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# Mutual Antagonism Between Sox10 and NFIA Regulates Diversification of Glial Lineages and Glioma Sub-Types

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## Abstract

Lineage progression and diversification is regulated by the coordinated action of unique sets of transcription factors. Oligodendrocytes (OL) and astrocytes (AS) comprise the glial sub-lineages in the central nervous system (CNS) and how their associated regulatory factors orchestrate lineage diversification during development and disease remains an open question. Sox10 and NFIA are key transcriptional regulators of gliogenesis associated with OL and AS. We found that NFIA inhibits Sox10 induction of OL differentiation through direct association and antagonism of its function. Conversely, we found that Sox10 antagonizes NFIA function and suppresses AS differentiation. Using this developmental paradigm as a model for glioma, we found that this relationship similarly regulates the generation of glioma sub-types. These studies describe the antagonistic relationship between Sox10/NFIA that regulates the balance of OL and AS fate during development and demonstrate for the first time that the transcriptional processes governing glial sub-lineage diversification oversee the generation of glioma sub-types.



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Author Contributions

BD and SG conceived the project, designed the experiments, and wrote the manuscript. SG and WZ performed all the experiments. BD, SG, WZ, and CM analyzed the data. CS, MW, TW, JN, JL, and FC provided essential reagents.

## Introduction

Progression of precursor populations through a developmental lineage is an ordered, stepwise process that culminates in the generation of a differentiated cell with a specific physiological function. A major challenge facing a precursor cell over the course of lineage development is assuring the timely expression of molecular components essential to the physiology and function of the differentiated derivative. Given the importance of transcription factors in the regulation of cell fate decisions, it follows that changes in the transcription factor milieu are an essential component of this tightly regulated process. As a consequence unique combinations of transcription factors are required to produce appropriate transcriptional outputs at different stages of lineage development.

Oligodendrocytes (OL) and astrocytes (AS) comprise the glial sublineages in the central nervous system (CNS) and how their associated regulatory factors orchestrate lineage diversification during development is a critical question. Previously we identified Nuclear Factor I-A (NFIA) as a key transcription factor in the specification of glial identity and differentiation of AS in the central nervous system (CNS) [1,2,3]. NFIA function during these distinct phases of astroglial development is mediated by interactions with different transcription factors, where during glial specification it associates with Sox9 and in differentiating astrocytes it collaborates with STAT3 [1,3]. Recently, we also found that NFIA suppresses OL differentiation by directly repressing myelin gene expression, though how it fits into the existing OL transcriptional network remains undefined [4]. Given the diverse functions of NFIA across glial sub-lineages, delineating its partnerships during OL development will further resolve how transcriptional networks operate during the compartmentalization of glial sub-lineages (ie. astrocyte v. oligodendrocyte).

In addition to central roles in development, transcriptional regulators of gliogenesis have also been implicated in glioma formation [5]. Previous studies have demonstrated roles for STAT3, Olig2, and NFIA in glioma formation, indicating common transcriptional requirements for glial development and glioma tumorigenesis [6,7,8]. While these studies established that glial fate determinants play a general role that supports tumoriogenesis, whether their developmental functions in specifying cell identity similarly influence the cellular constituency within glioma remains undefined. This is a key, unresolved question, as glioma is comprised of several sub-types, including astrocytoma and oligodendroglioma, which have vastly different clinical outcomes [9]. Therefore understanding how the generation of glioma tumor sub-types is linked to the developmental processes that regulate glial diversification has important implications for the understanding and treatment of this disease.

Given our previous findings that NFIA and Sox9 form a complex and cooperatively regulate a set of genes, we reasoned that NFIA might also have a functional relationship with Sox10 during oligodendrocyte precursor (OLP) differentiation. Using chick and mouse models, we found that NFIA directly antagonizes Sox10 regulation of myelin gene expression and that the reciprocal relationship exists during AS differentiation. Analysis of Sox10 knockout mice revealed a conversion of OLPs to AS, revealing a new role for Sox10 in the suppression of astrocyte fate. Applying this developmental relationship to the generation of

glioma sub-types, we used a novel mouse model of glioma, finding that overexpression of NFIA converts an oligodendroglioma to an astrocytoma. In sum, this study reveals that cross-antagonism between Sox10 and NFIA balances OL and AS fate decisions and in turn regulate the diversification of glial lineages during development and tumorigenesis.

## Results

### NFIA antagonizes Sox10 induction of myelin genes

NFIA has a dynamic expression pattern during OLP differentiation in the embryonic spinal cord, where it is expressed in the pMN domain and migrating OLPs, but is downregulated prior to myelin gene expression (Figure 1a and Figure S1) [4]. Conversely, Sox10 is expressed throughout OLP lineage development, beginning in the pMN domain at E12.5 and continuing in mature, myelin gene expressing oligodendrocytes (Figure 1a and Figure S1) [10,11,12]. These patterns of expression are complemented by functional differences between NFIA and Sox10, where NFIA represses- and Sox10 promotes- myelin gene expression [4,13].

That NFIA and Sox10 are co-expressed in OLP populations prior to the induction of myelin genes and regulate their expression in an opposing manner, led us to hypothesize that NFIA antagonizes Sox10 induction of myelin genes. To test this *in vivo* we made use of previous observations demonstrating that overexpression of Sox10 in the chick spinal cord promotes, early and ectopic expression of the myelin genes MBP and PLP [14]. As indicated in Figures 1b-e, overexpression of Sox10 results in the induction of MBP, PLP, and MAG at E4 in the chick spinal cord. Next we performed combined overexpression of Sox10 and NFIA, finding that inclusion of NFIA inhibits the early and ectopic induction of MBP, PLP, and MAG by Sox10 (Figure 1f-i, p). Moreover, ectopic Olig2 induction, by Sox10, was also inhibited by NFIA overexpression (Figure S2).

To confirm these findings in a cell system that models OLP differentiation we made use of *in vitro* OLP culture and lentiviral-mediated overexpression of Sox10 and NFIA (see methods) [4,15]. Consistent with our chick studies, we found that misexpression of lentiviruses containing Sox10 resulted in increased expression MBP, PLP, and MAG and that these effects were inhibited when Sox10 was combined with lentiviruses containing NFIA (Figure 1j-o, q). These *in vitro* and *in vivo* functional studies, in conjunction with the expression dynamics of NFIA and Sox10 during OLP development, indicate that NFIA antagonizes Sox10 induction of MBP, PLP, and MAG during OLP development.

### NFIA directly antagonizes Sox10 function

Having established that NFIA antagonizes Sox10 function during OLP differentiation, we next sought to decipher the biochemical basis of this relationship. Previously we found that NFIA forms a complex with Sox9 and positively co-regulates a set of genes associated with glial specification in the developing spinal cord [1]. Because Sox9 and Sox10 are closely related members of the SoxE sub-family of Sox genes, we reasoned that NFIA antagonism of Sox10 occurs through a direct mechanism. To examine this possibility, we first determined whether NFIA and Sox10 associate by performing immunoprecipitation (IP)

experiments in Oli-Neu on ectopically expressed, tagged versions of Sox10 and NFIA, as well as endogenous proteins. Our IP-western analysis of the protein lysates derived from these studies revealed that NFIA and Sox10 co-immunoprecipitate, indicating that they physically associate (Figure 2a).

That NFIA and Sox10 form a complex, suggests that NFIA directly antagonizes Sox10 induction of MBP, PLP and MAG. To further examine this relationship we determined whether this antagonism occurs on the promoter regions of MBP, PLP, and MAG regulated by NFIA and Sox10. Previously, we identified key NFIA binding sites in the MBP, PLP, and MAG promoters (Figure 2b-d; Figure S3) [4]. Subsequent bioinformatic analysis of these promoter regions identified Sox10 binding sites in relatively close proximity to the NFIA sites (Figure 2b-d; Figure S3). Chromatin immunoprecipitation (ChIP) analysis on E12.5 mouse spinal cord revealed that both NFIA and Sox10 associate with the regions containing their binding sites, suggesting that this antagonism occurs on these regulatory regions (Figure 2b-d). To confirm this we performed reporter assays with the promoter regions of MBP, PLP, and MAG that contain these NFIA and Sox10 binding sites and found that NFIA antagonizes Sox10 induction of each of these reporter constructs (Figure 2e-g). Together, these data indicate that NFIA directly antagonizes Sox10 transcriptional induction of MBP, PLP, and MAG.

## Sox10 antagonizes NFIA induction of astrocyte genes

That NFIA antagonizes Sox10 induction of myelin genes during OLP development raises the question of whether Sox10 antagonizes NFIA induction of astrocyte-related genes. Previously, we demonstrated that overexpression of NFIA in the chick spinal cord promotes the precocious migration of astrocyte-precursors and induction of GFAP, a marker of mature astrocytes (Figure 3a-e), both key aspects of astrocyte lineage differentiation [2]. To examine whether Sox10 antagonizes NFIA function during astrocyte differentiation, we combined NFIA and Sox10 misexpression in the chick spinal cord and assessed the impact on these NFIA induced, astrocyte-related phenotypes at E7. As indicated in Figures 3f-j, combined expression of Sox10 with NFIA blocked the precocious migration of GLAST-expressing astrocyte precursors and the induction of GFAP. Moreover, Sox10 blocked NFIA induction of Apcdd1, a key NFIA target that regulates astrocyte precursor migration (Figure 3d,i-k)[1]. To confirm Sox10 antagonism of NFIA induction of astrocyte-related genes, we employed a cortical progenitor culture system [2,16,17]. Previously, we demonstrated that overexpression of NFIA in these cortical progenitors promotes the formation of GFAP-expressing astrocytes (Fig. 3p,r). Consistent with our chick studies, we found that combined expression with Sox10 blocked NFIA induced GFAP-expressing astrocytes (Figure 3o-r; arrows). Together, these data indicate that Sox10 antagonizes NFIA induction of astrocyte-related genes.

Next we examined whether Sox10 antagonism of NFIA occurs on the regulatory regions of these astrocyte-related genes, by first determining whether the GFAP and Apcdd1 promoter regions also contain NFIA and Sox10 binding sites in close proximity. Using bioinformatics, we found that this was indeed the case and confirmed that both NFIA and Sox10 associate with these regulatory regions by performing ChIP assays on E12.5 spinal cord (Figure 3j-k; Figure S3). To determine whether this antagonism occurs on the Apcdd1 and GFAP

regulatory regions, we performed reporter assays with these regulatory elements in the presence of various combinations of Sox10 and NFIA. As indicated in Figures 3l-m, overexpression of NFIA induced activation of these reporter constructs, while combined expression with Sox10 resulted in either loss of activity (Apcdd1-Figure 3m) or attenuated activation (GFAP-Figure 3l). Together, our functional and biochemical studies indicate that Sox10 antagonizes NFIA induction of astrocyte-related genes.

That Sox10 antagonizes the ability of NFIA to promote astrocyte differentiation raises the possibility that Sox10 itself suppresses astrocyte differentiation. To examine this possibility we made use of the cortical progenitor culture system, where astrocyte differentiation can be induced via removal of FGF [2,17]. As expected overexpression of NFIA promoted astrocyte differentiation, which was reduced when combined with Sox10, indicating that Sox10 is capable of antagonizing NFIA function under these conditions as well. (Figure 3o, p, r; arrows). Next, we overexpressed Sox10 on its own and found that it reduced the generation of GFAP-expressing astrocytes by five-fold compared to control populations (Figure 3s, t, w; arrows; also see Figure S9 for single channel images), suggesting that it functions to suppress astrocyte differentiation *in vitro*.

## Sox10 suppresses astrocyte development

In the developing spinal cord Sox10 expression is restricted to the pMN domain and migrating OLP populations. While Sox10 is not expressed in migrating- or mature- astrocyte populations, multiple lineage tracing studies have indicated that a subset of pMN derived cells generate astrocytes in the ventral spinal cord, likely through radial glial transformation [18,19] (D. Rowitch, personal communication). These observations suggest that Sox10-expressing cells have the potential to give rise to astrocytes; indeed Sox10 is co-expressed with GLAST in the pMN domain, further supporting this possibility (Figure S1). To confirm this, we used a *Sox10-Cre;Rosa26-LacZ* mouse line to trace the fate of Sox10-expressing cells in the spinal cord and found that they similarly give rise to a subset of both grey- and white-matter astrocytes (GMA and WMA, respectively) in ventral and ventral-lateral regions of the spinal cord (Figure S4)[20].

That Sox10-expressing cells have the potential to give rise to astrocyte populations, coupled with our functional studies indicating that it antagonizes NFIA regulation of astrocyte-related genes, suggest that Sox10 functions early in OLP populations to ensure lineage integrity by suppressing astrocyte development programs. Therefore, we postulated that loss of Sox10 would disrupt repression of these astrocyte programs, resulting in enhanced astrocytogenesis or differentiation. To investigate this possibility, we made use of the *Sox10-LacZ* mouse line [21], where LacZ is inserted into the Sox10 locus and generated E16.5 and E18.5 embryos from the $Sox10^{LacZ/+}$ and $Sox10^{LacZ/LacZ}$ lines. To assess astrocyte differentiation, we analyzed expression of GFAP, Glutamine Synthase (GS), and AldoC, finding that expression of these markers was significantly enhanced in $Sox10^{LacZ/LacZ}$ embryos at both timepoints in grey- and white matter regions of the spinal cord (Figure 4a-r). This *in vivo* analysis was supplemented with *in vitro* studies, where we found increased astrocytogenesis from neural stem cells derived from $Sox10^{LacZ/LacZ}$ embryos and rescue of

these effects with ectopic expression of Sox10 (Figure S5). Together, these observations indicate that loss of Sox10 enhances astrocyte differentiation.

The enhanced astrocyte differentiation witnessed in the absence of Sox10 can be due to either accelerated differentiation of astrocyte precursor populations or a conversion of Sox10-expressing cells to the astrocyte fate. To distinguish between these models, we made use of the LacZ inserted into the Sox10 locus to assess the fate of the Sox10-LacZ expressing populations in *Sox10*$^{LacZ/LacZ}$ embryos. Double labeling experiments revealed minimal overlap between GFAP and β-gal in white matter regions in both *Sox10*$^{LacZ/+}$ and *Sox10*$^{LacZ/LacZ}$ animals (Figure 4c,l-arrow). Analysis of Pax6 and Nkx6.1, markers of ventral WMAs, revealed no change in their numbers, indicating that the observed increase in GFAP expression in white matter regions is not due to an increase in the number of WMAs, but rather an increase in its expression in individual astrocytes (Figure S6) [22]. Analysis of GS, AldoC, and Id3 in GMAs revealed an increase in the extent GS/β-gal+ and a six- and three- fold increase in the number of AldoC/β-gal+ and Id3/β-gal expressing cells in GMAs of *Sox10*$^{LacZ/LacZ}$ spinal cord, respectively (Figure 4d-r,s, t, w). Together these data suggest that increased expression of these markers in the grey matter is due to a conversion of OLP populations to the astrocyte fate in *Sox10*$^{LacZ/LacZ}$ embryos. Consistent with a conversion to astrocytes we found a 30% decrease in the number of OLPs (Olig2/β-gal+) in the grey matter of *Sox10*$^{LacZ/LacZ}$ embryos (Figure 4u-w). Finally these changes in cellular constituency were not accompanied by increases in cell death or proliferation (Figure. S6). Taken together, our analysis indicates that loss of Sox10 has differential effects on the differentiation of astrocyte populations in the spinal cord: in the white matter it accelerates the differentiation of individual GFAP-expressing astrocytes while in the grey matter it results in a conversion of OLPs to AldoC-, GS-, Id3-expressing astrocytes.

## Olig2 facilitates selectivity of NFIA and Sox-family interactions

Our finding that Sox10 and NFIA have an antagonistic relationship is in contrast to the cooperativity that exists between Sox9 and NFIA. That these seemingly similar complexes have opposing functional outcomes is paradoxical and led us to further investigate the nature of these relationships. Given that Sox9 and NFIA are also expressed in pMN domain, yet this domain does not robustly generate astrocyte precursors, we reasoned that factors specifically expressed in pMN domain interfere with the Sox9/NFIA relationship. Among transcription factors specifically expressed in the pMN domain, Olig2 plays a key role in the specification of the OL lineage during early embryogenesis. To test whether Olig2 can antagonize the cooperativity between Sox9/NFIA, we performed reporter assays on two key astrocyte-related genes, APCDD1 and GFAP. As indicated in figures 5a-b, Sox9/NFIA synergistically activates both reporters, while inclusion of Olig2 suppresses this activation. Next we performed co-IP studies, finding that Sox9/NFIA continue to associate in the presence of Olig2 and, indeed, co-IP with Olig2 (Figure 5e, lanes 2-3). These observations suggest that Olig2 associates with Sox9/NFIA complexes and neutralizes their capacity to cooperatively drive astrocyte gene expression. That Olig2 antagonizes Sox9/NFIA cooperativity raises the complementary question of whether it facilitates Sox10/NFIA antagonism. To address this question we performed reporter assays and found that Olig2 weakly attenuates NFIA suppression of Sox10 dependent induction of MBP-reporter

activity, while it potentiates Sox10 dependent repression of NFIA induced GFAP-reporter activity (Figure 5c-d). Together, these findings suggest Olig2 reinforces the antagonistic relationship that exists between Sox10/NFIA. To dissect the biochemical basis for this we performed co-IP studies, and, surprisingly, found that the extent of Sox10/NFIA association is significantly enhanced in the presence of Olig2 (Figure 5e, lanes 4-5). These data suggest that Olig2 facilitates the interaction between Sox10 and NFIA, reinforcing the antagonism that exists between these factors. To directly test this possibility we performed *in vitro* GST-pulldown studies, and found that NFIA binding to Sox10 is increased in the presence of Olig2 (Fig. 5f, lanes 2-3), indicating that Olig2 mediates the biochemical relationship between Sox10 and NFIA. Together, these studies indicate that Olig2 has a polarizing effect on Sox9/NFIA and Sox10/NFIA complexes that exist in pMN, it neutralizes the former and reinforces the latter, thus functioning to organize these transcriptional interrelationships that regulate glial sub-lineage fate decisions in the pMN domain during early development.

## NFIA regulates generation of glioma sub-types

Our foregoing data indicate that antagonism between Sox10 and NFIA regulates AS and OL fate choice. Given that many developmental programs are reutilized during malignancy, we examined whether this relationship between Sox10 and NFIA is applicable to the generation of glioma sub-types, astrocytoma and oligodendroglioma. Previous studies indicate that Sox10 is expressed in all glioma sub-types, while NFIA is highly expressed in astrocytoma and nominally expressed in oligodendroglioma [8,23,24,25,26]. That NFIA demonstrates very low expression in oligodendroglioma suggests a correlation between its suppression of oligodendrocyte development and its role in glioma formation. This, coupled with our observations that NFIA is highly expressed in astrocytoma and developmentally promotes astrocyte formation, led us to hypothesize that its overexpression in oligodendroglioma promotes a “conversion” to an astrocytoma-like tumor or “fate”. To test this hypothesis we developed a mouse model of oligodendroglioma that combines in utero electroporation (IUE) with PiggyBac technology to target the oligodendrocyte lineage with the *RasV12* oncogene in the developing mouse cortex (Figure. 6a; see methods). IUE with these constructs at E16.5 results in the generation of oligodendroglioma tumors by P14 that bear many of the pathological hallmarks of these tumors including, small monomorphic nuclei lacking processes, perineuronal satellitosis, and invasion into perivascular and subpial spaces (Figure 6g; Figure S7). Furthermore, this mouse model recapitulates the patterns of NFIA and Sox10 expression found in human oligodendroglioma, where Sox10 is highly expressed, while NFIA demonstrates nominal expression (Figure 6b-e).

To examine whether NFIA overexpression influences the generation of glioma sub-types in this mouse model of oligodendroglioma, we combined IUE-mediated PiggyBac overexpression of RasV12 with HA-NFIA and harvested mouse brains at P14. After confirmation of ectopic NFIA expression through immunostaining for HA (Figure 6j; Figure S7), analysis of these tumors was performed using molecular and pathological criteria. Molecular analysis revealed a dramatic increase in the expression of the astrocyte marker, GFAP, and endovascular marker, PECAM/CD31, indicating that overexpression of NFIA promotes both astroglial and vascular properties of these tumors (Figure 6l-m v. 6h-i). At the pathological level, numerous nodular foci were identified containing tumor cells with more

elongated and pleomorphic nuclei consistent with astrocytic differentiation and astrocytoma (Fig 6g v. 6k). Together these molecular and pathological criteria indicate that overexpression of NFIA promotes the conversion of oligodendroglioma to an astrocytoma-like sub-type of glioma.

## Discussion

Our study describes for the first time the relationship between Sox10 and NFIA during glial development in the CNS. We found that NFIA antagonizes the ability of Sox10 to induce the expression of myelin genes. An analogous relationship exists during astrocyte development as Sox10 directly antagonizes NFIA induction of astrocyte-related genes. Moreover, loss of Sox10 results in enhanced differentiation of white matter astrocytes and increased generation of grey matter astrocytes, suggesting that Sox10 functions early in OLP development to suppress AS developmental programs. Application of these findings to glioma reveals that overexpression of NFIA in a mouse model of oligodendroglioma results in the conversion to astrocytoma-like tumors. These observations provide the first evidence that transcriptional regulators of glial fate oversee the generation of glioma sub-types.

### NFIA and Sox genes: Gliogenic Partners

Our previous studies revealed that NFIA suppresses OLP differentiation by directly repressing myelin gene expression [4]. Here we found that NFIA associates with Sox10 and antagonizes its ability to promote myelin gene expression. This mode of antagonism is likely to occur through direct association of these proteins on the promoter regions of these myelin genes as both NFIA and Sox10 ChIP these regions at E12.5 in the spinal cord. Subsequent downregulation of NFIA prior to OLP differentiation (between E16.5-E18.5) frees Sox10 of this repression and allows it drive myelin gene expression in a timely manner. These observations suggest that downregulation of NFIA is a key event regulating the timing of OLP differentiation as it triggers Sox10 induction of myelin gene expression. Interestingly, Sox9 directly induces NFIA expression and is similarly downregulated in OLP populations, implicating this regulatory axis in the timing of OLP differentiation [1]. In the future it will be important to fully dissect the mechanism of NFIA downregulation during OLP differentiation.

Understanding the assembly and regulation of activator complexes that control the timely induction of myelin genes is crucial because their constituents are often expressed in OLPs prior to their differentiation. Our results indicate that NFIA plays a key role in regulating these complexes by antagonizing Sox10 function in OLPs. This relationship between Sox10 and NFIA is further reinforced by Olig2, which facilitates Sox10/NFIA complex formation. The involvement of Olig2 with this complex serves two key functions: 1) Ensures timely myelin gene expression by promoting NFIA association with Sox10 and 2) Inhibits NFIA from promoting astrocyte fate. The inhibition of astrocyte fate in pMN domain is a key function of Olig2 that has remained very poorly defined. Our mechanistic studies on this matter have revealed that in addition to facilitating Sox10/NFIA interactions, Olig2 also neutralizes Sox9/NFIA cooperativity. Thus Olig2 interaction with the Sox-family/NFIA

complexes represents a new layer to the intricate transcriptional interplay that regulates both patterning and cell fate decisions during early gliogenesis.

The premise for examining the relationship between Sox10 and NFIA is we previously found that Sox9 and NFIA form a complex and cooperate to regulate a set of genes expressed during the initiation of gliogenesis [1]. While NFIA associates with both Sox9 and Sox10, two closely related members of the SoxE-subfamily, these relationships have drastically different consequences; NFIA and Sox9 have a cooperative relationship, whereas the NFIA and Sox10 relationship is antagonistic. Given that Sox9 and Sox10 generally function in a pro-glial manner, coupled with the redundant nature of many Sox genes, it is surprising that their association with NFIA results in such contrasting outcomes. These vastly different outcomes are mediated in part by interaction with Olig2, however it is likely that Sox10/NFIA complexes have different constituents than Sox9/NFIA complexes and these other factors also contribute to these contrasting effects. Indeed, another mode of Sox10 regulation involves sequestration and subsequent inhibition of myelin gene activation by Hes5 and Sox5/6 [28]. These observations, coupled with our findings point to a more nuanced role for Sox genes and NFIA in glial specification and the compartmentalization and differentiation of glial sub-lineages. Moreover, given that several other members of the Sox- and NFI-families are expressed during gliogenesis in the developing spinal cord and other regions of the CNS, a Sox/NFI transcriptional regulatory code for glial development may exist and warrant further investigation [29].

## Sox10 and the Suppression of Astrocyte Fate

Our studies on the Sox10/NFIA relationship in OLP development led us to investigate the nature of this relationship on astrocyte-development programs, where we found that Sox10 antagonizes the ability of NFIA to promote astrocyte development. NFIA is expressed in the pMN domain and in OLP populations therefore its ability to promote the astrocyte fate must be suppressed in these populations. Our findings indicate that Sox10 plays a key role in suppressing the ability of NFIA to promote astrocyte-development programs. Previously, we found that Olig2 suppresses NFIA induction of astrocyte fate, thus it seems likely that Sox10 and Olig2 collaborate to suppress global astrocyte-programs in OLPs [27]. Suppression of astrocyte fate represents a new function for Sox10 and may resolve a long-standing paradox surrounding its role in OLPs. Sox10 is expressed in pMN domain, yet its function has been primarily linked to myelination, which occurs several days after specification. Given that NFIA is expressed in pMN as well, our data indicate that Sox10 functions early in OLP development to suppress astrocyte development and preserve the integrity of the OLP lineage. The mutually cross-repressive nature of Sox10/NFIA on oligodendrocyte and astrocyte-development programs is a form of transcriptional “checks and balances” that ensures proper compartmentalization of the sublineages early in glial development (Figure S8). Indeed, the dynamics of these relationships may explain why small subsets of pMN derived cells generate astrocytes (see Figure S4).

To further investigate this role of Sox10 we examined how genetic deletion impacts astrocyte development, finding that its loss results in increased GFAP expression in WMAs and increased production of Id3-, AldoC-, and GS-expressing GMAs. Our data indicate that

the increased GFAP expression in WMA is not due to a conversion of Sox10-expressing cells to WMAs or increased production of WMAs. This argues that the increase in GFAP expression is occurring in individual WMAs and is likely due to accelerated differentiation from pMN populations where NFIA is left "unchecked" by the absence of Sox10. The effects are strikingly different for GMA astrocytes, where the numbers of Id3- and AldoC-expressing astrocytes are increased due to a conversion of Sox10-LacZ cells to the astrocyte fate. These results indicate that WMAs and GMAs have different requirements for Sox10 function, suggesting that these populations have unique transcriptional requirements. This notion is supported by previous studies showing that GMA and WMA in the cortex have differential requirements for Olig2, with GMAs demonstrating increased GFAP expression in its absence [30,31]. Because Sox10 and Olig2 both suppress NFIA function and GMA development, it will be important to uncover how their functions are coordinated and identify the associated transcriptional networks supporting this function.

That Sox10-LacZ cells convert to GMAs raises the question of which populations are undergoing this conversion. One possible source is NG2 cells, which are OLP populations that generate oligodendrocytes and GMAs in the ventral forebrain and spinal cord in the adult [32,33]. Given this link between NG2 cells and GMAs it may be interesting to investigate whether Sox10 also suppresses GMA production from NG2 cells in the adult and whether its relationship with NFIA regulates the balance between oligodendrocyte and astrocyte generation.

## Linking Glial Diversification and Glioma Sub-Types

Our observations that NFIA function and expression is strongly correlated with astrocyte formation and astrocytoma, respectively, and not OLPs and oligodendroglioma, led us to hypothesize that it can influence the generation of glioma sub-types. Using a mouse model of oligodendroglioma, we found that overexpression of NFIA converts an oligodendroglioma to an astrocytoma. Together these studies provide the first evidence that developmental paradigms regulating glial sub-lineage diversification similarly regulate the generation of glioma sub-types.

It is becoming increasingly clear that tumorigenesis is the convergence of genetic mutation and developmental context. This tenet was established at the cellular level in glioma using the RCAS-tva system, where targeting Ras and Akt to neural progenitors resulted in glioblastoma, while targeting these same oncogenes to astrocytes resulted in decreased tumor penetrance and malignancy[34]. Furthermore, targeting these same lineages with PDGF-B resulted in the generation of oligodendroglioma [35]. While targeting different oncogenic stimuli to distinct cell populations results in specific sub-types and grades of glioma, the molecular basis for how developmental and cellular context influences the generation of tumor sub-types remains poorly defined. The longstanding view on developmental contributions to tumorigenesis has correlated differentiative status with proliferation and malignancy. Our results provide a new perspective on the role of developmental processes in tumorigenesis, where the transcriptional processes that govern lineage diversification similarly regulate the generation of tumor sub-types. Here, we found that overexpression of the astrocyte fate determinant NFIA alters glioma sub-type

independent of cell context or oncogenic stimuli, suggesting that specific interactions between oncogenes and developmental regulators of glial sub-lineages influences the generation of glioma sub-types. More broadly, such specific relationships likely toggle oncogenic (or tumor suppressor) signaling pathways and are key components of how developmental context regulates tumorigenesis. In glioma, analogous relationships have been established for STAT3: in the absence of PTEN, STAT3 suppresses tumorigenesis, while in the presence of EGFR-vIII overexpression STAT3 promotes tumorigenesis [6].

Previous studies have established that transcriptional regulators of developmental gliogenesis also contribute to glioma tumorigenesis; these include Olig2, STAT3, and NFIA[6,7,8]. In each of these studies the given factor was found to contribute to gross tumorigenesis (see above); whether it influenced the generation of specific glioma sub-types was not determined, reflecting perhaps the limits of the model system used or the mode of gene manipulation. Our studies are unique in that we combine overexpression with a developmental model of glioma, revealing novel roles and insight into NFIA function during glioma formation. It is likely that employing analogous experimental approaches for STAT3 and Olig2 will also reveal new insight into their respective functions during glioma formation. Finally, prior studies on Sox10 using the RCAS-tva model revealed that its overexpression did not alter glioma sub-types and only modestly increased tumor penetrance in the PDGF-B model of oligodendroglioma[25]. Analogous experiments in our IUE/MBP-Ras model gave similar results, indicating that increased Sox10 expression in oligodendroglioma models does not influence the generation of glioma sub-types.

Our study has identified a novel developmental relationship regulating the diversification of glial sublineages during CNS development that also manages the generation of glioma subtypes. The conservation of glial developmental relationships in tumorigenesis has important implications in the treatment of glioma, as the ability to manipulate cell fates within tumors to provoke more differentiated or less malignant phenotypes is a potential therapeutic approach to this largely fatal disease. A deeper understanding of how NFIA and Sox10 function is coordinated with other determinants of glial fate to orchestrate glial diversification during development and tumorigenesis will be important in determining whether malignant gliomas can be managed through conversion to less malignant sub-types.

## On-Line Methods and Supplemental Information

### Chick and Mouse Experiments

Expression constructs were cloned into the RCAS(B) [36] or pCIG vectors and injected into the chick spinal cord at stage HH13-15 (~E2). Harvested embryos were fixed in 4% PFA for 2-4 hours, depending on stage. Electroporation was carried out with a BTX Electro Square Porator [37]. The *Sox10-LacZ* [21] and *Sox10-Cre* [20] mouse lines were used in these studies. In situ hybridization and immunohistochemistry analysis was performed as described [1].

### ChIP, Immunoprecipitation, and Reporter Assays

Mouse E12.5 spinal cords were dissected, dissociated, and processed for ChIP assays. The samples were pre-cleared with protein G beads and immunoprecipitated using NFIA

antibody (abcam), Sox10 (rabbit-polyclonal), or control IgG (Santa Cruz). The DNA was purified and PCR was preformed using region specific primers. See supplemental information for primer sequences. HEK293 or HEK293T cell lines cells were transfected with pGL3-reporter constructs and a CMV-β-galactosidase vector using Superfect transfection reagent (Qiagen). Cells were harvested and analyzed for luciferase activity; β-galactosidase was used to normalize for transfection efficiency.

Co-immunoprecipitation was performed by transfecting P19 or Oli-Neu cells with Flag-NFIA and/or HA-Sox10; harvested cell lysates were subject to immunoprecipitation using a specific antibody or IgG control and protein G agarose beads. See supplemental information for additional information.

## GST Pulldown Experiments

For GST pulldown experiments, GST or GST fusion proteins with portions of Sox10 were produced in the Escherichia Coli strain Rosetta (Novagen) uopn induction with 0.5mM isopropyl-β-D-thiogalactopyranoside (IPTG). After 4h of induction at 25°C, cells were collected by centrifugation, resuspended in lysis buffer supplemented with 1mM dithiothreitol and protease inhibitors, and sonicated for two 30s pulses at 4°C. Bacterial debris was removed by centrifugation at 15000rpm for 20min. Equal amounts of GST or GST fusions were immobilized on glutathione agarose beads (Life Technologies). GST bound beads were incubated for 2 h at 4°C with *in vitro*-translated 35S-labeled proteins (Promega) or non-radio labeled “cold” proteins in binding buffer (1XPBS, 1%Triton-X, 25mg/ml BSA, 1mM DTT, and protease inhibitors). After extensive washing, resin-bound proteins were separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels. The Sox10 plasmids used in these studies were cloned into the PGEX-KG vectors[40].

## OLP and cortical culture

Oligosphere cultures were performed as previously described [4,38]. For viral infection of OLPs, cells were dissociated and plated on PDL-coated coverslips at a density of $1.5\times10^4$ cells/$cm^2$ in OPM media subsequently infected with NFIAFUIGW, FUIGW-Sox10, or control GFP virus for 14 hours. Rat cortical progenitor cells were isolated via dissection of E13.5 rat embryonic cortex and dissociation with papain. Cells were grown in DMEM/F12 supplemented with N2 and B27 (GIBCO) and 20 ng/ml bFGF (R&D systems), described in [2,16]. Cells were transfected with Lipofectamine 2000 using pCS+ or pcDNA plasmids containing GFP, Myc-mSox10, HA-mNFIA, and/or myc-mOlig2. In experiments that required the removal of bFGF to promote astrocyte differentiation, bFGF was removed 24 hours post-transfection and replaced with media described above supplemented with 2% FCS [16].

## Oligosphere differentiation

Mouse embryonic E14.5 cortex were dissected, dissociated, then plated in neurosphere proliferation media (NPM) which was comprised of DMEM/F12, B27 supplement (Gibco), and 10ng/mL EGF and 10ng/mL bFGF. Neurospheres were allowed to form 4-6 days. To generate oligospheres, whole neurospheres were then plated on Poly-D-lysine (PDL)-coated coverslips in oligosphere proliferation media (OPM) that was composed of NPM

supplemented with 10ng/mL platelet derived growth factor (PDGF), but without EGF. After 2 days oligospheres were induced to differentiate by replacing OPM with basal chemically defined medium supplemented with 15nM triiodthyronine, 10ng/mL CNTF, and 5mg/mL NAC. For viral infection of oligodendrocyte precursors, neurospheres were dissociated and plated on PDL-coated coverslips at a density of $1.5\times10^4$ cells/$cm^2$ in OPM media. Cells were allowed to attach for 3 hours before exposure to virus. Cells were transduced with either NFIA-FUIGW or control GFP virus for 14 hours, followed by replacement of media with OPM. After 48 hours differentiation was induced as described above.

## In Utero Electroporation and Glioma Formation

In utero electroporation was performed as previously described [39]. Electroporation was performed at embryonic day 16 and gestation age was confirmed during surgery. All plasmids were used at the final concentration of 2.0 **μ**g/**μ**l. Selective targeting of oligodendrocytes is achieved by co-electroporating a 'helper plasmid' which regulates *pBase* transposase expression by an upstream *Glast promotor* (*pGlast-PBase*) while a 'donor plasmid' carries a bicistronic *GFP-t2a-RasV12* transgene driven by the MBP promoter flanked by terminal repeat (TR) sequences, which transposase recognizes (*MBP-GFPt2aRas*). For the IUE/oligodendroglioma-NFIA overexpression studies, HA-NFIA was cloned downstream of a constitutive CAG-HA-NFIA promoter, flanked by TR sequences. This plasmid (or the empty control) was co-electroporated along with the *pGlast-PBase* and MBP-*GFPt2aRas*. Animals were harvested at P14 and brains were fixed in 4%PFA. After fixation, brains were embedded in paraffin, sectioned, and subjected to molecular and pathological analysis via immunostaining or H&E staining. All mouse experiments were approved by the Baylor College of Medicine IACUC committee.

## Antibodies

The following antibodies were used for ChIP, western blot, and immunofluorescence: GFAP (Chemicon, 1:1000), GFAP (DAKO, 1:1000), HA (Roche, Covance, SCBT), LacZ (Abcam, 1:1000), LacZ (MP, 1:1000), MBP (Covance, 1:500), NFIA (1:2000), Nkx6.1 (DSHB, 1:5), Olig2 (R&D, 1:2000), Pax6 (Abcam, 1:500). PLP (MP, 1:200), S100 (DAKO, 1:1000), Sox10 (1:10000), Sox10 (SCBT 1:100),

## Chromatin Immunoprecipitation (ChIP) Assay

Mouse E12.5 spinal cords were dissected, dissociated, and processed for ChIP assays. Harvested cells were fixed with 1% formaldehyde for 10 minutes. Cross-linked chromatin was then sheared by sonication and cleared by centrifugation. The samples were pre-cleared with protein G beads and immunoprecipitated using appropriate antibody or control IgG (Santa Cruz Biotechnology). Immunoprecipitated complexes were isolated, the cross-links reversed, and proteins digested with proteinase K. The DNA was purified and PCR was performed using region specific primers. The following primers were used: MBP Forward 5'- TACAGGCCCACATTCATATCTC, Reverse 5'-TTCTTGGATGGTCTGAAGCTC; MBP-Control Forward 5'-CACAACACACAAGGAAAAGGAT, Reverse 5'-GGGGAAGAATGCTTCACTTAAT; PLP Forward-5'-TGGTCACACACAGTCTGTTCAT, Reverse 5'-GGGTCTGAATCAAAAGCCTACT;

PLP-Control Forward 5'-TCTGTAAACACGGCTATTCAGC, Reverse 5'-TCAGGCCTCTTTTCTCAACATA; MAG Forward 5'-TTGATTCCTGGGTCCTACTAGC, Reverse 5'-AACTAGGAGAGGGTGTGTTTCC; MAG-Control 5'-ATGCCAGTCTAGACCCATTCTT, Reverse 5'-CGTGCAAAGCACATATACACAT; GFAP Forward 5'-CAGGGCCTCCTCTTCATG, Reverse 5'-TAGAGCCTTGTTCTCCACC; GFAP Control Forward 5'-AGTTACCAGGAGGCACTTGC, Reverse 5'-CGGTTTTCTTCGCCCTCCA; APCDD1 Forward 5'ATTAAAGAAAGGCAGGACAGGA, Reverse 5'-ATGCCTCCAAAATATCCAGCTA; APCDD1-Control Forward 5'GGTTCATGATTCTGCACTCTGT, Reverse 5'AGAGACAACCCTGTGAAGACAA.

### Culture and transfection of cortical progenitors

Rat embryonic E14.5 cortex were dissected, dissociated, then plated at a density of $2\times10^4$ cells/$cm^2$ on plates coated with 15ug/ml of polyornithine (Sigma) and 1ug/ml human fibronectin (Biochemical Technologies). Cells were grown in DMEM/F12 supplemented with N2 and B27 (GIBCO) and 20ng.ml bFGF (R&D Systems). Cells were expanded for 4 days followed by transfection with Lipofectamine 2000. In experiments requiring removal of bFGF to promote astrocyte differentiation, bFGF was removed 24 hours post-transfection and replaced with media described above supplemented with 2% FBS.

### Statistics

Anova was used to analyze the luciferase reporter assays to determine the differences between group means; ttest was used to compare individual means and is reported as astericks in associated figure graphs. No statistical methods were used to pre-determine sample sizes, but our sample sizes are similar to those reported in previous publications[1,2,12,21]. Data distribution was assumed to be normal, but this was not formally tested. Blinding and randomization of samples was not used in the data analysis. A methods checklist is available with the supplementary materials

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## References

1. Kang P, L. H.-K. Glasgow S, Finley M, Garber Z, Foster AE, Novitch BG, Gronostajski RM, Deneen B. Sox9 and NFIA coordinate a transcriptional regulatory cascade during the onset of gliogenesis. Neuron. 2012; 74:79–94. [PubMed: 22500632]
2. Deneen B, et al. The Transcription Factor NFIA Controls the Onset of Gliogenesis in the Developing Spinal Cord. Neuron. 2006; 52:953–968. [PubMed: 17178400]

3. Namihira M, et al. Committed Neuronal Precursors Confer Astrocytic Potential on Residual Neural Precursor Cells. 2009; 16:245–255.

4. Fancy SPJ, G. S. G. Finley M, Rowitch DH, Deneen B. Evidence that Nuclear Factor IA Inhibits Repair after White Matter Injury. Annals of Neurology. 2012; 72:224–233. [PubMed: 22807310]

5. Stiles CD, Rowitch DH. Glioma stem cells: a midterm exam. Neuron. 2008; 58:832–846. [PubMed: 18579075]

6. de la Iglesia N, et al. Identification of a PTEN-regulated STAT3 brain tumor suppressor pathway. Genes Dev. 2008; 22:449–462. [PubMed: 18258752]

7. Ligon KL, et al. Olig2-regulated lineage-restricted pathway controls replication competence in neural stem cells and malignant glioma. Neuron. 2007; 53:503–517. [PubMed: 17296553]

8. Glasgow SM, et al. The miR-223/nuclear factor I-A axis regulates glial precursor proliferation and tumorigenesis in the CNS. J Neurosci. 2013; 33:13560–13568. [PubMed: 23946414]

9. Maher EA, et al. Malignant glioma: genetics and biology of a grave matter. Genes Dev. 2001; 15:1311–1333. doi:10.1101/gad.891601. [PubMed: 11390353]

10. Stolt CC, R. S. Ader M, Lommes P, Riethmacher D, Schachner M, Bartsch U, Wegner M. Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. Genes & Dev. 2002; 16:165–170. [PubMed: 11799060]

11. Kuhlbrodt K, H. B. Sock E. Borgmeyer-Hermans I, and Wegner M. Sox10, a Novel Transcriptional Modulator in Glial Cells. The Journal of Neuroscience. 1998; 18:237–250. [PubMed: 9412504]

12. Zhou Q, W. S. Anderson DJ. Identification of a novel family of Oligodendrocyte Lineage-Specific Basic Helix-Loop-Helix Transcription Factors. Neuron. 2000; 25:331–343. [PubMed: 10719889]

13. Li H, Lu Y, Smith HK, Richardson WD. Olig1 and Sox10 Interact Synergistically to Drive Myelin Basic Protein Transcription in Oligodendrocytes. J. Neurosci. 2007; 27:14375–14382. doi: 10.1523/jneurosci.4456-07.2007. [PubMed: 18160645]

14. Liu Z, et al. Induction of oligodendrocyte differentiation by Olig2 and Sox10: Evidence for reciprocal interactions and dosage-dependent mechanisms. Developmental Biology. 2007; 302:683–693. [PubMed: 17098222]

15. Pedraza CE, M. R. Lei J, Hao Q, Macklin WB. Production, characterization, and efficient transfection of highly pure oligodendrocyte precursor cultures from mouse embryonic neural progenitors. Glia. 2008; 56:1339–1352. [PubMed: 18512250]

16. Gabay L, L. S. Rubin L, Anderson DJ. Deregulation of Dorsoventral pattering by FGF confers Trilineage Differentiation Capacity on CNS Stem Cells In vitro. Neuron. 2003; 40:485–499. [PubMed: 14642274]

17. Gabay L, L. S. Rubin L, Anderson DJ. Deregulation of Dorsoventral pattering by FGF confers Trilineage Differentiation Capacity on CNS Stem Cells In vitro. Neuron. 2003; 40:485–499. [PubMed: 14642274]

18. Tsai HH, L. H. Fuentealba LC, Molofsky AV, Taveira-Marques R, Zhuang H, Tenney A, Murnen AT, Fancy SP, Merkle F, Kessaris N, Alvarez-Buylla A, Richardson WD, Rowitch DH. Regional astrocyte allocation regulates CNS synaptogenesis and repair. Science. 2012; 337:358–362. [PubMed: 22745251]

19. Masahira N, T. H. Ono K, Watanbe K, Ding L, Furusho M, Ogawa Y, Nabesgima Y, Alavarez-Buylla A, Shimizu K, Ikenaka K. Olig2-positive progenitors in the embryonic spinal cord give rise not only to motoneurons and oligodendrocytes, but also to a subset of astrocytes and ependymal cells. Developmental Biology. 2006; 293:358–369. [PubMed: 16581057]

20. Matsuoka T, et al. Neural crest origins of the neck and shoulder. Nature. 2005; 436:347–355. [PubMed: 16034409]

21. Britsch S, G. D. Riethmacher D, Peirano RI, Rossner M, Nave K-A, Birchmeier C, Wegner M. The transcription factor Sox10 is a key regulator of peripheral glial development. Genes Dev. 2001; 15:66–78. [PubMed: 11156606]

22. Hochstim C, Deneen B, Lukaszewicz A, Zhou Q, Anderson DJ. Identification of Positionally Distinct Astrocyte Subtypes whose Identities Are†Specified by a Homeodomain Code. Cell. 2008; 133:510–522. [PubMed: 18455991]

23. Bannykh SI, Stolt CC, Kim J, Perry A, Wegner M. Oligodendroglial-specific transcriptional factor SOX10 is ubiquitously expressed in human gliomas. J Neurooncol. 2006; 76:115–127. doi: 10.1007/s11060-005-5533-x. [PubMed: 16205963]
24. Rousseau A, et al. Expression of oligodendroglial and astrocytic lineage markers in diffuse gliomas: use of YKL-40, ApoE, ASCL1, and NKX2-2. J Neuropathol Exp Neurol. 2006; 65:1149–1156. [PubMed: 17146289]
25. Ferletta M, Uhrbom L, Olofsson T, Ponten F, Westermark B. Sox10 has a broad expression pattern in gliomas and enhances platelet-derived growth factor-B--induced gliomagenesis. Mol Cancer Res. 2007; 5:891–897. [PubMed: 17855658]
26. Song HR, et al. Nuclear factor IA is expressed in astrocytomas and is associated with improved survival. Neuro Oncol. 2010; 12:122–132. [PubMed: 20150379]
27. Deneen B, et al. The transcription factor NFIA controls the onset of gliogenesis in the developing spinal cord. Neuron. 2006; 52:953–968. [PubMed: 17178400]
28. Liu A, L. J. Marin-Husstege M, Kageyama R, Fan Y, Gelinas C, Casaccia-Bonnefil P. A molecular insight of Hes5-dependent inhibition of myelin gene expression: old partners and new players. EMBO. 2006; 25:4833–4842.
29. Heng YH, M. R. Harvey TJ, Smith AG, Barry G, Cato K, Plachez C, Little E, Mason S, Dixon C, Gronostajski RM, Bailey TL, Richards LJ, Piper M. NFIX Regulates Neural Progenitor Cell Differentiation During Hippocampal Morphogenesis. Cereb Cortex. 2012 epub Oct 2012.
30. Cai J, C. Y. Cai WH, Hurlock EC, Wu H, Kernie SG, Parada LF, Lu QR. A crucial role for Olig2 in white matter astrocyte development. Development. 2007; 137:1887–1899. [PubMed: 17428828]
31. Ono K, T. H. Ikeda K, Furusho M, Nishizawa T, Watanabe K, Ikenaka K. Regional- and temporal-dependent changes in the differentiation of Olig2 progenitors in the forebrain, and the impact on astrocyte development in the dorsal pallium. Developmental Biology. 2008; 320:456–480. [PubMed: 18582453]
32. Zhu X, B. D. Nishiyama A. NG2 cells generate both oligodendrocytes and gray matter astrocytes. Development. 2008; 135:145–157. [PubMed: 18045844]
33. Zhu X, H. R. Nishiyama A. NG2 cells generate oligodendrocytes and gray matter astrocytes in the spinal cord. Neuron Glia Biology. 2008; 4:19–26. [PubMed: 19006598]
34. Holland EC, et al. Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. Nat Genet. 2000; 25:55–57. [PubMed: 10802656]
35. Dai C, et al. PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. Genes Dev. 2001; 15:1913–1925. [PubMed: 11485986]
36. Morgan BA, F. D. Manipulating gene expression with replication-competent retroviruses. Methods Cell. Biol. 1996; 51:185–218. [PubMed: 8722477]
37. Momose T, et al. Efficient targeting of gene expression in chick embryos by microelectroporation. Development, Growth & Differentiation. 1999; 41
38. Pedraza CE, Monk R, Lei J, Hao Q, Macklin WB. Production, characterization, and efficient transfection of highly pure oligodendrocyte precursor cultures from mouse embryonic neural progenitors. Glia. 2008; 56:1339–1352. [PubMed: 18512250]
39. Chen F, LoTurco J. A method for stable transgenesis of radial glia lineage in rat neocortex by piggyBac mediated transposition. J Neurosci Methods. 2012; 207:172–180. [PubMed: 22521325]
40. Wissmüller S, Kosian T, Wolf M, Finzsch M, Wegner M. The high-mobility-group domain of Sox proteins interacts with DNA-binding domains of many transcription factors. Nucleic Acids Res. 2006; 34(6):1735–44. [PubMed: 16582099]

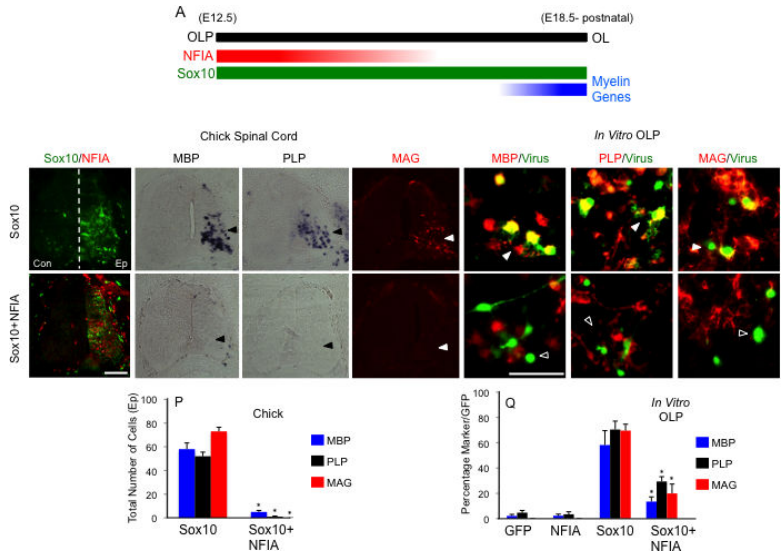


**Figure 1. NFIA antagonizes Sox10 induction of myelin genes**

(A) Schematic summarizing Sox10, NFIA, and myelin gene expression during OLP differentiation. Overexpression of Sox10 (B-E) and Sox10+NFIA (F-I) in the chick spinal cord. Ectopic expression in B and E was detected using αHA (Sox10) or αFlag (NFIA) (also see supplemental figure S2). Arrows in C-E denote ectopic induction of myelin genes. Overexpression of Sox10 (J-L) and Sox10+NFIA (M-O) in OLP cultures; arrows in J-L denote Sox10 induction of myelin gene expression, unfilled arrows in M-O denote non-overlapping expression of transgene and marker. (P) Quantification of chick studies is derived from 8 independent spinal cords, 8 sections/spinal cord. Unpaired t-test values (MBP, $p=2.4\times10^{-12}$; PLP, $p=2.58\times10^{-12}$; MAG, $p=1.67\times10^{-15}$). Q) Quantification of *in vitro* studies; performed in triplicate on embryos derived from three independent litters. Unpaired t-test values (MBP, p=0.001; PLP, p=0.00009; MAG, p=0.003). For all graphs, *p<.001; All error bars indicate SD. Scale bars in I and M are 100um.

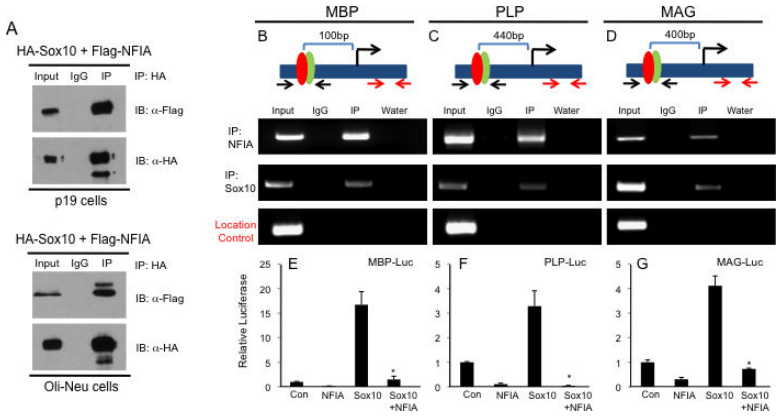


**Figure 2. NFIA associates with Sox10 and inhibits its activity**

(A) Sox10 and NFIA co-IP from Oli-neu cell extracts. (B-D) Schematic of Sox10 and NFIA binding sites in MBP, PLP, and MAG promoters (also see supplemental figure S3). E12.5 mouse spinal cord ChIP demonstrating that Sox10 and NFIA associate with these promoters. Red ovals represent NFIA sites and green oval represent Sox9 sites. Location controls are intronic regions at least 5kb from the promoter binding sites. (E-G) NFIA antagonizes Sox10 activation of myelin gene promoters. Values presented are the average of three independent experiments performed in triplicate. Unpaired t-test values for Sox10 compared to Sox10+NFIA in (E) p=0.008; in (F) p=0.001, in (G) p=0.005. ChIP gel images are representative of three independent experiments. For all graphs *p<.001; All error bars indicate SD. Gel and blot images in A-D are cropped; full-length images are presented in supplementary figure S10.

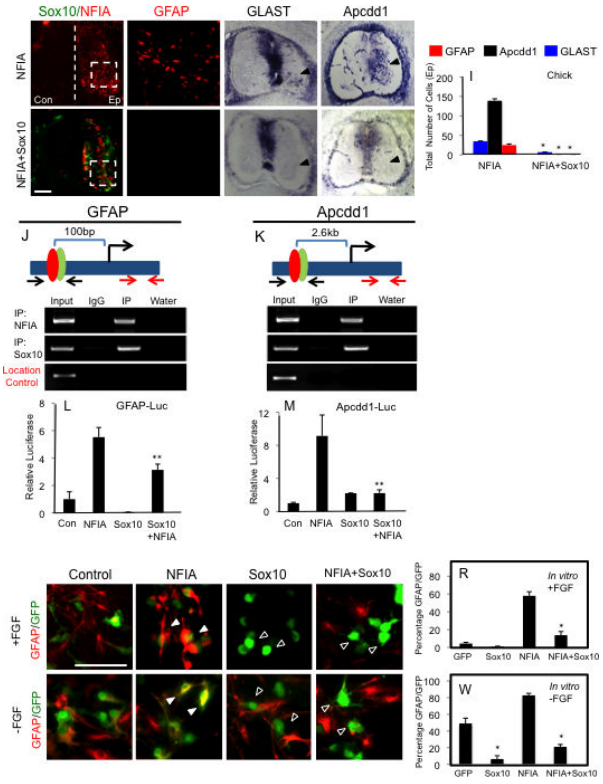


**Figure 3. Sox10 antagonizes NFIA induction of astrocyte-related genes**

Overexpression of NFIA (A-D) and Sox10+NFIA (E-H) in the chick spinal cord. Region in dashed box in A, E is shown at higher magnification in B, G. Ectopic expression in A and F was detected using αHA (Sox10) or αFlag (NFIA). Arrows in C-E denote ectopic induction of astrocyte-related genes by NFIA. (I) Quantification is derived from 8 independent spinal cords, 8 sections/spinal cord. Unpaired t-test values (GFAP, $p=5.6\times10^{-12}$; APCDD1, $p=3.4\times10^{-16}$; GLAST, $p=1.89\times10^{-36}$). (J-K) Schematic of Sox10 and NFIA binding sites in GFAP and Apcdd1 promoters; E12.5 mouse spinal cord ChIP. (L-M) Sox10 antagonizes NFIA activation of GFAP and Apcdd1 promoters. For L and M, experiments were performed on three independent occasions, in triplicate. Comparison of Sox10 and Sox10+NFIA resulted in unpaired t-test values p=0.01 for both GFAP and Apcdd1 promoters. ChIP gel images are representative of three independent experiments. Location controls are intronic regions at least 5kb from the promoter binding sites. (N-W) Overexpression of Sox10 and/or NFIA in embryonic rat cortical culture, with FGF (N-Q) and without FGF (R-U); filled arrows denote overlapping expression, unfilled arrows denote non-overlapping expression. (V-W) Quantification of cortical cultures; values presented are the average of five independent experiments performed in triplicate. In V, the unpaired t-test value, p=0.00001; in W the unpaired t-test value, (GFP compared to Sox10 p=0.001; NFIA compared to Sox10+NFIA p=0.000001). For all graphs, *p<.001, **p<.01 All error bars indicate SD. Scales bars in F and P are 100um. Gel images in L-M are cropped; full-length images are presented in supplementary figure S10.

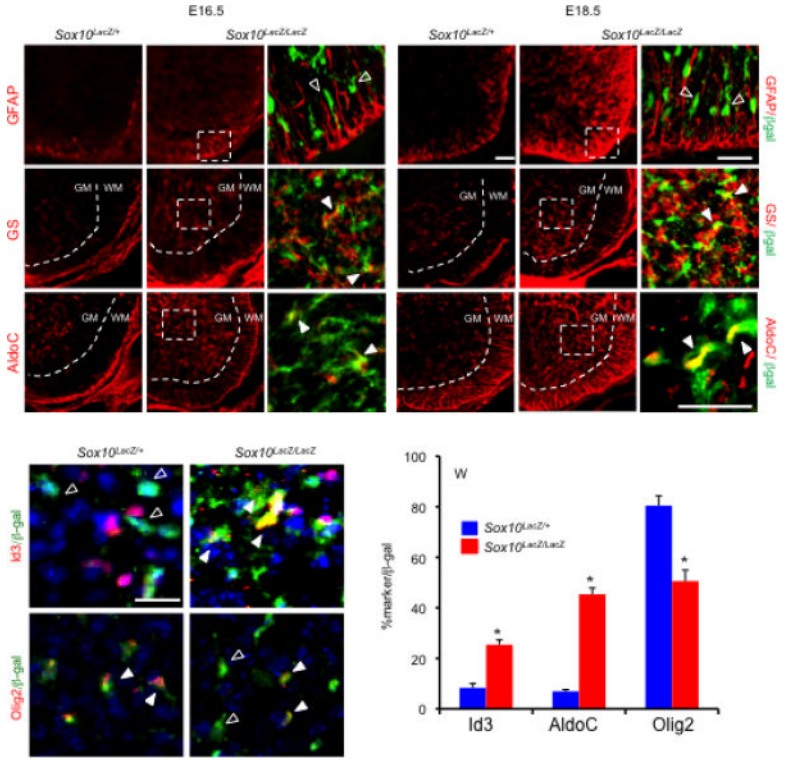


**Figure 4. Loss of Sox10 promotes astrocyte development**

Analysis of GFAP (A-C, J-L), GS (D-F, M-O), and AldoC (G-I, P-R) expression in spinal cord from $Sox10^{LacZ/+}$ and $Sox10^{LacZ/LacZ}$ embryos. Dashed line denotes white matter (WM) and grey matter (GM) boundary. Double-labeling with GFAP/β-gal (C,L), GS/β-gal (F,O), and AldoC/β-gal (I,R); Boxed regions are magnified in adjacent panels. Filled arrows denote overlap; unfilled arrows denote no overlap. Double labeling with Id3/β-gal (S-T) and Olig2/β-gal (U-V); quantification in W is derived from three embryos of each genotype from two independent litters; the unpaired t-test values in W, (Id3, p=0.0001; AldoC, p=0.00001; Olig2, p=.006). In W *p<.006; All error bars indicate SEM. Panels C, F, L, O are imaged at 20X. Panels I, R are imaged at 40X. Scale bar in D is 50um, in F, R, and S is 100um

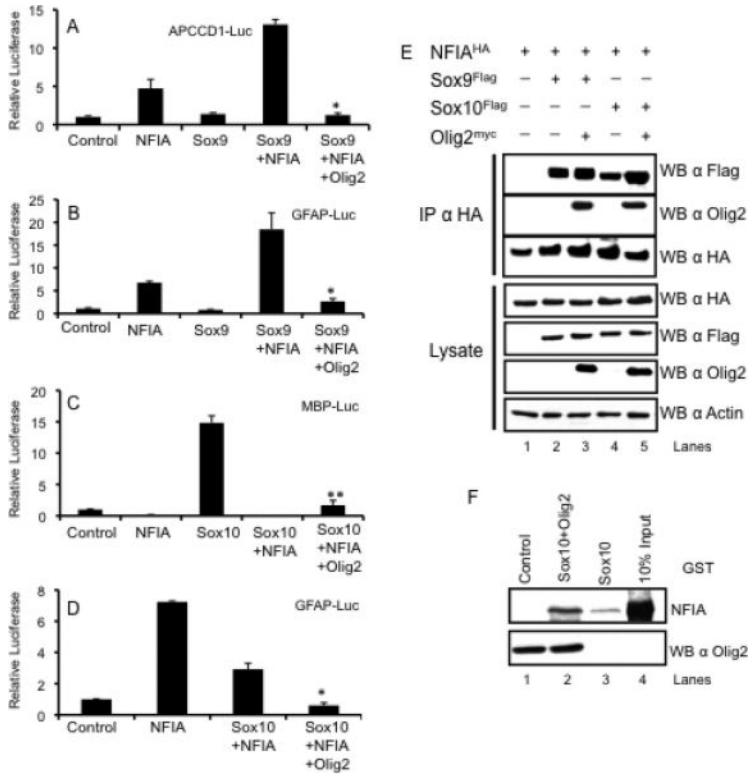


**Figure 5. Olig2 facilitates Sox10/NFIA interactions**

NFIA- and Sox- activity on astrocyte and myelin-gene promoters (A-C). (A-B) Olig2 antagonizes NFIA/Sox9 activation of APCCD1 and GFAP1 promoters. Unpaired t-test values comparing Sox9+NFIA and Sox9+NFIA+Olig2 resulted in p=0.0007 in (A) and p=0.01 in (B). (C) NFIA suppresses the ability of Sox10 to activate the MBP promoter in luciferase assays. The addition of Olig2 mildly alleviates this repression. (D) Olig2 potentiates Sox10 suppression of NFIA-induced GFAP induction. Unpaired t-test values comparing Sox10+NFIA and Sox10+NFIA+Olig2 resulted in p=0.0007 in (C) and p=0.001 in (D). Immunoblot (IB) of IP extracts from 293 cells expressing HA-NFIA and either Flag-Sox9 or Flag-Sox10 in the presence or absence of Myc-Olig2. (E) The enhancement of Sox10/NFIA Co-IP in the presence of Olig2 is depicted in lanes 4-5. Olig2 does not affect Sox9/NFIA interaction, lanes 2-3. (F) Interaction of NFIA, Olig2, and Sox10 in GST pulldown experiments. The amount of radiolabeled-NFIA present in one-tenth of the extract before pulldown (1/10 input) is shown in lane 4. NFIA directly binds Sox10 HMG domain, lane 3. Binding of Sox10 is enhanced in the presence of non-radio labeled “cold” Olig2, lane 2. The presence of *in vitro* translated Olig2 was verified by IB. Values in A-D presented are the average of three independent experiments performed in triplicate. Images in E-F are representative of three independent experiments. For all graphs, ** p<0.01, *p<0.001. All error bars represent SD. Blot images in E-F are cropped; full-length images are presented in supplementary figure S10.

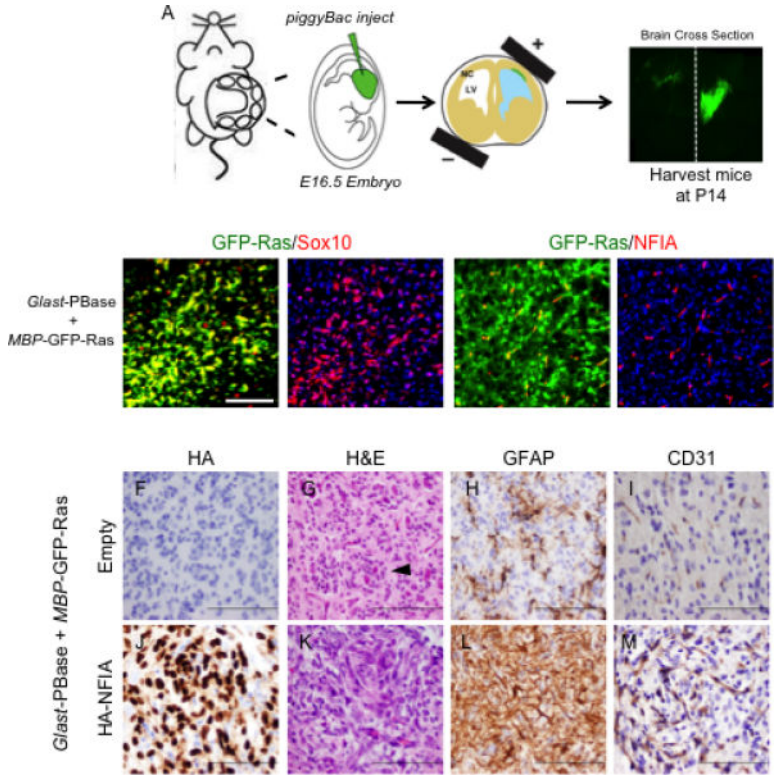


**Figure 6. NFIA regulates generation of glioma sub-types**

(A) Schematic of IUE mouse glioma model; dashed line in cross section represents cortical midline. (B-E) Expression of NFIA and Sox10 in mouse oligodendrogliomas. In F-M, E16.5 embryos were injected and subjected to IUE with GLAST-pBase, Pb-MBP-GFP-Ras and either Pb-CAG-empty (F-I) or Pb-CAG-HA-NFIA (J-M). (F-I) Representative oligodendroglioma tumors harvested from P14 mice that were electroporated with Pb-CAG-empty; (J-M) Representative astrocytoma-like tumors harvested from P14 mice that were electroporated with Pb-CAG-HA-NFIA. Arrow in G denotes perineuronal satellitosis, a hallmark of oligodendroglioma tumors (see supplemental figure S7). Images are representative of 10 mice/10 tumors for each condition, derived from three independent IUE experiments/litters for each condition. Scale bar in B is 100um and F-M is 250um.