Reduced Axonopathy and Enhanced Remyelination After Chronic Demyelination in Fibroblast Growth Factor 2 (Fgf2)–Null Mice: Differential Detection With Diffusion Tensor Imaging

Jennifer E. Tobin, PhD, Mingqiang Xie, PhD, Tuan Q. Le, BA, Sheng-Kwei Song, PhD, and Regina C. Armstrong, PhD

Abstract

Chronic central nervous system demyelinating diseases result in long-term disability because of limited remyelination capacity and cumulative damage to axons. Corpus callosum demyelination in mice fed cuprizone provides a reproducible model of chronic demyelination in which the demyelinating agent can be removed to test modifications that promote recovery and to develop noninvasive neuroimaging techniques for monitoring changes in myelin and axons. We used the cuprizone model in mice with genetic deletion of fibroblast growth factor 2 (Fgf2) to determine the impact of FGF2 on axon pathology and remyelination after chronic demyelination. We also evaluated the ability of quantitative magnetic resonance diffusion tensor imaging (DTI) to distinguish the corresponding pathological changes in axons and myelin during the progression of demyelination and remyelination. During the recovery period after chronic demyelination, Fgf2-null mice exhibited enhanced remyelination that was detected using DTI measures of radial diffusivity and confirmed by electron microscopic analysis of the proportion of remyelinated axons. Ultrastructural analysis also demonstrated reduced axonal atrophy in chronically demyelinated Fgf2-null versus wild-type mice. This difference in axon atrophy was further demonstrated as reduced immunohistochemical detection of neurofilament dephosphorylation in Fgf2-null mice. Diffusion tensor imaging axial and radial diffusivity measures did not differentiate Fgf2-null mice from wild-type mice to correlate with changes in axonal atrophy during chronic demyelination. Overall, these findings demonstrate that attenuation of FGF2 signaling promotes neuroprotection of axons and remyelination, suggesting that FGF2 is an important negative regulator of recovery after chronic demyelination.

Key Words: Axon damage, Diffusion tensor imaging, Fibroblast growth factor, Oligodendrocyte progenitors, Regeneration, Remyelination.

INTRODUCTION

Multiple sclerosis (MS) is the most common human demyelinating disease and the main cause of nontraumatic neurological disability in young adults in North America and Europe (1). In MS, spontaneous remyelination of demyelinated lesions can be effective, especially early in the disease course. However, repeated or prolonged episodes of demyelination can stifle the capacity for repair as well as increase damage to axons. Multiple factors may contribute to poor repair of chronically demyelinated lesions, including depletion of oligodendrocyte progenitor (OP) cells and signals in the lesion environment that inhibit OP differentiation, such as fibroblast growth factor 2 (FGF2), Jagged-1, Lingo-1, and hyaluronic acid (2–5).

Interpreting the effects of FGF2 on the oligodendrocyte lineage during remyelination has been controversial. Contradictory findings have resulted from in vivo studies of FGF2 administration in central nervous system white matter. For example, FGF2 administration caused oligodendrocyte tauopathy in normal white matter, whereas FGF2 overexpression increased generation of oligodendrocyte lineage cells in a model of autoimmune demyelination (6, 7). Alternatively, studies in Fgf2-null mice indicate that endogenous FGF2 inhibits terminal differentiation of OP cells into remyelinating oligodendrocytes (8). In Fgf2-null mice, oligodendrocyte repopulation of lesions is enhanced after viral demyelination of the spinal cord or cuprizone demyelination of the corpus callosum (CC) (2, 9). Furthermore, remyelination is significantly improved in Fgf2-null mice (2). These studies in Fgf2-null mice uncover enhanced repair capacity of the endogenous OP population after chronic demyelination.

The current studies test the ability of magnetic resonance diffusion tensor imaging (DTI) to detect the evolution of pathological features associated with chronic demyelination and recovery in Fgf2-null versus wild-type (WT) mice. In addition, because FGF2 upregulation promotes neuronal
survival in diverse forms of central nervous system injury (10, 11), we evaluated the consequence of FGF2 absence on axon integrity during chronic demyelination. Cuprizone-induced CC demyelination is used to facilitate analysis of remyelination and neuroprotection in Fgf2-null mice. Cuprizone ingestion in mice induces reproducible and extensive demyelination especially within the caudal CC (12, 13), with pathological characteristics of oligodendrocyte loss that are shared with certain MS lesions (14, 15). In addition, MS lesions exhibit 2 main forms of axonopathy. During acute injury in MS, most axons develop swellings from disrupted axonal transport, followed by recovery during resolution of the inflammatory response (1). Subsequent progression to a chronic disease stage is associated with demyelination, atrophy (i.e. reduced diameter), and slow continuous axon loss (1). The cuprizone model exhibits axonal swellings during the acute stage that resolve after the initial episode of demyelination (13). After continued cuprizone feeding, axons become chronically demyelinated and atrophic, yet most axons remain viable and recover after a return to normal chow without toxin (13). Importantly, our analysis of regenerative processes is facilitated by this ability to remove the demyelinating agent from the diet.

MATERIALS AND METHODS

Mice and Cuprizone Demyelination

All experimental procedures using mice were approved by the Uniformed Services University of the Health Sciences Institutional Animal Care and Use Committee and/or the Washington University Animal Studies Committee. C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The Fgf2-null (−/−) and WT (+/+) mice were derived from breeding pairs provided by Dr Thomas Doetschman (University of Cincinnati, Cincinnati, OH) (16). All mouse lines were backcrossed to the C57BL/6 background. Mice were fed ad libitum a diet of 0.2% (wt/wt) cuprizone (oxalic bis-[cyclohexylidenehydrazide]; Sigma-Aldrich, St Louis, MO), mixed into milled chow pellets (Harlan Teklad, Madison, WI) beginning at 8 weeks of age. Analysis time points during cuprizone treatment and recovery were selected based on previous studies, documenting axon and myelin pathology throughout the time course of cuprizone treatment and recovery in C57BL/6 mice and in the Fgf2-null line (2, 9, 13, 17, 18). Mice were examined at 8 weeks of age before the start of cuprizone feeding (0 week, pretreatment), during the period of initial oligodendrocyte loss and demyelination at 4 weeks of cuprizone treatment (4 weeks, acute demyelination), after 12 weeks of cuprizone feeding to produce chronic demyelination (12 weeks, chronic demyelination), and after 12 weeks of cuprizone treatment followed by a 6-week recovery period on normal chow (12 weeks of cuprizone treatment + 6 weeks off). All mice used were males, with the exception of the longitudinal imaging studies in which genders were mixed to facilitate comparisons between littermates because gender should not have an effect in the cuprizone model in C57BL/6 background mice (19).

DTI Analysis of Fgf2 Mice

Fgf2 WT and -null mice (n = 6 of each genotype) underwent a longitudinal DTI study, with scans at selected stages of the disease: pretreatment (0 week), acute demyelination (4 weeks), chronic demyelination (12 weeks), and after recovery on normal chow (12 weeks of cuprizone treatment + 6 weeks off) based on our previous studies (2, 13, 18). An additional cohort of mice was not treated with cuprizone and was studied at 8 weeks of age (age-matched to 0 week pretreatment) and 26 weeks of age (age-matched to 12 weeks of cuprizone treatment + 6 weeks off) to assess age-dependent effects. Diffusion tensor images were acquired at each time point using a 4.7 T Oxford Instrument magnet (Oxfordshire, UK) as described (13). Coronal images (eleven 0.5-mm slices) were acquired throughout the rostral-caudal extent of the CC and were coregistered between scans according to the anatomical position at which the anterior commissure crossed the midline. A multiple-echo spin-echo imaging sequence was used to acquire diffusion-weighted images with the acquisition parameters: TR = 1.5 s, TE = 50 ms, Δ = 25 ms, δ = 8 ms, number of average = 2, number of echo = 3, slice thickness = 0.5 mm, field-of-view = 3 × 3 cm², and data matrix = 256 × 256 (zero filled to 512 × 512). Diffusion sensitizing gradients applied along 6 directions: [Gx,Gy,Gz] = [1,1,0], [1,0,1], [0,1,1], [−1,1,0], [0, −1,1], and [1,0, −1]. Two b values (0 and 0.768 ms/μm²) were applied. As previously reported (18), the eigenvalues (λ₁, λ₂, and λ₃) of the diffusion tensor were derived by matrix diagonalization and on a pixel × pixel basis, and axial (λ₃) and radial diffusivity (λ₃) values were derived using software written in Matlab (MathWorks, Natick, MA). The CC from the midline to under the peak of the cingulum was manually defined as the region of interest on the color-coded relative anisotropy maps (Fig. 1E, F) using National Institutes of Health ImageJ, as in our previous study (13).

Analysis of Myelin and Axon Pathology in Fgf2 Mice

Fgf2 WT and -null mice (n = 3 mice of each genotype at each time point) were perfused with 2% paraformaldehyde/3% acrolein for processing of alternate sections from the same mouse for electron microscopy and immunohistochemistry (13). Cuprizone-induced abnormalities differ along the rostrocaudal extent of the CC, with the most extensive alterations observed in the caudal CC (12, 13). Therefore, regions of the CC were examined separately for quantitative analysis corresponding (approximated relative to bregma) as rostral (+0.25 to +0.75 mm), middle (−0.5 to −1.0 mm), and caudal (−1.5 to −2.0 mm) (13). Parasagittal sections were processed for electron microscopy, as described (13). Sections were examined in a Philips CM100 transmission electron microscope with 3 micrographs of each region of each mouse acquired by an investigator blinded to the genotype and conditions. Images were imported into MetaMorph (Molecular Devices, Downingtown, PA) for quantitative analysis, including axon diameter and myelin thickness. Within each micrograph, all
axons within a randomized area were analyzed with at least 100 axons examined per region. Axons with diameters typical of unmyelinated fibers (<0.3 μm) were excluded from this analysis to follow more specifically the changes associated with demyelination and remyelination within the myelinated fiber population (13, 20). Therefore, the percentage of myelinated axons for each region was calculated as the percentage of myelinated axons among the total number of axons counted per region, excluding any axons of diameters less than 0.3 μm.

Adjacent parasagittal floating 40-μm sections were double immunostained for total neurofilament (NF200, rabbit polyclonal pan-neurofilament antibody; Chemicon, Billerica, MA) and nonphosphorylated neurofilament (SMI-32, mouse monoclonal IgG; Covance, Princeton, NJ) to evaluate axonal integrity (21). NF200-positive and SMI-32-positive
cross-sectional profiles were counted on high-magnification images acquired on a Zeiss Pascal confocal microscope with a $63 \times$ objective and then imported into Adobe Photoshop 7. Axonal damage was estimated as the percentage of SMI-32–positive profiles among all neurofilament-positive profiles counted (13).

**Statistical Analysis**

Comparisons for a single mouse genotype or condition across cuprizone time points were examined using 1-way analysis of variance with a Newman-Keuls post hoc test. Comparisons across 2 genotypes or conditions and multiple cuprizone time points were examined using a 2-way analysis of variance. Values of 2 genotypes or conditions at a single time point were compared using a $t$-test. Scattergraph data were analyzed by linear regression and comparison of the slopes and intercepts. Double immunofluorescence quantification of the proportion of SMI-32 profiles among those detected with NF200 was assessed using $\chi^2$ analysis. A level of 0.05 was used for the overall test of significance. Values are expressed as the mean ± SEM.

**RESULTS**

**DTI Screening of the CC Throughout the Cuprizone Time Course of Demyelination Through Early Remyelination in Fgf2-null and WT Mice**

Longitudinal DTI analysis of cohorts of Fgf2-null and Fgf2 WT mice was performed to assess overall changes associated with the distinct cuprizone disease stages that we previously characterized histopathologically in C57BL/6 mice (Fig. 1) (13, 17, 18). Differential analysis of axial and radial diffusivity values focused on the caudal CC in which cuprizone-induced pathology is most extensive (12, 13).

Axial diffusivity values reflected a significant effect of cuprizone treatment across time points ($p < 0.0001$; Fig. 1A). In both Fgf2-null and WT mice, cuprizone treatment resulted in decreased axial diffusivity values from pretreatment to 4 weeks of cuprizone treatment (WT, $p < 0.01$; null, $p < 0.001$). These results are consistent with our previous studies in C57BL/6 mice in which reduced axial diffusivity values were observed in regions of axon swellings and variocities, as well as high cell densities from reactive changes in the CC during the onset of cuprizone demyelination (13, 18). The Fgf2 genotype did not have an overall effect on axial diffusivity across the time points examined ($p = 0.1368$).

Radial diffusivity values reflected a significant effect of cuprizone treatment across time points ($p < 0.0001$; Fig. 1B). Importantly, an effect of Fgf2 genotype was observed across time points ($p < 0.01$). In both Fgf2-null and WT mice, radial diffusivity values were unchanged from pretreatment to 4 weeks of cuprizone treatment but were significantly increased by 12 weeks of cuprizone treatment (WT and null, $p < 0.001$ for each genotype at 12 weeks vs 4 weeks or pre-cuprizone time points). After chronic demyelination and 6 weeks of recovery, radial diffusivity values remained significantly elevated in Fgf2 WT mice ($p < 0.001$), whereas in Fgf2-null mice, they were significantly reduced ($p < 0.05$ vs 12 weeks) and similar to nontreated levels ($p > 0.05$ vs pre-cuprizone). Although Fgf2-null and WT mice did not exhibit overt differences in the CC on relative anisotropy maps (Fig. 1E, F) during this recovery period, the specific changes detected in the quantitative analysis were evident in the radial diffusivity maps (Fig. 1G, H). The Fgf2 WT values are similar to those observed in our previous studies in which demyelinated regions of the CC exhibit increased radial diffusivity values that did not recover fully after chronic demyelination even when followed up to 12 weeks after return to normal chow (13, 18). Therefore, the normalization of radial diffusivity in Fgf2-null mice during the recovery period suggests a significant improvement in remyelination associated with Fgf2 genotype.

**Ultrastructural Analysis of Remyelination**

To examine ultrastructural differences for correlations with the DTI changes that may indicate improved remyelination in Fgf2-null mice, we performed quantitative electron microscopy on the CC. The Fgf2-null mice have a higher proportion of myelinated axons at 12 weeks of cup treatment and during the 6-week recovery period compared with the Fgf2 WT mice ($p < 0.05$ between genotypes across all time points). *$p < 0.05$ for comparisons of genotypes at each time point.

**FIGURE 2.** Ultrastructural analysis of myelination in fibroblast growth factor 2 (Fgf2)–null and wild-type (WT) mice. (A–F) Electron micrographs of corpus callosum (CC) in Fgf2 WT (A–C) and Fgf2-null (D–F) mice illustrate normal myelination in nontreated (26 weeks) controls (A, D), demyelination after 12 weeks of cuprizone (cup) treatment (B, E), and remyelination after 12 weeks of cup treatment and 6 weeks on normal chow (C, F). (G) The percentage of myelinated axons quantified in the caudal region of the CC. The Fgf2-null mice have a higher proportion of myelinated axons at 12 weeks of cup treatment and during the 6-week recovery period compared with the Fgf2 WT mice ($p < 0.05$ between genotypes across all time points). *$p < 0.05$ for comparisons of genotypes at each time point.
microscopic analysis of the myelination status in the CC (Fig. 2). After 12 weeks of cuprizone treatment, marked demyelination was noted in mice of both Fgf2-null and Fgf2 WT genotypes (Fig. 2B, E), the percentage of myelinated fibers increased during the 6-week recovery period on normal chow, suggesting partial remyelination (Fig. 2C, F). The Fgf2-null mice had a higher proportion of myelinated axons at 12 weeks of cuprizone treatment (p < 0.05) and during the recovery period (p < 0.05) as compared with Fgf2 WT mice (Fig. 2G).

Ultrastructural demonstration of remyelination is based on evidence of reduced myelin thickness relative to axon diameter. For analysis of CC remyelination after cuprizone, the g ratio value (an overall population average value of the ratio of axon diameter to myelinated fiber diameter) is not as sensitive as population distribution analysis using the slope of myelin thickness relative to axon diameter, which displays the distribution of individual fiber changes in myelin thickness relative to axon diameter (13, 22). After chronic cuprizone demyelination and a 6-week recovery, both Fgf2 WT and -null mice showed remyelination in a significant proportion of axons when compared with nontreated mice of the same genotype (Fig. 3; comparison of slopes: Fgf2 WT, p < 0.05 for no cuprizone vs 12 weeks of cuprizone treatment + 6 weeks off; Fgf2-null, p < 0.05 for no cuprizone vs 12 weeks of cuprizone treatment + 6 weeks off). After this recovery period, Fgf2 WT and -null mice also show similar myelin thickness relative to axon diameter (Fig. 3C, F; 12 weeks of cuprizone treatment + 6 weeks off; p > 0.05 for WT vs null). Importantly, before the recovery period, there was an Fgf2 genotype effect (Fig. 3B, E; 12 weeks of cuprizone treatment, p < 0.05 for Fgf2 WT vs Fgf2-null mice). Specifically, the myelinated fiber population in Fgf2-null mice was associated with reduced myelin thickness relative to axon diameter (Fig. 3D, E; Fgf2-null mice, p < 0.05 for no cuprizone vs 12 weeks of cuprizone treatment). These data indicate remyelination at 12 weeks of cuprizone treatment in Fgf2-null mice that was not detected in Fgf2 WT mice (Fig. 3B, E; comparison of slopes; Fgf2 WT, p > 0.05 for no cuprizone vs 12 weeks of cuprizone treatment).

Our ultrastructural data on the percentage of myelinated axons (Fig. 2G) combined with the population analysis of the distribution of myelin thickness relative to axon diameter (Fig. 3) facilitate distinctions between myelinated populations

![FIGURE 3. Relationship of myelin thickness to axon diameter in fibroblast growth factor 2 (Fgf2)-null and wild-type (WT) mice. Scatter plots of individual axons in the caudal corpus callosum for Fgf2 WT (A, D) and Fgf2-null (B, E) mice in nontreated (26 weeks) controls (A, D), demyelination after 12 weeks of cuprizone (cup) treatment (B, E), and after 12 weeks of cup treatment and 6 weeks on normal chow (C, F). (A–C) In Fgf2 WT mice, thinner myelin relative to axon diameter (indicative of remyelination) is demonstrated by the significantly reduced slope observed during the recovery period (a, Fgf2 WT, p < 0.05, no cup treatment vs 12 weeks of cup treatment + 6 weeks off). (D–F) In comparison with Fgf2 WT mice, Fgf2-null mice have thinner myelin before cup treatment (b, no cup treatment p < 0.05, WT vs null). The Fgf2-null mice exhibit significantly reduced slopes from nontreated Fgf2-null mice after both 12 weeks of cup (c, p < 0.05, no cup vs 12 weeks of cup treatment) and during the recovery period (e, p < 0.05, no cup vs 12 weeks of cup treatment + 6 weeks off), indicating significant remyelination at the 12-week time point and continuing during the recovery period. Importantly, the slopes are also significantly different for the Fgf2-null mice vs Fgf2 WT mice at 12 weeks of cup treatment (d, WT vs null, p < 0.05 at 12 weeks of cup treatment), indicating earlier remyelination in Fgf2-null mice than in WT mice. The slopes are not different for Fgf2-null versus WT mice after the recovery period (f, WT vs null, p > 0.05), indicating that the myelin thickness of remyelinated fibers is similar between genotypes, although Fgf2-null mice started with thinner myelin before cup treatment (b).](http://jnen.oxfordjournals.org/)
that have not demyelinated and those that have undergone remyelination. Specifically, after 12 weeks of cuprizone treatment, the Fgf2 WT mice had a majority of axons without myelin (Fig. 2G; 15.83% ± 7.6% myelinated) and a slope that is not significantly different from the nontreated Fgf2 WT population (Fig. 3B). Therefore, in Fgf2 WT mice, most of the axon population with myelin after 12 weeks of cuprizone treatment is composed of fibers that have myelin thickness similar to those in nontreated mice; presumably, these represent normally myelinated axons that did not undergo

FIGURE 4. Axon damage in fibroblast growth factor 2 (Fgf2)-null and wild-type (WT) mice. (A, B) Representative confocal microscopic images of double immunolabeling with NF200 (green; pan-neurofilament marker) and SMI-32 (red; non-phosphorylated neurofilament epitope) in the caudal corpus callosum (CC) of Fgf2 WT mice without cuprizone (cup) treatment (A) and after 12 weeks of cup treatment to illustrate the increase of SMI-32 immunolabeling (B). Scale bars = 20 μm. (C, D) Quantification of the proportion of SMI-32-labeled axons in the rostral, middle, and caudal CC of Fgf2 WT (C) and -null (D) mice that are nontreated (26 weeks) controls (no cup treatment), fed cup for 12 weeks (12 weeks of cup treatment), or fed cup for 12 weeks followed by 6 weeks on normal chow (12 weeks of cup treatment + 6 off). After 12 weeks of cup treatment, significantly fewer axons are SMI-32 positive in Fgf2-null mice (D) versus Fgf2 WT mice (C) (2-way analysis of variance p = 0.0059; rostral p < 0.01, middle p < 0.01, caudal p < 0.001). (E, F) Quantification of axon diameter in electron micrographs from Fgf2 WT (E) and -null (F) mice. (Representative ultrastructural images are shown in Fig. 2). After 12 weeks of cup treatment, mean axon diameters are significantly reduced in Fgf2 WT mice; this atrophy is attenuated in Fgf2-null mice (F) (middle p < 0.05, caudal p < 0.05).
Reduced Axonal Damage in Fgf2-Null Mice

We next examined axons in Fgf2-null and WT mice to characterize the effect of the Fgf2-null phenotype on axonopathy with chronic demyelination and to evaluate the contribution of axonal pathology to the DTI findings. SMI-32 immunostaining was combined with immunolabeling for NF200, a pan-neurofilament epitope, to detect the total population of axons with myelin after 12 weeks of cuprizone treatment. SMI-32 immunofluorescence in the CC of Fgf2-null mice exhibited an increase in SMI-32 immunostaining in the CC of Fgf2-null mice, a significant proportion of axons with myelin after 12 weeks of cuprizone treatment and that remyelination was ongoing during chronic demyelination at 12 weeks of cuprizone ingestion. Electron microscopy confirmed that a greater proportion of demyelinated axons underwent spontaneous remyelination in Fgf2-null mice, and that remyelination was ongoing during chronic demyelination after 12 weeks of cuprizone treatment in Fgf2 WT mice that is greatly reduced in Fgf2-null mice. The effect of Fgf2 absence in the protection from chronic axonopathy was further supported by immunohistochemical studies showing reduced neurofilament dephosphorylation during chronic demyelination in Fgf2-null mice. However, DTI analysis did not detect this attenuation of chronic axonopathy in Fgf2-null mice.

DISCUSSION

The current studies extend our previous work in Fgf2-null mice by adding 2 independent approaches; both indicate that remyelination is significantly enhanced after chronic demyelination. Importantly, in Fgf2-null mice, we can now detect remyelination at an earlier time point and now reveal a dramatic reduction of chronic axonal abnormalities. Furthermore, we demonstrate the capabilities and limitations of DTI for distinguishing these pathological effects. Specifically, the extent of enhanced remyelination in Fgf2-null mice is sufficiently robust and generalized to be detectable non-invasively with longitudinal DTI analysis. Electron microscopy confirmed that a greater proportion of demyelinated axons underwent spontaneous remyelination in Fgf2-null mice, and that remyelination was ongoing during chronic demyelination after 12 weeks of cuprizone treatment in Fgf2 WT mice that is greatly reduced in Fgf2-null mice. The effect of FGF2 absence in the protection from chronic axonopathy was further supported by immunohistochemical studies showing reduced neurofilament dephosphorylation during chronic demyelination in Fgf2-null mice. However, DTI analysis did not detect this attenuation of chronic axonopathy in Fgf2-null mice.

The neuroprotective effects in Fgf2-null mice are an important clue to understanding how to prevent early-stage axon damage from progressing to discontinuity and permanent neurological deficits in demyelinating diseases. Fibroblast growth factor 2 can be synthesized by reactive astrocytes and by activated microglia and macrophages in demyelinated lesions. Diverse cellular components in the lesion environment including axons, astrocytes, microglia, as well as oligodendrocyte lineage cells can express multiple FGF receptor (FGFR) isoforms that may respond differentially to FGF2 (24, 25). Given this complexity, the improved axon integrity observed in the absence of FGF2 may result from direct effects on axons or indirect effects through multiple cell types. For example, FGF2 has been reported to act through FGFR1 on astrocytes to induce scarring characteristics (24), which could drive the lesion environment to be less favorable for axon homeostasis. Oligodendrocytes may have trophic effects on axons and remyelination may protect denuded axons from further insult (1, 26), processes that may also contribute indirectly to the axon protection observed in Fgf2-null mice. In the peripheral nervous system, increased axon size and myelination were observed in Fgf2-null mice after sciatic nerve injury (27), indicating potential common beneficial effects of FGF2 removal for axons in both the central and peripheral nervous systems. Combining these findings with those of beneficial effects of FGF2 on neuronal survival (10, 11), we propose a model of differential effects of FGF2 upregulation after injury in which FGF2 directly promotes neuronal, that is, cell body, survival while indirectly impairing axonal recovery. In white matter tracts, FGF2 may act indirectly to induce an astroglial scarring phenotype and/or inhibit OP differentiation and remyelination, which may create conditions that leave axons vulnerable to further damage and do not sufficiently support axon health or recovery.
Electron microscopy provides a more detailed characterization of remyelination in Fgf2-null versus WT mice than was possible with our previous immunohistochemical analysis of myelin-associated glycoprotein (MOG) (2). Myelin-associated glycoprotein immunostaining of the CC in cuprizone-treated mice has advantages for analyzing large lesion areas and correlates well with Luxol fast blue myelin staining (28). However, both immunohistochemistry for MOG and LFB staining are less sensitive than electron microscopy for detecting remyelination (28). Consistent with this difference in technique sensitivity, the ongoing remyelination in Fgf2-null mice was detected at 12 weeks by electron microscopy, but not by quantification of MOG immunostaining (2).

Whether FGF2 limits remyelination by acting directly on oligodendrocyte lineage cells or indirectly through other cells in the lesion environment is not yet clear. Retroviral lineage tracing, to monitor differentiation of endogenous cycling cells during the course of cuprizone demyelination and remyelination, demonstrated increased differentiation of OP cells into oligodendrocytes in Fgf2-null mice, indicating that FGF2 in lesions may limit remyelination by inhibiting OP differentiation (8). RNA interference studies of cultured OP cells show that FGF2 can act through FGFR1 to regulate OP differentiation into oligodendrocytes (29). In the context of the demyelinated lesion environment, FGF2 may also activate FGFR1 on astrocytes to induce glial scarring, thereby indirectly inhibiting remyelination.

This is the first demonstration of the use of DTI to detect changes associated with a genetic manipulation that improved recovery from demyelinating disease. The work complements a similar study that demonstrated DTI detection of improved remyelination pharmacologically stimulated by thyroid hormone treatment during a recovery period after chronic demyelination (30). These studies provide proof of principle for noninvasive longitudinal DTI assessments that can be applied to help evaluate interventions for promoting remyelination in translational studies. Longitudinal functional and imaging assessments provide critical comparisons across disease stages to assess the extent of disease severity and the subsequent recovery achieved. Our earlier studies in C57BL/6 mice with scans every other week throughout the cuprizone course identified 4 weeks of cuprizone treatment as the peak of reduced axial diffusivity, whereas radial diffusivity values were elevated from 6 to 12 weeks of cuprizone treatment and remained elevated during the recovery period (17, 18). The decrease of axial diffusivity at 4 weeks of cuprizone treatment correlates with early-stage axonal damage,axon swelling, and neurofilament dephosphorylation, along with a high density of CD11b-immunolabeled microglia/macrophage cells in the CC (13). During the chronic demyelination stage, axonal swellings resolve to relatively uniform axonal profiles, although axonopathy is still indicated by axon diameter reduction (13). Diffusion tensor imaging axial diffusivity values during chronic demyelination were not significantly different from controls, possibly reflecting dissipation of microglia/macrophage populations (13) or increased extracellular volume masking axonal damage effects of decreasing axial diffusivity. Accordingly, the reduction of chronic-stage axonopathy in Fgf2-null mice was not detected by DTI axial diffusivity. The DTI-detected signal reflects the volume average effect of the environment on the image voxel in the anatomical region of interest quantified. Because the CC contains both myelinated and unmyelinated axons, potential changes in unmyelinated axons during chronic demyelination might also be undetected with current techniques. In contrast, the periods of elevated radial diffusivity generally correspond to histological and ultrastructural evidence of demyelination in the CC (13). Importantly, we distinguished a significant normalization of radial diffusivity values after removal of cuprizone from the diet in the Fgf2-null mice versus the WT mice. This difference of radial diffusivity during the recovery period correlated with ultrastructural evidence of significantly improved remyelination in Fgf2-null mice.

These studies support the expectation that recovery from chronic demyelination may be enhanced through therapeutic strategies that reduce signals inhibiting OP differentiation. Fibroblast growth factor 2 signaling is an effective component limiting remyelination of chronically demyelinated lesions. The pleiotrophic effects of FGF2 create an unfavorable profile for therapeutic development so that further studies are needed to characterize the relevant FGFR components and more specific therapeutic targets. The dramatic neuroprotective effect observed with genetic removal of FGF2 requires further study to identify the specific molecular mechanisms that may be involved and potentially exploited to prevent accrual of axonal damage and loss during chronic demyelination. Diffusion tensor imaging will be an important tool for longitudinal monitoring and screening of reagents to promote remyelination but will require complementary imaging techniques to evaluate chronic axonopathy.

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