TGFβ superfamily

(a) TGFβ superfamily members are classified into TGFβ, Activin/inhibin/Nodal group and BMP groups. In the canonical signaling pathway, shown here, TGFβ family members signal through type I and type II receptors for each member to receptor-associated SMAD proteins (R-SMAD), SMAD2 and SMAD3. Activin/inhibin/Nodal proteins signal through distinct type I and II receptors: ACVR1B or ALK4 acts as a transducer of Activin/inhibin/Nodal signals. These Activin ligands bind to either ACVR2A or ACVR2B and then bind ACVR1B and then activate (phosphorylate) SMAD2/SMAD3. BMP signals are transduced through their distinct type I and type II receptors for each member to R-SMAD proteins SMAD1, SMAD5 and SMAD8. Phosphorylated R-SMADs associate with SMAD4 and are then translocated to the nucleus to activate transcription of target genes. GDF family members signal through both Activin and BMP pathways, except for GDF10, which signals through TGFβ receptors. This summary is simplified and, for example, does not represent the full complexity of signaling that can come through heterotetrameric binding between TGFβ, Activin/inhibin/Nodal and BMP families.

(b) The genetic relationship of GDF/BMP proteins as established by sequence homology. Figures modified from 12,14.
Supplementary Figure 2

GDF10 is upregulated in peri-infarct cortex.

(a) GDF10 protein expression in the ipsilateral hemisphere after stroke. This low magnification photomicrograph of the ipsilesional hemisphere shows axonal loss, as indicated by loss of MAP2, in the stroke core. GDF10 is induced in the cortex bordering the stroke core, as seen by the increased intensity of staining (arrows). The stroke core contains an abundance of microglia and macrophages, labeled by CD11b. (b) GDF10 expression in the peri-infarct cortex. Image is taken from the region of interest indicated by box in panel (a) GDF10 protein expression is upregulated compared to contralateral control in panel 1(c). (b) Higher magnification view of peri-infarct cortex shows that GDF10 is present in MAP2+ neuronal dendrites (arrows) in addition to neuronal cell bodies (Fig. 1) and not present in CD11b+ microglia/macrophages. (c) GDF10 expression in the non-injured cortex from the contralateral hemisphere. GDF10 has a sparse low level of endogenous expression. This indicates that GDF10 induction is specific to cortex immediately adjacent to the site of stroke injury. In the absence of stroke, CD11b is not induced in the control cortex. (d) GDF10 expression in the peri-infarct colocalizes to neurons labeled with MAP2 and excludes microglia and macrophages. GDF10 immunoreactivity is present in MAP2+ neuronal somas and in dendrites (arrows). GDF10 does not colocalize with CD11b+ microglia/macrophages. (e) Secreted GDF10 is also found in the extracellular space in the peri-infarct cortex near surviving processes (MAP2).
Supplementary Figure 3

GDF10 protein levels and siRNA knockdown in vivo, P4 neuron outgrowth, and siRNA protein knockdown

(a) GDF10 siRNA, vehicle or scrambled siRNA was delivered into the stroke cavity and peri-infarct tissue processed for Western blot. X axis shows days after stroke and siRNA delivery. * = p< 0.05, ** = p< 0.01 compared to the scrambled siRNA; # = p<0.05 compared to Stroke only. Lower panel shows representative Western blot images from (a). N = 3 for each experiment. (b) Both myelin and CSPGs inhibit the outgrowth or P4 cortical neurons. *** = p<0.001, multiple comparisons ANOVA, Tukey-Kramer post-hoc. (c) Western blot of GDF10 protein level with two-day treatment of siRNA in P4 neurons. Numbers in the columns indicate the distinct siRNA construct. Lower panel shows a representative blot from each experiment. Each experiment represents 4 samples and 2 technical replicates. (d) Western blot results for knockdown of TGFβRI, II and Smads1, 2, 3, 5. Same conventions as in (a). ** = p<0.01 compared to the scrambled siRNA.
(a) Primary Cortical Neurons express GDF10

![Image](image_url)

(b) High magnification of neuronal GDF10

![Image](image_url)

Supplementary Figure 4

Primary cortical neurons plated in vitro express GDF10

(a) Primary cortical neurons stained with GDF10 and MAP2 antibody demonstrates neuronal expression of GDF10 in vitro. This substantiates the in vitro GDF10 knockdown experiments using siRNAs to study axonal outgrowth, without a need for addition of GDF10 to the system to mimic induction. In contrast to the low endogenous expression of GDF10 in uninjured cortical neurons in vivo, GDF10 expression in vitro is likely induced by mechanical stress of dissection and plating of primary neurons. (b) High magnification of neuronal soma expressing GDF10. GDF10 immunoreactivity is also evident along neuronal processes (arrows).
Supplementary Figure 5

pSmad2/3 quantification in peri-infarct cortex after TGFβ antagonism in vivo

Stroked animals were treated with two different TGFβ antagonists, SB431542 and Losartan at 10mg/kg and 100mg/kg, respectively, based on published i.p. doses\textsuperscript{16,17} for 5 days after stroke (n=3 mice per group). pSmad puncta of 0.45μm were quantified at 100x imaging fields in two sections per animal, and mean puncta values were statistically compared. As seen with in vitro axon outgrowth studies in primary cortical neurons from mouse (Fig. 2) and human iPSC-derived neurons (Fig. 3), in vivo administration of TGFβ antagonists, SB431542 (p=0.0056) and Losartan (p=0.0244), each significantly decreases pSmad2/3 signaling within 300μm of the infarct core. *=p<0.05.
Supplementary Figure 6

Quantitative connectional mapping and axonal connections in peri-infarct cortex

(a) Left panel. Quantitative connectional map of neurons back-labeled from a retrograde tracer injection (cholera toxin b subunit) into cervical level 5 spinal cord (n = 5). This labels all neurons in motor, somatosensory and premotor cortex that send projections to the spinal cord. The location of all neurons is plotted in tangential sections and the x y coordinates are made relative to bregma. Middle panel. Axonal label plotted from BDA injections into forelimb motor cortex in stroke+GDF10 and Cyto C+stroke (protein control). This is the same experiment as in Fig 4a. Right panel: alignment of corticospinal projections with GDF10/stroke map, to show location of areas of axonal sprouting relative to motor, premotor and somatosensory areas. Note that a substantial region of new connections formed in Stroke+GDF10 is in cortex rostral to the corticospinal populations of neurons.

(b) The effect of stroke on motor cortex connections. Left panel: quantitative connectional map of connections from tracer injections into forelimb motor cortex in stroke only (red label) and in control, non-stroke (light blue label) mice. The cortical areas with dense overlap of connections in stroke and control are in dark blue. Note the presence of connections only in the stroke condition in posterior cortical areas that localize to motor cortex and second somatosensory cortex in registering to the corticospinal map and to primary somatosensory cortex by cytochrome oxidase stain (not shown). P value is significant (Hotellings T² test). Middle panel: Polar plot of connections in stroke (red) and control (blue). Conventions as in Fig. 4b. Right panel: quantification of neuronal connections in linear array through cortical hemisphere.
Supplementary Figure 7

Infarct volume, BDA volume and injection locations.

(a) Left Y axis: BDA injection volume, right Y axis infarct volume. Columns are for *in vivo* axonal tracing studies. There are no significant differences among groups. (b) Location of BDA injections for each group relative to the midline of the cortical hemisphere and to the rostral pole of the brain. There are no significant differences among groups. Error bars are SEM.
Quantitative connectional mapping and axonal connections in GDF10 controls compared with normal control.

(a-c) Cortical connections of forelimb motor cortex in normal control animals only compared to stroke+protein control, cytochrome C delivery from the infarct core via biopolymer hydrogel. (n = 8 for each group). Figure conventions are as in Supplementary Figure 8 and Figure 4. There is a significant difference in the cortical connectional map (a), polar plots (b) and linear measurement of neuronal connections (c) between normal control forelimb motor cortex and stroke+scrambled siRNA. This indicates that the normal mode axonal sprouting that is seen in stroke compared to control brains is also seen in the control conditions for GDF10 delivery. (d-f) Cortical connections of forelimb motor area in normal control compared to scrambled siRNA delivered into infarct core. siRNA GDF10 knocks down GDF10 protein levels (Supplementary Fig 5a) and blocks post-stroke axonal sprouting (Fig. 4d-f). To verify that the siRNA is not having effects outside of GDF10 knock down, scrambled sequence siRNA was tested. Figure conventions are as in Supplementary Fig. 8 and Fig. 4. There is a significant difference in cortical connection map (a), polar plots (b) or linear measurement of neuronal connections (c) between control and scrambled siRNA (n = 7-8 for each group). * = p<0.05. Note that Cyto C+stroke and scrambled siRNA+stroke produce very similar maps of forelimb motor cortex connections, and are comparable to stroke only (Supplementary Fig. 6b).
Supplementary Figure 9

Quantitative connectional mapping and axonal connections in GDF10 controls compared with stroke only.

(a-c) Cortical connections of forelimb motor area in stroke only compared to scrambled siRNA delivered into infarct core. siRNA GDF10 knocks down GDF10 protein levels (Supplementary Fig 3a) and blocks post-stroke axonal sprouting (Fig. 4d-f). To verify that the siRNA is not having effects outside of GDF10 knock down, scrambled sequence siRNA was tested. Figure conventions are as in Supplementary Fig. 8 and Fig. 4. There is no significant difference in cortical connection map (a), polar plots (b) or linear measurement of neuronal connections (c) between stroke and scrambled siRNA. (d-f) Cortical connections of forelimb motor cortex in stroke only compared to stroke+protein control, cytochrome C delivery from the infarct core via biopolymer hydrogel (n = 8 each group). Figure conventions are as in Supplementary Fig 8 and Figure 4. There is no significant difference in cortical connection map (a), polar plots (b) or linear measurement of neuronal connections (c) between stroke and Cyto C. Cohorts (n=8) of mice treated with GDF10 (red) or control protein (Cytochrome C) (light blue).
Supplementary Figure 10

Synaptic protein localization in peri-infarct cortex axons that have undergone axonal sprouting after GDF-10 treatment.

(a) Synaptic protein analysis was performed in the same peri-infarct tissues from animals used for BDA axonal sprouting maps in Fig. 4(a) and (b). Presynaptic VGLUT2 and postsynaptic Homer1 antibodies were used for identification of synaptic contacts. Marker colocalization analyses were performed on Imaris Imaging software to uniquely identify the synaptic connections formed by GDF10-induced sprouting cortical neurons after stroke (mapped in Fig. 4). (b) Video through a 10.5μm thick section of peri-infarct cortex taken at 100x. BDA surface is shown in light blue. VGLUT2 presynaptic marker is shown by green spots, and Homer1 postsynaptic marker in red spots.
**Supplementary Figure 11**

**Comparison of forelimb motor system connections in CytoC + stroke versus GDF10 siRNA + stroke.**

Conventions as in Supplementary Figures 10 and 11.
Supplementary Figure 12

Pipeline for incorporating microarray and RNA-seq data sets for neurodevelopmental and CNS injury experiments.

Raw data from different platforms are processed to have gene symbols which were subsequently used to merge the datasets. Merged datasets were normalized, then batch effect was adjusted. In one case, the datasets have very few common genes on different array platforms and these datasets were combined (unionized) instead of taking intersections.
Supplementary Figure 13

Neuronal numbers in *in vitro* axonal outgrowth assays.

The numbers of neurons used for each *in vitro* experiment to generate the data on axonal outgrowth effect of GDF10 and other experimental manipulations in Figures 2 and 3. There is no significant difference in neuronal sampling number across experiments.