pathology and cognitive impairment. Similar to findings in aging and stroke models, increasing circulating GDF11 concentrations appears to be associated with substantial benefits in the brains of Alzheimer's disease models, which may be dependent on vascular adaptations.

GDF11 in circulation

Local production and action of GDF11 and its signaling partners undoubtedly plays a central role in its influence on brain fate and function throughout the lifespan. As evidenced by many experiments demonstrating brain outcomes following peripheral delivery, circulating GDF11 may also regulate brain homeodynamics. Since GDF11 is produced and secreted systemically, whether endogenous GDF11 concentrations change in circulation throughout the lifespan and in disease states is an important question. Until very recently, investigations of the endogenous contributions of circulating GDF11 were thwarted by insufficiently specific detection methods, due to the high homology between GDF11 and MSTN/GDF8. Prior reports exploring associative roles between circulating GDF11 and brain-related outcomes or aging have predominantly relied on antibody or aptamer detection, which may not accurately resolve GDF11 from MSTN/GDF8 (Egerman et al. 2015; Loffredo et al. 2013; Yang et al. 2017). Indeed, an initial study utilizing non-discerning aptamer and antibody quantification demonstrated that GDF11 levels in blood decrease with advanced age in mice (Loffredo et al. 2013). The age-related plasma concentration decrease was subsequently refuted and demonstrated to potentially increase with age in rodents and humans, also using aptamer and antibody-based quantification (Egerman et al. 2015). With the goal of accurately assessing GDF11 and MSTN/GDF8 concentrations in circulation, we developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay capable of precisely quantifying GDF11 and MSTN/GDF8 levels in

biological samples and found that circulating GDF11 concentrations do not decrease throughout the human lifespan. However, MSTN/GDF8 levels decrease with age in men (Schafer et al. 2016). A recent study confirmed our findings of no age-related changes in GDF11 using LC-MS/MS (Semba et al. 2018). Thus, we speculate that prior observations of age-dependent decreases in circulating GDF11 concentrations may partly reflect decreases in MSTN/GDF8.

Interestingly, an antibody-based screen of secreted proteins in plasma from individuals with sporadic Alzheimer's disease versus control identified a cluster of TGF β superfamily signaling receptors and ligands as associated with cognitive performance, although a specific role for GDF11 was not investigated (Jaeger et al. 2016). Another antibody-reliant study measured plasma GDF11 levels in limited samples of older adults with vascular mild cognitive impairment, vascular dementia, Alzheimer's disease mild cognitive impairment, and Alzheimer's disease dementia, in addition to both younger and older cognitively unimpaired controls and found no correlations between circulating GDF11 levels and cognitive status or age (Yang et al. 2017). Investigation of GDF11 in cerebrospinal fluid (CSF) may be more informative for understanding brain dynamics but as of yet, remains extremely understudied. One study compared GDF11 levels in CSF from individuals with amyotrophic lateral sclerosis (ALS), neurological controls (possessing a range of neurologic conditions other than ALS), and normal controls using antibody-based quantification and found that GDF11 is non-significantly lower in individuals with ALS, and among participants with ALS, higher CSF GDF11 concentrations are significantly correlated with less severe disease status (Drannik et al. 2017). Given the concerning limitations of antibody-based methods, however, further studies are required to determine whether and how GDF11 in blood and CSF may influence brain health in aging and neurodegenerative disease, and recently developed LC-MS/MS assays may be very useful in these pursuits.

Summary

Past decades have illuminated important and dynamic contributions of GDF11 to brain development and function throughout the lifespan. Its broad expression throughout rodent brain development suggests

important regulatory roles in patterning and neurogenesis (Nakashima et al. 1999; McPherron et al. 1999; Kim et al. 2005; Shi and Liu 2011), and mechanistic experiments have supported functional influence. GDF11 may regulate stem cell specification (Vanbekbergen et al. 2014) and appears to inhibit neurogenesis in neural epithelium and retina (Kim et al. 2005; Kawauchi et al. 2009; Wu et al. 2003; Gokoffski et al. 2011), while facilitating neurogenesis in the spinal cord (Shi and Liu 2011). This suggests that, in the developing CNS, GDF11 may largely act as a negative regulator of cell formation, which is similar to MSTN/GDF8's function in muscle, but may also provide pro-neurogenic influence in certain scenarios. Differentiation and functional morphology are also influenced by GDF11. In *Xenopus*, the coordinated action of GDF11 and additional TGF β superfamily signaling factors appears to stimulate neurite outgrowth (Hocking et al. 2008), which suggests that GDF11 acts cooperatively with other TGF β factors in certain contexts. In cultured neuronal stem cells, GDF11 enhances neurite growth and induces expression of lineage markers (Wang et al. 2018); contrastingly, in rat cortical neuron cultures, GDF11 decreases neurite area but increases the number of excitatory synapses (Augustin et al. 2017). *Drosophila* experiments also demonstrate a modulatory role for the GDF11 orthologo, MYO, in NMJ synaptic plasticity and activity (Augustin et al. 2017; Lo and Frasch 1999; Awasaki et al. 2011). Therefore, GDF11 has broad effects on neurogenesis, differentiation, maturation, and physiology in the developing CNS of distinct model systems.

In contrast to its predominantly negative role in developmental neurogenesis, GDF11 may enhance neurogenesis in the adult brain (Katsimpardi et al. 2014; Ozek et al. 2018; Zhang et al. 2018a) and provide trophic and rejuvenative benefits in brain injury and neuropathology models (Lu et al. 2018; Ma et al. 2016; Ma et al. 2018; Zhang et al. 2018b; Wang et al. 2015). Peripheral administration of recombinant GDF11 stimulates neurogenesis in the aged SVZ and SGZ, which may be dependent on cerebrovascular enhancements, rather than direct effects on neurons (Katsimpardi et al. 2014; Ozek et al. 2018). Critically, exogenously increasing GDF11 does not increase neurogenesis in young mouse brain, which further supports distinct influence of GDF11 throughout the lifespan (Ozek et al. 2018). Given its pro-neurogenic and pro-angiogenic effects, it is not surprising that

GDF11 treatment appears to ubiquitously improve outcomes in stroke models (Lu et al. 2018; Ma et al. 2016; Ma et al. 2018). Furthermore, vascular and reduced pathology benefits have been observed in association with increasing circulating GDF11 concentrations in Alzheimer's mouse models (Zhang et al. 2018b; Wang et al. 2015).

Most of the studies exploring GDF11's effects in the aged or challenged brain, however, have artificially increased circulating GDF11 levels, rather than modulating endogenous and/or local brain function. This makes direct comparisons with developmental studies, which often relied on transgenic models difficult. Indeed, it is possible that peripherally administered, recombinant GDF11 may not cross the blood-brain barrier and may instead exert its influence through direct effects on the cerebrovasculature, at least in the aged brain (Ozek et al. 2018). The effects of potentially non-brain-penetrable GDF11, therefore, may exert distinct influence relative to GDF11 produced in varying brain cell types in a defined temporal pattern. Changes in expression patterns and subsequent interactions with cognate receptors and inhibitory proteins throughout aging may also play an important role in regulation of GDF11 signaling. While these factors may explain aspects of antithetical influence in the context of young versus aged neurogenesis, yet undetermined mechanism responsible for differential influence may also play a role.

Furthermore, "normal" brain and circulating concentrations of endogenous GDF11 throughout aging and in disease contexts remain relatively undefined, due to the challenges inherent to accurate measurement of GDF11, resolved from its homolog MSTN/GDF8. Since GDF11 is known to have dose-dependent effects, achieving physiologic levels in treatment studies may be an ideal goal for teasing out its mechanistic roles, as well as considering translation into humans. Moreover, although GDF11 may play important growth and trophic roles in the aged brain, particularly in neuropathology models, studies in other organ systems, including skeletal muscle and the heart, suggest that it maintains negative growth regulatory properties (Egerman et al. 2015; Hammers et al. 2017; Hinken et al. 2016; Smith et al. 2015; Zimmers et al. 2017). Indeed, we have previously found that physically frail older adults with cardiovascular disease possess higher circulating levels of GDF11, relative to non-frail counterparts (Schafer et al. 2016). Similarly, GDF11 treatment alters metabolic and catabolic processes, leading to reduced body

mass, leading to reduced body mass, which is an outcome that should be avoided in older organisms at risk for age-related physical frailty (Ozek et al. 2018; Jones et al. 2018). These concerns caution the use of systemic GDF11 administration as a means to enhance brain health in aging and disease. However, GDF11-dependent changes in body composition appear to be dose-dependent (Ozek et al. 2018; Jones et al. 2018). Whether it is possible to titrate GDF11 administration to achieve brain benefits while minimizing adverse outcomes in peripheral systems remains to be determined. Testing the safety and efficacy of distinct GDF11 delivery methods is another promising therapeutic avenue to pursue.

In summary, GDF11 has potent effects on brain health throughout the lifespan. Many unanswered questions remain, however, with respect to the regulation of GDF11 action, its CNS compared to peripheral effects, the impact of systemic versus local delivery, the implications of distinct interactions with inhibitory proteins and receptors in diverse contexts, whether recombinant and endogenous forms exert differential activity, and how concentration may influence its action. Importantly, advanced technologies for specific quantification of GDF11 and manipulation of its abundance through multiple avenues provide a foundation from which to pursue these avenues. The history of GDF11 also reminds us of the mandate that careful selection of methods and interpretation of results are necessary to disentangle its mechanisms of action from those of MSTN/GDF8.

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