Foxg1 Antagonizes Neocortical Stem Cell Progression to Astrogenesis

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Abstract

Neocortical astrogenesis follows neuronogenesis and precedes oligogenesis. Among key factors dictating its temporal articulation, there are progression rates of pallial stem cells (SCs) towards astroglial lineages as well as activation rates of astrocyte differentiation programs in response to extrinsic gliogenic cues. In this study, we showed that high Foxg1 SC expression antagonizes astrocyte generation, while stimulating SC self-renewal and committing SCs to neuronogenesis. We found that mechanisms underlying this activity are mainly cell autonomous and highly pleiotropic. They include a concerted downregulation of 4 key effectors channeling neural SCs to astroglial fates, as well as defective activation of core molecular machineries implementing astroglial differentiation programs. Next, we found that SC Foxg1 levels specifically decline during the neuronogenic-to-gliogenic transition, pointing to a pivotal Foxg1 role in temporal modulation of astrogenesis. Finally, we showed that Foxg1 inhibits astrogenesis from human neocortical precursors, suggesting that this is an evolutionarily ancient trait.

Key words: astrogenesis, commitment, differentiation, Foxg1, NSC

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been implicated in a dense and intricate functional network (Mallamaci et al. 2019; Kanski et al. 2014; Sloan and Barres 2014; Takouda et al. 2017). A small group of transcription factors, including CoupTFII, Zbβ20, Sox9, and Nfa, promote the astrogenesis onset, partly by changing the epigenetic state of astroglial genes (Naka et al. 2008; Namihira et al. 2010; Kang et al. 2012; Namihira et al. 2016). Once chromatin of genes active in astroglia gets permissive, then its transcription rate results from the interaction among dedicated signaling pathways, impinging on astroglial promoters. Some of them inhibit astroglial gene transcription (e.g., Nrg1/ErbB4ICD-NCoR (Hermanson et al. 2002; Sardi et al. 2006; Schlessinger and Lemmon 2006; Miller and Gauthier 2007)), some others promote it (e.g., IL6/Jak2/Stat1,3 (Derouet et al. 2004; Barnabé-Heider et al. 2005; He et al. 2005), Bmp/Smad1,5,8 (Hakamshi 1999; Sun et al. 2001), Dl1/Notch1rd (Ge et al. 2002; Kamakura et al. 2004).

We previously found that pallial NSC-restricted overexpression of Foxg1, an ancient transcription factor controlling telencephalic specification (Hannashima et al. 2007), subpallial/pallial fate (Manuel et al. 2010; Mariani et al. 2011; Patriarchi et al. 2016), hippocampal programs (Muzio and Mallamaci 2005), and neurogenesis progression (Miyoshi and Fishell 2007; Toma et al. 2013; Chiola et al. 2019), leads to a substantial decrease of astrocyte generation (Branccaccio et al. 2010). However, we demonstrated the occurrence of this phenomenon only in a murine, heterochrono system in vitro, we did not address its physiological relevance and, last, we did not focus on molecular mechanisms underlying it (Branccaccio et al. 2010).

In this study we showed that Foxg1 overexpression within neocortical stem cells commits these cells to neuronogenesis rather than astrogenesis, in vivo as well as in vitro, both in mouse and human. Interestingly, we found that Foxg1 inhibition of astrogenesis stems from variegated mechanisms. These include a direct trans-repression of genes biasing NSCs to astroglial fates, as well as an articulated impact on key pathways which modulate astroglial gene transcription, resulting into a robust dampening of it. Finally, we provided evidence that Foxg1 levels decline, while neocortical NSCs move from neuronogenesis to gliogenesis.

These findings point to an evolutionarily conserved, pivotal role of Foxg1 in fine temporal regulation of astrogenesis. They also suggest that neurological symptoms observed in syndromes with altered Foxg1 allele dosage (Guerrini and Pannini 2012) might be partially caused by an unbalanced astrocyte generation.

### Materials and Methods

#### Animal Handling and Embryo Dissection


Wild type (strain CD1, purchased from Envigo, Italy) and Foxg1+/− strain (Hébert and McConnell 2000) were maintained at the SSIA animal facility. Embryos were staged by timed breeding and vaginal plug inspection. Pregnant females were sacrificed by cervical dislocation. Embryonic cortices were dissected out in cold 1X-phosphate buffered saline (PBS), under sterile conditions.

#### Derivation of Human Neocortical Precursor Line

NPCs were derived from the cerebral cortex of a single 10.2 post conception week (PCW) human fetus, collected from routine termination of pregnancies under full ethical approval in line with Department of Health guidelines (LREC 96/085;96/085—in vitro study of postmortem human fetal neural tissue, blood and haematopoietic organs, approved by Cambridge Central Ethics Committee). Cells were grown and expanded in a chemically defined, serum-free medium in the presence of Fibroblast Growth Factor 2 (Fgf2) and Epidermal Growth Factor (Egf) (10 and 20 ng/mL, respectively) and routinely assessed for multipotency, as described (Pluchino et al. 2009).

#### Results

**Foxg1 Overexpression in Murine Neocortical NSCs Antagonizes Astrogenesis**

Previous investigations in our lab gave evidence for a reduction of the astroglial output following Foxg1 overexpression in NSCs (Branccaccio et al. 2010). However, this phenomenon was only documented in vitro, as well as in a temporal frame delayed as compared with the standard astrogliogenic schedule (Okano and Temple 2000). Based on these findings, we hypothesized that Foxg1 might also control the physiological, timed progression of neocortical NSCs towards glial fates. To test this hypothesis, we decided to assess if Foxg1 overexpression impacts the in vivo astroglial output of genetically manipulated NSCs transplanted into wild type recipient brains, according to a developmentally plausible schedule. For this purpose, we engineered dissociated E12.5 corticocerebral precursors for conditional, TetON-driven Foxg1 overexpression, under the control of aNestin gene-derivative promoter (pNes) selectively firing in NSCs (Branccaccio et al. 2010). We acutely activated the transgene via doxycyclin administration and we maintained cells in a proliferative medium for 7 days. Then, we transplanted cells into the parietal corticocerebral parenchyma of P0 isochronic mouse pups. Specifically, we injected a 1:1 mix of cells, made alternatively gain-of-function (GOF) for Foxg1 or a control, and labeled with EGF and mCherry, respectively. Four days later, we sacrificed the pups and scored their brains for the astroglial outputs of the 2 different, transplanted precursor types (Fig. 1A,D). For this purpose, we took advantage of S100b, a Ca2+-binding protein shared by ependymal and glial lineages, mainly expressed by astroglial cells of perinatal neocortical tissue (Delouille et al. 2004; Raponi et al. 2007; Falcone et al. 2015). We found that, compared with controls, S100b+ derivatives of Foxg1-GOF cells were reduced by 19.25 ± 6.94% (P < 9.60 × 10−6, n = 8, paired t-test) (Fig. 1E, right and Fig. S1B). As Foxg1 also promotes NSCs self-renewal (Branccaccio et al., 2010),
we reasoned that this might lead us to underestimate the actual impact of Foxg1 overexpression on the NSC astrogenic bias. Therefore, to compensate for such an effect, first, we evaluated the frequency of Nestin−NSCs within sister cell preparations, engineered like the transplanted ones (but not labeled by EGFP or mCherry) and kept in proliferative medium for 7 days (Fig. 1A,D). Then, we normalized the in vivo astroglial output of transplanted cells against such frequency. We found that Foxg1 overexpression induced a 2.5-folds increase of Nestin−cells (+149.76 ± 6.34%, P < 4.55 × 10−3, n = 3,3) (Fig. 1E, left and Fig. S1A), meaning that the average, NSCs-normalized S100β astrocytic output was decreased by as much as 67.67%.

To corroborate these results, we performed a peculiar loss-of-function assay, employing neural precursors alternatively engineered by an aFoxg1-shRNA-expressing-LV or a control (Fig. 1B,D). Here we expected an enlargement of the astroglial output. Consequently, to increase the sensitivity of the assay, we interrogated E12.5 precursor derivatives kept in vitro for only 4 days being therefore further from the astrogenic peak. Specifically, we co-transplanted these cells as a 1:1 mix into heterochronic P0 pups and we assessed their final glial output at P4. The S100β cells frequency did not change upon Foxg1 manipulation (n = 4,4) (Fig. 1F, right and Fig. S1D). However, on the day of transplantation, the frequency of Nestin−cells was decreased by 32.14 ± 5.26% (P < 1.12 × 10−3, n = 3,3) in aFoxg1-shRNA samples compared with controls (Fig. 1F, left and Fig. S1C). This means that aFoxg1-shRNA manipulation upregulated the average, NSC-normalized astrocytic output by 39.75%.

To further validate these results, we repeated our GOF assay restricting the manipulation of Foxg1 NSC levels to the in vivo environment. To this aim, we kept dissociated E11.5 corticocerebral precursors, made acutely Foxg1-GOF under pNes/TetON control, for 3 days in a doxy-free proliferative medium. Then, we transplanted a 1:1 mix of these cells (EGFP-labeled) and their controls (mCherry-labeled) into the lateral ventricle of E14.5 isochronic mouse embryos. Last, we activated Foxg1 and control transgenes via oral doxycyclin administration to pregnant females. We allowed the embryos to be born, sacrificed the pups at P4, and we evaluated the astroglial outputs of the 2 different, transplanted precursor types (Fig. 1C,D). Similarly to the first Foxg1-GOF assay, we found that S100β derivatives of Foxg1-GOF precursors were robustly reduced compared with controls (−24.08 ± 11.03%, P < 0.01, n = 4,4, paired t-test) (Figs 1G and S1E), so definitively pointing to a genuine Foxg1 antiastrogenic activity.

To model molecular mechanisms underlying this activity, we considered to investigate them in dissociated neural cultures. To confirm the feasibility of this approach, we first verified if the outcome of Foxg1 manipulation could be fully replicated in these cultures, within a biologically acceptable temporal framework. For this purpose, we engineered dissociated E12.5 corticocerebral precursors for conditional Foxg1 overexpression as described above for transplantation assays, we maintained these cells in a proliferative medium for 7 days, and we allowed them to differentiate on poly-γ-L-lysinated coverslips for 4 additional days. We kept the Foxg1 transgene on during the entire procedure. Following Foxg1 overactivation, we found a pronounced loss of S100β astrocytes (−63.89 ± 9.14%, P < 0.003, n = 4,4) as well as a consistent reduction of Gfap+ cells (−37.32 ± 20.10%, P < 0.002, n = 3,3) (Figs 1H,I and S1G,H) (Gfap is an intermediate filament protein, mainly confined to astrocytes of rodent neocortex (Malatesta et al. 2008)). Remarkably, this anti-differentiative effect was specific to the astroglial lineage, as, within the same cultures, the frequency of Tubb3+ neurons was almost doubled (+90.45 ± 4.53%, P < 0.03, n = 3,3) (Figs 1H,I and S1I). In a second test, astrogial cultures were conversely prepared from Foxg1−/− (Hébert and McConnell 2000) mice-derived corticocerebral precursors. In this case, no S100β−output change was detected compared with wild type controls (Figs 1J,K and S1K). As in previous in vivo assays, we also evaluated the frequencies of NSCs in both Foxg1-GOF and -LOF cultures and used them to normalize the number of S100β−and Gfap+ cells. (Here, NSCs were identified as expressing Sox2 but not a mCherry reporter under the control of the neuronogenic-lineage-specific Tubulin-α1 promoter (Fig. 1H,J)). Interestingly, we found that, at day in vitro (DIV), NSCs were augmented by 89.64 ± 10.67% (P < 0.0001, n = 4,4) in Foxg1-GOF cultures and decreased by 26.95% in Foxg1-LOF cultures (Figs 1J,K and S1F,J). This implicates that the average, NSC-normalized astroctic output varied by −80.96% (S100β+ cells) and −52.41% (Gfap+ cells) in Foxg1-GOF cultures, as well as by +32.93% (S100β+ cells) in Foxg1-LOF cultures.

It is possible that what we observed in our assays did not depend on NSC fate choice, but it alternatively reflected an altered kinetic behavior of astrocyte-committed progenitors originating from engineered NSCs. To fix this issue, we repeated the in vitro Foxg1-GOF assay adopting 3 ad hoc devices. First, we ran it over a shorter temporal window (4 days for proliferation and 2 days for differentiation). Second, we took advantage of LIF stimulation to unmask early astroglial committed precursors. Third, we engineered both Foxg1-GOF and control preparations by lentiviruses harboring an additional IRES-EGFP module under pNes/TetON control, labeling NSCs and their immediate progenies, and we evaluated the Gfap+EGFP/EGFP+ ratio of each experimental preparation, as a more direct index of NSC-to-astroblast transition. Interestingly, we found that this ratio was diminished by 52.51 ± 3.61% in Foxg1-GOF samples compared with controls (P < 4.7 × 10−5, n = 4,4) (Figs 1L,M and S1L), so confirming the negative impact exerted by Foxg1 on the NSC astrogenic bias.

To secure this interpretation, we scored Foxg1-mutant NSCs by a classical clonal assay. Specifically, we made E11.5 corticocerebral precursors Foxg1-GOF or -LOF by dedicated lentiviral effectors, we kept them as floating neurospheres for 4 days, we let them attach on poly-γ-L-lysinated coverslips at clonal density, and we evaluated their clonal outputs 3 days later (Fig. 1N). We observed a decrease of neuron-astrocyte-mixed clones in Foxg1-GOF assays (5.27 ± 0.94% in mutants vs. 25.45 ± 1.24% in controls, P < 10−5, n = 4,4) and an increase of these clones in -LOF assays (32.83 ± 2.33% in mutants vs. 24.81 ± 3.19% in controls, P < 0.04, n = 4,4). In case of Foxg1-GOF assays, we also found a decrease of astrocyte-only clones (1.38 ± 0.48% in mutants vs. 5.07 ± 0.75% in controls, P < 3.1 × 10−3, n = 4,4) as well as a concomitant increase of neuron-only clones (72.55 ± 1.33% in mutants vs. 60.56 ± 2.14% in controls, P < 1.6 × 10−3, n = 4,4) (Figs 1O,P and S1M,N). Altogether, these data show that, in addition to stimulating NSC self-renewal, Foxg1 antagonizes the NSC shift from neuronogenic towards astrogenic fates.

Finally, before investigating specific antiastrogenic mechanisms driven by Foxg1, we wondered about their general articulation, cell-autonomous or not cell-autonomous. As Foxg1-GOF precursors gave rise to reduced astroglial outputs upon their transplantation into wild type brains (Fig. 1A,E), we expected that cell-autonomous mechanisms should play a likely prevalent role in this context. To corroborate this inference and unveil possible, collateral nonautonomous processes, we repeated the test shown in Figure 1H,J, with some ad hoc modifications. Specifically, we monitored the histogenetic behavior of aliquots of sensor (S)
Figure 1. Foxg1 antagonizes astrogenic progression of murine corticocerebral stem cells. (A–G) In vivo analysis. (A–C) Experimental strategies, (D) lentiviral vectors employed, and (E–G) results. (E, left) (ctr)-normalized frequencies of Nestin+ derivatives of E12.5 neocortical (ncx) precursors, acutely infected as in (A, analysis 1) (absolute frequency of Nestin+ cells in [ctr] samples, 10.38 ± 0.39%). Analysis performed on cells acutely attached on poly-L-lysin-coated coverslips. (E, right) Absolute frequencies of S100β+ astrocytes, evaluated within the parietal cortex of P4 pups among derivatives of cells engineered as in (A, analysis 2), and cotransplanted as a 1:1 mix into the cortical parietal parenchyma of isochronic P0 pups (average parameter value in [ctr] samples, 30.38 ± 2.24%). (F, left) (ctr)-normalized frequencies of Nestin+ derivatives of E12.5 neocortical precursors, acutely infected as in (B, analysis 1) (absolute frequency of Nestin+ cells in [ctr] samples, 22.45 ± 1.21%). Analysis performed on cells acutely attached on poly-L-lysin-coated coverslips. (F, right) Absolute frequencies of S100β+ astrocytes, evaluated within the parietal cortex of P4 pups among derivatives of cells engineered as in (B, analysis 2), and cotransplanted as a 1:1 mix into the cortical parietal parenchyma of heterochronic P0 pups (average parameter value in [ctr] samples, 25.04 ± 2.85%). (G) Absolute frequencies of S100β+ astrocytes, evaluated within the parietal cortex of P4 pups among derivatives of cells engineered as in (C), and cotransplanted as a 1:1 mix into the cortical parietal parenchyma of isochronic E14.5 wild-type embryos (average parameter value in [ctr] samples, 36.27 ± 4.77%). (H–K) In vitro analysis: neural cell frequencies, long protocol. (H, J) Experimental strategy and lentiviral vectors employed for its implementation, and (I, K) results. (I) Results of GOF analysis referred to in (H). Analysis 1: ctr-normalized frequencies of Sox2-mCherry− cells at DIV4 (absolute [ctr] frequency, 15.05 ± 0.79%). Cells acutely attached on poly-L-lysin-coated coverslips. Analysis 2 results: ctr-normalized frequencies of S100β+, Gfap+, and Tubb3+ cells at DIV11 (absolute [ctr] frequency, 14.95 ± 1.27%, 21.67 ± 1.03% and 16.85 ± 3.00%, respectively). (K) Results of LOF analysis referred to in (J). Analysis 1: ctr-normalized frequencies of Sox2-mCherry− cells at DIV4 (absolute [ctr] frequency, 14.36 ± 0.10%). Cells acutely attached on poly-L-lysin-coated coverslips. Analysis 2: ctr-normalized frequencies of S100β− cells at DIV11 (absolute [ctr] frequency, 25.36 ± 1.70%). (L, M) In vitro analysis: neural cell frequencies, short protocol. (L) Experimental strategy, lentiviral vectors employed, and (M) results. Here shown are (ctr)-normalized frequencies of Gfp+ cells among Egfp-expressing derivatives of...
E12.5 wild type pallial precursors, acutely labeled by a constitutively expressed mCherry transgene, following their 1:4 coculture with isochronous conditioner (C) precursors, pre-made GOF for Foxg1 or a control (Fig. 1Q). It turned out that the ratio among Gapa*mCherry* astrocytes and total mCherry* cells was not affected by the “genotype” of the cocultured C-derivative population, while Foxg1 overexpression by C-founders deregulated the fraction of their descendants expressing Gapa (−19.44 ± 2.69%, P < 2.3 × 10⁻⁸, n = 6,5) (Fig. 1R). All that confirms that Foxg1 inhibition of astrogenesis largely relies on cell-autonomous mechanisms and suggests that noncell-autonomous processes hardly contribute to it.

**Foxg1 Antagonizes the NSC Astrogenic Progression by Downregulating Key Transcription Factors Channeling NSCs to Astroglial Fates**

We hypothesized that the antiastrogenic activity of Foxg1 could primarily originate from its ability to down-regulate a small set of key transcription factors genes promoting the neurogenic-versus-astrogenic switch: Couptf1, Sox9, Nfia, and Zbhb20 (Naka et al. 2008; Kang et al. 2012; Nagao et al. 2016). In fact, Foxg1 is a well known transcriptional inhibitor (Li et al. 1995; Yao et al. 2001). Moreover, as resulting from Jaspal analysis (Mathelier et al. 2014), Couptf1, Sox9, Nfia, and Zbhb20 loci harbor a number of putative Foxg1-binding sites, among which a few high-score, evolutionarily conserved ones (Fig. S2A–D). We tested this prediction in pre-gliogenic neurocortical precursors made Foxg1-GOF by somatic lentiviral transgenesis (Fig. 2A–C, control-norm-[Foxg1-mRNA] = 4.42 ± 0.64, not shown). As expected, all 4 genes resulted to be downregulated upon Foxg1 overexpression, by −41.5 ± 7.01% (P < 0.045, n = 4,4), −39.8 ± 5.05% (P < 0.046, n = 6,6), −55.8 ± 4.73% (P < 0.003, n = 6,6), and −24.80 ± 9.34% (P < 0.038, n = 6,6), respectively (Fig. 2D, E). No changes were conversely detectable in Foxg1-knockdown cultures (Fig. 2B, C, F; control-norm-[Foxg1-mRNA] = 0.65 ± 0.04, not shown), suggesting that gene downregulation observed in Foxg1-GOF preparations did not reflect a dominant-negative effect.

Then, to assess functional relevance of these phenomena to Foxg1 antiastrogenic activity, as a proof-of-principle, we overexpressed Zbhb20 and Nfia in wild type (Fig. S3A–C) and Foxg1-overexpressing precursors and we evaluated the histogenetic outcome of these manipulations by dedicated clonal assays (Fig. 2G). While not fully rescuing the Foxg1-GOF phenotype, the transduction of a constitutively active Zbhb20 transgene into Foxg1-GOF cultures mitigated the absolute frequency decrease of neuronal-astroglial and astroglial clones evoked by Foxg1 overexpression compared with controls (from −25.37% to −13.11%, P < 0.009, n = 4,4, and from −7.51% to −4.55%, P < 0.021, n = 4,4, respectively). [Here, control frequencies of neuronal—astroglial and astroglial clones were 36.25 ± 2.14% and 8.89 ± 1.70%, respectively]. On the other side, Zbhb20 alone did not significantly alter the frequencies of the different clone types. This suggests that, rather than simply compensating for it, Zbhb20 transduction partially rescued a key molecular event mediating Foxg1 impact on NSC fate choice (Figs 2H and S4A). As for Nfia, its overexpression in Foxg1-GOF NSCs fully restored the drop of mixed clones caused by Foxg1 upregulation (these clones were as little as 5.63 ± 0.48% in Foxg1-GOF cultures, compared with 21.69 ± 3.53% in controls [P < 0.002, n = 4,4]), while not affecting the prevalence of these clones if elicited in control NSCs. Moreover, Nfia also abolished the slight increase of neuronal clones (75.17 ± 1.73% vs. 69.49 ± 2.43%, P < 0.049, n = 4,4) as well as the decrease of astroglial ones (1.24 ± 0.46% vs. 4.62 ± 0.79%, P < 0.005, n = 4,4) caused by Foxg1 overexpression. Remarkably, however, its overactivation in control NSCs almost halved the frequency of neuronal clones (40.19 ± 2.03% vs. 69.49 ± 2.43%, P < 0.001, n = 4,4) and elicited a massive increase of astroglial ones (24.99 ± 1.17% vs. 4.62 ± 0.79%, P < 0.001, n = 4,4) (Fig. 2I and S4B). All this indicates that, in addition to rescuing the Foxg1-GOF phenotype, the Nfia transgene also overcompensated for it.

Finally, to cast light on molecular mechanisms mediating Foxg1-driven downregulation of Couptf1, Sox9, Nfia, and Zbhb20, we profiled chromatin of mid-neuronogenic neurocortical precursors, both control and Foxg1-GOF, for Foxg1 recruitment at selected regions of the corresponding loci, by Chromatin-Immunoprecipitation (ChIP)-qPCR (Fig. 2J). Specifically, nine Foxg1-BSs were inspected, 2 for Couptf1, 3 for Zbhb20, 2 for Sox9, and 2 for Nfia. These BSs included 4 putative ones selected by Jaspars software (Mathelier et al. 2014), with score index above 14 (14⇑Foxg1-BSs), and shared by mice and humans, as well 5 experimentally verified ones, reported by the NCRI-GEO database (15⇑Foxg1-BSs) (Fig. S2A–D). In general, all these BSs were specifically enriched in aFoxg1-immunoprecipitates compared with IgG-treated samples (however, this does not apply to Couptf1-Foxg1-BSs.h1 and Nfia1-Foxg1-BSs.h1 in control aFoxg1-immunoprecipitates) (Fig. 2K). Interestingly, in case of Couptf1, Sox9 and Nfia loci, there was at least one BS significantly over-enriched in Foxg1-GOF compared with control aFoxg1-immunoprecipitates (BSs displaying Foxg1-responsive enrichment included: Couptf1-Foxg1-BSs.h1 [3.83 ± 0.43% vs. 1.60 ± 0.24%, P < 0.005, n = 4,3], Couptf1-Foxg1-BSs.a [1.36 ± 0.15% vs. 0.88 ± 0.19%, P < 0.050, n = 4,4], Sox9-Foxg1-BSs.h1 [2.02 ± 0.25% vs. 1.25 ± 0.26%, P < 0.025, n = 4,3], and Nfia1-Foxg1-BSs.h1 [4.56 ± 0.47% vs. 2.66 ± 0.41%, P < 0.017, n = 4,4]) (Fig. 2K). This suggests that Foxg1 directly downregulates Couptf1, Sox9, and Zbhb20 transcript variants, and that such trans-expression may contribute to the decline of Couptf1, Sox9, and Nfia transcripts occurring in Foxg1-GOF precursors.

**Foxg1 Antagonizes Astrogenesis by Directly Transrepressing Astroglial Genes**

We hypothesized that Foxg1 inhibition of astrogenesis could be further strengthened by a direct impact of Foxg1 on genes implementing the astrogial differentiation program. To assess this issue, first we investigated the response of selected astrogial genes, Gapa, S100b, and Agrp4, to Foxg1 manipulation. For this purpose, we employed E14.5 corticocerebral precursors engineered

engineered neocortical precursors (absolute [ctrl] parameter value, 19.06 ± 0.85%) in vitro analysis: clonal assays. (N) Experimental strategy, lentiviral vectors employed, and (OP) results. Here shown are absolute frequencies of Tub33*Gapa* (neuron-only), Tub33*Gapa* (astrocyte-only), Tub33*Gapa* (neuron-astrocyte-mixed) clones, as evaluated among total clones originating from E11.5 neocortical precursors, infected and processed as in (N). (Q, R) In vitro analysis: cell-autonomous versus noncell-autonomous mechanisms. (Q) Experimental strategy, lentiviral vectors employed, and (R) results. Here, shown are (ctrl)-normalized Gapa*mCherry*mCherry* and Gapa*mCherry*mCherry* cell number ratios, as well as (ctrl)-normalized mCherry* cell frequencies, as evaluated in DIV11 cultures originating from of E12.5 neocortical (troc) precursors, acutely infected, and processed as in (Q). (absolute [ctrl] parameter values, 56.50 ± 1.70%, 41.57 ± 1.78%, and 16.12 ± 1.49%, respectively). Error bar = s.e.m. n is the number of biological replicates (i.e., independently transduced cell samples, or—case (E, right), (F, right), and (G)—cortransplanted brains). P-value calculated by t-test (one-tail, unpaired, or—case (E, right), (F, right), and (G)—one-tail, paired).
for TetON-controlled Foxg1 overexpression, kept in the presence of growth factors and terminally pulsed by LIF (Fig. 3A). As expected, we found that Gfap-, S100b-, and Aqp4-mRNA levels were significantly decreased, by −49.92 ± 13.25% ($P < 0.03$, $n = 6,6$), −46.79 ± 16.91% ($P < 0.03$, $n = 5,5$), and −43.98 ± 14.70% ($P < 0.03$, $n = 5,5$), respectively (Fig. 3B, left). No statistically significant changes of these mRNAs were observed in a specular Foxg1-LOF assay (Fig. 3B, right), so ruling out any dominant negative effects.

Next, to cast light on mechanisms mediating Foxg1-dependent repression of these genes, we selected a set of putative Foxg1-binding sites (BSs) within Gfap, S100b, and Aqp4 promoters (2 for Gfap, 3 for S100b and 2 for Aqp4), by Jaspar software.
Figure 3. Foxg1 represses astroglial-lineage active genes. (A, B) Regulation of astroglial Gfap, S100b and Aqp4 genes by Foxg1: (A) experimental strategy, lentiviral vectors employed, and (B) results. Data double-normalized, against endogenous Gapdh-mRNA and (ctr) values. (C, D) Assaying direct regulation mechanisms: Chromatin ImmunoPrecipitation-PCR (qChIP-PCR) quantification of Foxg1-enrichment at putative Foxg1 binding sites within Gfap, S100b and Aqp4 loci (named as in Fig. S2E–G). (C) experimental strategy, lentiviral vectors employed, and (D) results. Data normalized, against input chromatin. (E–J) Assaying indirect regulation mechanisms. (E, G) Modulation of nuclear pStat3, pSmad1,5,8, ErbB4ICD, NCoR1, Notch1ICD protein levels in Nestin+ E12.5+DIV7 neural stem cells (NSCs), evaluation by quantitative immunofluorescence. (E) Experimental protocol, lentiviral vectors employed, and (G) results. Data normalized against (ctr). (F, H) Functional relevance of pStat3, pSmad1,5,8, ErbB4ICD, NCoR1, and Notch1ICD misregulation to Foxg1 antiastrogenic activity, assessed by counteracting Foxg1-driven changes of these effectors and evaluating the resulting S100b+ cell frequency. Constitutively active Stat3 and Smad1 (caStat3 and caSmad1) were overexpressed one by one or combined; functional relevance of ErbB4 and NCoR1 was investigated by dampening their essential Tab2 cofactor. (F) Experimental protocol, lentiviral vectors employed, and (H) results. Data normalized against (ctr); absolute frequencies of S100b+ cells: 11.84 ± 2.30% (ctr), 8.58 ± 0.43% (ctr−caStat3), 8.87 ± 0.62% (ctr−caStat3−caSmad1), 21.36 ± 5.27% (ctr−Tab2); absolute frequencies of Gfap+ cells: 37.59 ± 3.14% (ctr−Notch1ICD). Data normalized against (ctr). Error bar = s.e.m. n is the number of biological replicates. These are: (B, D, H) independently transduced neural cultures; (G) single cells, randomly and evenly picked from 4,4 independently transduced neural cultures. P-values calculated by t-test (one-tail, unpaired).
Foxg1 Antagonizes Astrogenesis Via a Pleiotropic Impact on Key Pathways Tuning Astroglial Genes

In addition to its direct effect on astroglial genes, we hypothesized that Foxg1 might further dampen their activity indirectly, by impacting transactive modulators of their transcription. In particular, we focused our attention on 4 key pathways involved in fundamental control of astroglial gene transcription: IL6/Jak2/Stat1,3; Bmp/Smad1,5,8; Nrg1/ErbB4 ICD-NCoR; Dll1/Notch1 ICD. For these pathways, we measured nuclear levels of their ultimate nuclear effectors (modulating gene transcription) within Foxg1-GOF, Nestin+ NSCs. We also evaluated nuclear NSC levels of a key antagonist of the IL6/Jak2/Stat1,3 pathway, Neurog1. We acutely engineered E12.5 corticocerebral precursors, making them to overexpress Foxg1 in the neurostem compartment. We kept these precursors in culture under growth factors for 7 days, and we finally immunoprofiled them for Nestin and the 6 effectors in order, each evaluated by quantitative immunofluorescence (qIF): p[Tyr705]Stat3, p[Ser463/465]Smad1,5,8, Neurog1, ErbB4, NCoR1, and Notch1 (Fig. 3E). We found that p[Tyr705]Stat3, p[Ser463/465]Smad1,5,8, Neurog1, ErbB4, NCoR1, and Notch1 were slightly, albeit significantly upregulated, by +8.75 ± 3.56% (P = 0.032, n = 182 ± 150), and +14.8 ± 2.83% (P < 0.0002, n = 212 ± 281), respectively (Figs 3G and S4C–H).

To address the relevance of these changes to astrogial gene expression, we functionally counteracted them by transducing E12.5 neocortical precursors, made acutely Foxg1-GOF, with prevalidated (Fig. S3D–G), “rescuing” XI lentiviruses. We kept engineered cells as proliferating neurospheres for 7 days under growth factors. We allowed them to differentiate for 4 more days. Finally, we evaluated their S100β astroglial output (Fig. 3F). We found that constitutively active Stat3 (caStat3) (Hillion et al. 2008) lessened the astroglial deficit elicited by Foxg1 overexpression, however only to a limited extent (normalized against controls, this deficit moved from −66.46 ± 3.15% to −49.70 ± 6.29%, P < 0.03, n = 4). Conversely, constitutively active Smad1 (Fuentenbalba et al. 2007) (caSmad1), alone or in combination with caStat3, fully restored the normal astroglial output. In a similar way, functional NCoR1 inhibition, via knockdown of its necessary Tab2 cofactor (Sardi et al. 2006), considerably reduced the Foxg1-dependent astrogenic deficit (Figs 3H and S6A–D). Remarkably, in all these cases the delivery of the “rescuing agent” to control cultures did not upregulate astrogenesis rates (caStat3 and caSmad1) reduced the output of these cultures (Fig. 3H), possibly because of premature shrinkage of their neurostem compartment (Fig. S5A).

All this suggests that each of these agents did not simply mask the astrogenic deficit elicited by Foxg1 overexpression, via compensatory mechanisms independent from Foxg1 regulation. It rather indicates that they abolished molecular abnormalities which specifically mediate the impact of Foxg1 overexpression on astrogenesis. Last, NSC transduction of a constitutively active Notch1ICD transgene ( Cassidy et al. 2014) failed to rescue the hypoastrogenic Foxg1-GOF phenotype. Moreover, it reduced the Gfap+ astrogial output of both control and Foxg1-GOF cultures, from 100 ± 8.35% to 21.64 ± 2.48% (control-normalized frequencies, P < 0.001, n = 4)., and from 40.28 ± 5.63% to 26.34 ± 4.71% (control-normalized frequencies, P < 0.001, n = 4), respectively (Figs 3H and S6E). This phenomenon was unexpected. It likely reflected the defective capability of derivatives of Notch1ICD-overpressing NSCs to respond to astrocytic cytokines and activate the mature astroglial marker Gfap (Fig. S5B).

Next, we investigated basic mechanisms leading to pStat3 and pSmad1,5,8 deficits. We evaluated the impact of Foxg1 manipulations on key downregulators implicated in the corresponding signaling machineries (Fig. 4A). As for the IL6/Jak2/Stat1,3 axis, we found a significant downregulation of Gp130-, Jak2-, and Stat3-mRNAs (Gp130: −24.51 ± 6.53%, P < 0.05, n = 6; Jak2: −54.33 ± 6.52%, P < 0.0002, n = 6; Stat3: −32.26 ± 8.88%, P < 0.03, n = 6) and no changes for Il6Ra-mRNA in Foxg1-GOF cultures (Fig. 4B). Jak2- and Stat3-mRNA were conversely upregulated in Foxg1-LOF cultures (Jak2: +13.32 ± 3.80%, P < 0.02, n = 6; Stat3: +10.59 ± 2.44%, P < 0.02, n = 6), whereas Gp130- and Il6Ra-RNA were unaffected (Fig. 4C). Concerning the Bmp/Smad1,5,8 pathway, BmpRII-mRNA was downregulated in Foxg1-GOF samples (−29.59 ± 11.44%, P < 0.03, n = 6) (Fig. 4B) and unaffected in Foxg1-LOF ones (Fig. 4C). Bmp4 was not affected at all. Last, NCoR1 mRNA levels, implicated in the balance between NotchICD and ErbB4ICD-mediated signaling, did not show any significant change either in Foxg1-GOF and -LOF cultures (Fig. 4B). Finally, as a proof-of-principle, to assess relevance of the changes described above to the Foxg1-GOF astroglial phenotype, we counteracted the jak2-mRNA decline peculiar to Foxg1-GOF neocortical precursors by a lentiviral expression vector encoding for the constitutively active, JAK2V617F mutant kinase (Vainchenker 2005), and we evaluated the impact of this manipulation on Gfap+ cell frequency (Fig. 4D). Interestingly, the introduction of a JAK2V617F transgene in Foxg1-GOF cultures mitigated the decline of Gfap+ cells caused by Foxg1 overexpression, which moved from −43.13 ± 4.02% to −30.26 ± 2.84% (control-normalized frequencies, P < 0.02, n = 4).

The frequency of these cells was conversely unaffected, when JAK2V617F was delivered to controls (Figs 4E and S6F). All this suggests that the downregulation of Jak2 (and, possibly, other Fox1-sensitive, IL6/Jak2/Stat1,3- and Bmp/Smad1,5,8-signal mediators reported above) may contribute to Fox1 inhibition of astrogial genes expression.

Foxg1 Impairs Transactivating Abilities of the pStat3-pSmad1,5,8 Complex

It was previously shown that Foxg1 chelates Smad1 and Smad4 (Rodriguez et al. 2001). Therefore, we wondered if it may antagonize astrogenesis, by impairing intrinsic transactivating abilities of these Bmp signaling effectors. To address this issue, we acutely engineered E12.5 neocortical precursors with 2 transgenes, encoding for constitutively active Stat3 (caStat3) and Smad1 (caSmad1), driven by a constitutive promoter (Pgk1-p). To sense the activity of the resulting caStat3-caSmad1 complex
in a way independent of possible local epigenetic effects of Foxg1 on astroglial gene chromatin, we employed a synthetic Bmp-signaling sensor (\textit{BmpRE-ZsGreen}), delivered via a randomly integrating lentivector. To so-engineered neural cells, we concomitantly delivered a Foxg1-expressing lentivirus or a control. We kept these cells for 10 days in proproliferative conditions and, lastly, we profiled them for EGFP fluorescence by cytofluorimetry (Fig. 4F). We found that both the frequency of ZsGreen$^+$ cells and the median fluorescence intensity of ZsGreen$^+$ cells were reduced in Foxg1-GOF samples, by 38.38 $\pm$ 6.63% (control-normalized value, $P < 0.05$, $n = 4,4$), and 26.88 $\pm$ 2.78% (control-normalized value, $P < 0.001$, $n = 4,4$), respectively.

Figure 4. Molecular details of Foxg1 impact on different astrogenic pathways. (A–C) Consequences of Foxg1 manipulation on mRNA levels of IL6Ra, Gp130, Jak2, Stat3, Bmp4, BmpRII, NCoR1. (A) Experimental protocol and lentiviral vectors employed. (B, C) Results. Data double-normalized, against endogenous Gapdh-mRNA and (ctr) values. (D, E) Functional relevance of Jak2 misregulation to Foxg1 antiastrogenic activity. This was assessed by counteracting Foxg1-driven change of Jak2-mRNA and evaluating the resulting S100b$^+$ cell frequency. (D) Experimental protocol and lentiviral vectors employed. (E) Results. Data normalized against (ctr); absolute frequency of S100b$^+$ cells in (ctr) samples: 28.70 $\pm$ 0.81%. (F, G) Foxg1 impact on transactivating abilities of BMP/Jak-Stat pathways effectors. This was assessed by expressing caStat3 and caSmad1 in E12.5 neocortical precursor cultures and evaluating their ability to transactivate a lentivector-delivered, randomly integrating fluorescent reporter gene, associated to Bmp responsive elements (BMP-RE), upon Foxg1 overexpression. (F) Experimental protocol, lentiviral vectors employed, and (G) (ctr)-normalized results. Analysis by cytofluorimetry. Absolute (ctr) frequency of ZsGreen$^+$ cells: 78.50 $\pm$ 5.33%. Error bar = s.e.m. $n$ is the number of biological replicates. $P$-values calculated by $t$-test (one-tail, unpaired).
Foxg1 NSC Expression Levels Progressively Decline Before the Perinatal Astrogenic Burst

We wondered if Foxg1 antiastrogenic activity might be instrumental in the proper temporal progression of astrogenesis. Specifically, we hypothesized that high Foxg1 levels in early NSCs could refrain them from differentiating into astroblasts, while lower levels peculiar to later NSCs could be permissive to such differentiation. To address this issue, we measured Foxg1-mRNA and -protein levels within pallial stem cells of different ages.

In a first assay, we employed derivatives of E11.5 pallial precursors, cultured in proliferative medium for 2 up to 7 days. From these cultures we FACsorted samples of NSCs (identified as pNes-EGFP-"pTa1-mCherry"- upon lentiviral transduction of these reporters) at DIV2 and DIV7, approximately corresponding to in vivo E13.5 and E18.5, respectively. We analyzed their RNA and we found a significant decrease of Foxg1-mRNA level in DIV7 compared with DIV2 samples (−59.47 ± 5.11%, P < 0.01, n = 5.5). Here, as controls, we also measured expression of Zbtb20 and Neurog1, 2 genes active in NSCs according to opposite temporal progressions (Hirabayashi et al. 2009; Ohtsuka et al. 2011). As expected, Zbtb20 and Neurog1 were increased and decreased, respectively, in more aged samples (Fig. 5A,B). In a second assay, we evaluated Foxg1 protein level in DIV2 and DIV6 Nestin+ NSC derivatives of E12.5 acutely dissociated neocortices, by double aNestin-aFoxg1 qIF. Compared with DIV2 samples, Foxg1 level was diminished by 52.55 ± 1.2% at DIV6 (P < 3.6 × 10⁻⁴, n = 288,229) (Fig. 5C,D), confirming that a robust Foxg1 expression decline occurs in neocortical stem cells concomitantly with the neurogenic-to-astrogenic transition.

To corroborate these findings, we compared in vivo Foxg1 expression at late versus early neuronogenic stages of neocortical development. As expected, we found that periventricular Foxg1 levels overtly declined at E16.5 with respect to E12.5 (Fig. 5F). Conversely, in a parallel control assay where the BmpRE-ZsGreen Bmp-signaling sensor was replaced by a pSK1-mCherry transgene, no mCherry expression decline was detectable in Foxg1-GOF samples (not shown). These results suggest that Foxg1 may dampen the intrinsic, pSmad1/4 transactivating power.

(Fig. 4G). Conversely, in a parallel control assay where the BmpRE-ZsGreen Bmp-signaling sensor was replaced by a pSK1-mCherry transgene, no mCherry expression decline was detectable in Foxg1-GOF samples (not shown). These results suggest that Foxg1 may dampen the intrinsic, pSmad1/4 transactivating power.
Foxg1 Antiastrogenic Activity is Conserved in Human Pallial Precursors

To assess if FOXG1 antagonizes astrogenesis progression in humans like in rodents, we run an ad hoc GOF assay in late pallial precursors derived from a legal, human PCW10 abortion, pre-expanded in vitro over about 150 days. At the beginning of the procedure (DIV0), we transduced these precursors with 2 lentiviruses, expressing the rtTAM2 transactivator under the constitutive Pkgl promoter and Foxg1 (or a control) under the rtTAM2/doxycycline-responsive TREt promoter (Fig. 6A). We kept the engineered cells for 7 days in proliferation medium and 7 more days in differentiation medium. We exposed them to 9 ng/ml doxycyclin from DIV0 to DIV11 (so eliciting a final expression gain about 4, not shown) and we immunoprofiled their derivatives at DIV15 for S100b (which is mainly confined to cells expressing the pan-astrocytic marker AldoC in these cultures, not shown). It turned out that S100b+ astrocyte frequency was reduced by ~51.29 ± 8.05% in Foxg1-GOF samples compared with controls (P < 0.006, n = 4,4) (Figs 6A and 7A). To get a closer insight into FOXG1 role in human NSC fate choice, we run an additional GOF assay, differing from the previous 1 in 3 aspects. We restricted Foxg1 overexpression to the NSC compartment, under the control of the pNes promoter; we shortened the differentiation phase of the procedure by 4 days; we exposed the engineered cells to a terminal, 24 h pulse of LIF. Then, we evaluated the frequency of cells expressing GFAP (specifically detectable in astroglial, but also neurostem human cells (Malatesta et al. 2008)) as a proxy of the NSC astrogenic bias (Fig. 6B). Immunoprofile of these cultures at DIV10 showed that such frequency was reduced by ~27.66 ± 5.41% in Foxg1-GOF samples compared with controls (P < 0.003, n = 4,4) (Figs 6B and 7B). All this supports the hypothesis that Foxg1 may antagonize the NSC astrogenic progression in humans like in rodents.

Next, to corroborate these results and rule out that they originated from a dominant negative effect, we interrogated a sister preparation of the PCW10 neocortical precursors referred to above, pre-expanded in vitro over about 120 days, by a NSC-restricted FOXG1-LOF approach (Figs 6C and 7C). For this purpose, we transduced such precursors by a lentiviral mix encoding for pNes-driven expression of mir-aFoxg1.1690 (this is an RNAi effector decreasing Foxg1-mRNA levels by about 42% upon constitutive, Pkgl1-driven expression in mixed neural cultures; data not shown). Then, we kept transduced cells 7 days in proliferative medium and 3 more days in a differentiative medium, terminally supplemented by Lif. Finally, we immunoprofiled cells for neurostem/astroglial markers. Frequency of Egfp-GFAP+ astrocytes was unaffected. Conversely, normalized against controls, Egfp+GFAP+ NSCs were reduced by ~26.97 ± 7.82% (P < 0.025, n = 3,3). All this points to a robust increase of the NSC-normalized GFAP+ astroglial output (normalized against controls, +58.59 ± 3.83%, P < 0.003, n = 3,3) (Fig. 6C). It further suggests that the negative Foxg1 impact on NSC astroglial differentiation emerged upon both gain- and loss-of-function approaches, suggesting that this process is sensitive to even subtle changes of Foxg1 levels around baseline. Noticeably, these observations were performed upon transplantation of neural precursors premanipulated in vitro by lentiviral transgenesis, an approach allowing for stable and accurate control of gene expression levels.

Next, the astrogenesis decline evoked by Foxg1 overexpression in NSCs was not simply due to a general differentiation deficit of neocortical precursors. In fact, it was associated to a pronounced, absolute increase of neurogenesis (Fig. 1H,J). As emerging from clonal assays (Fig. 1N–P), this did reflect a net, consistent shift in the NSC histogenetic choice. This means that channeling NSCs towards neuronal rather than glial fates is a genuine, key role exerted by Foxg1 in normal development.

Last, an antiastrogenic activity was observed when NSC-restricted Foxg1 overexpression was switched on in vivo, in a late-neurorgenic, isochronic environment (Fig. 1C,G). Moreover, clone founder cells showed an altered astrogenic bias following Foxg1 manipulation during the neurorgenic phase (Fig. 1N–P). Not least, Foxg1 NSC levels (both mRNA and protein) turned out to progressively decline, while moving from early neurorgenic stages to subsequent gliogenic phases (Fig. 5). All this suggests
that a progressive decline of Foxg1 levels is among key factors dictating age-dependent, NSC developmental choices (Ohtsuka et al. 2011; Okamoto et al. 2016).

As for cellular articulation of Foxg1 activity, it has to be emphasized that astrogenesis inhibition was detectable upon NSC-restricted Foxg1 overexpression (Figs 1A,C,D,H,N and 6B), by scoring progenies of engineered neural precursors transplanted in wild type brains (Fig. 1E,G) or allowed to differentiate in vitro (Figs 1I,O and 6B). It is known that, despite the relevance of environmental signals to the progression of early pallial precursors towards gliogenesis (Morrow et al. 2001), such progression can largely occur in a clone-autonomous way (Qian et al. 2009).
et al. 2000). Our data indicate that Foxg1 largely acts via cell-autonomous mechanisms and contributes to intraclonal astrogenesis control (Fig. 1Q,R).

Concerning molecular articulation of Foxg1 activity, this was quite complex. Foxg1 targets included genes involved in both choice and implementation of the astrogenic program. Moreover, its impact on these targets was direct as well as indirect (Fig. 7).

First of all, Foxg1 downregulated selected transcription factor genes which normally promote NSC acquisition of astrogenic competence (Fig. 2A–F). These were: Zbtb20 and its CoupTf1 target, stimulating the histogenetic progression of neocortical precursors and specifically increasing their responsivity to astrogenic cytokines (Naka et al. 2008; Nagao et al. 2016; Tonchev et al. 2016), as well as Sox9 and its Nfia target, both promoting the respiratory increase associated to the NSC-to-astrocyte progenitor transition (Kang et al. 2012), the latter sustaining astroglial gene promoter demethylation (Namihira et al. 2009). Apparently, all 4 genes were directly transrepressed by Foxg1 (Fig. 2J,K). However, indirect mechanisms also likely contributed to their dynamics. For example, the drop of Sox9-mRNA might have been exacerbated by the Foxg1-dependent collapse of the IL6/Jak2/Stat3 pathway, which normally sustains its expression (Hall et al. 2017; Jeselsohn et al. 2017). In a similar way, the decrease of Nfia mRNA might reflect the downregulation of both Sox9, acting upstream of it (Kang et al. 2012), and nuclear Notch\(^{\text{ICD}}\), also promoting its transcription (Namihira et al. 2009). Conversely, Zbtb20, insensitive to caStat3, Bmp4, Notch\(^{\text{ICD}}\), Sox9, or Nfia (Nagao et al. 2016), might have been suppressed by Foxg1 via prevalently direct mechanisms. Intriguingly, Zbtb20 and Foxg1 play contrasting roles in other key neuro-developmental scenarios too, e.g., in medial-lateral pallial specification, as promoters of archicortical and neocortical fates, respectively (Muzio and Mallamaci 2005; Nielsen et al. 2007, 2010).

Next, Foxg1 further repressed genes which implement the astroglial program within committed progenitors and their mature progenies, such as Gfap, S100b, Aqp4 (Fig. 3A,B). Even in this cases, gene downregulation was likely mediated by direct (Fig. 3C,D) as well as indirect (Fig. 3E–H) mechanisms. Among the most prominent molecular changes underlying Gfap downregulation, there was the pronounced pSmad1,5,8 decrease evoked by Foxg1 overexpression in NSCs (Fig. 3F,G), possibly as a consequence of reduced sensitivity of these cells to Bmp ligands (Fig. 4A,B). Moreover, Foxg1 apparently jeopardized the transactivating abilities of the “active Smad1-active Stat3”-containing complex (Fig. 4F,G), likely by chelating Smad1, as already described in heterologous systems (Rodriguez et al. 2001). However, the Smad1,5,8 machinery is limiting to both neuronogenesis and astrogenesis progression (Nakashima 1999; Sun et al. 2001; Hirabayashi et al. 2009). Therefore, a Bmp signaling decrease should be able to simply exacerbate the absolute astrogenic deficit evoked by Foxg1, not cause it. We propose that this deficit primarily stem from the collapse of pStat3 (Fig. 3F,G), in the presence of sustained Neurog1 expression (Fig. 3G). The former was reasonably due to the downregulation of at least 3 key components of the corresponding signaling cascade (Fig. 4A,
that an abnormal human pallial precursors. In this respect, it has been shown that, both these factors compete for the same RBPJk bridge (Foxg1 expression levels are manipulated in Foxg1–GOF NSCs. In fact, both these factors compete for the same RBPJk bridge (Kao et al. 1998), mediating their interaction with astroglial genes (Ge et al. 2002; Hermanson et al. 2002). In this way, the increase of the NCoR1/NotchICD ratio occurring in Foxg1–GOF NSC nuclei (114.18/86.22:1) × 100% = +32.42%, Fig. 3G) might severely unbalance this competition, so resulting in prevailing inhibitory, RBPJk-mediated inputs to astroglial promoters. To note, the moderate upregulation of nuclear NCoR1 occurring in Foxg1–GOF samples (Fig. 3G), not due to NCoR1 gene transactivation (Foxg1 expression levels in NSCs modulate astrogenesis rates by regulating Egfr and Fgfr9. Glia. 63:412–422.


Funding
Telethon Italy (Grant GGP13034 to A.M.) and SISSA (intramural funding to A.M.).

Notes
We thank Nika Bleichl who contributed to early setup of in vitro histogenetic assays. We are grateful to Marco Bestagno and Nicoletta Caronni for technical assistance in FACsorting procedures, as well as to Alberto Tommasini for supporting us with analytic cytofluorimetric assays. Conflict of Interest: The authors declare no competing interests.

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