Received: 5 April 2017 | Revised: 27 September 2017 | Accepted: 9 October 2017

DOI: 10.1002/glia.23253

GLIA
WILEY

RESEARCH ARTICLE

TGF-β pro-oligodendrogenic effects on adult SVZ progenitor cultures and its interaction with the Notch signaling pathway

Laura I. Gómez Pinto[1] | Debora Rodríguez[2] | Ana M. Adamo[1*] | Patricia A. Mathieu[1*]

[1]Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, CABA C1113AAD, Argentina

[2]Departamento de Ciencias Básicas, Universidad Nacional de Luján, Ruta 5 and Av Constitución, 6700 Luján, Buenos Aires, Argentina

Correspondence
Patricia A. Mathieu, Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, C1113AAD CABA, Argentina.
Email: pmathieu@qb.ffyb.uba.ar

Funding information
Fondo para la Investigación Científica y Tecnológica, Préstamo BID, Grant Number: PICT 2013 2772; Consejo Nacional de Investigaciones Científicas y Técnicas, Grant Number: PIP 0567, Universidad de Buenos Aires, Grant Number: UBACYT 20020120100132BA

Abstract

Adult neural progenitor cells (NPCs) are capable of differentiating into neurons, astrocytes, and oligodendrocytes throughout life. Notch and transforming growth factor β1 (TGF-β) signaling pathways play critical roles in controlling these cell fate decisions. TGF-β has been previously shown to exert pro-neurogenic effects on hippocampal and subventricular zone (SVZ) NPCs *in vitro* and to interact with Notch in different cellular types. Therefore, the aim of our work was to study the effect of TGF-β on adult rat brain SVZ NPC glial commitment and its interaction with Notch signaling. Initial cell characterization revealed a large proportion of Olig2+, Nestin+, and glial fibrillary acidic protein (GFAP+) cells, a low percentage of platelet-derived growth factor receptor α (PDGFRα+) or NG2+ cells, and <1% Tuj1+ cells. Immunocytochemical analyses showed a significant increase in the percentage of PDGFRα+, NG2+, and GFAP+ cells upon four-day TGF-β treatment, which demonstrates the pro-gliogenic effect of this growth factor on adult brain SVZ NPCs. Real-time polymerase chain reaction analyses showed that TGF-β induced the expression of Notch ligand Jagged1 and downstream gene Hes1. Notch signaling inhibition in cultures treated with TGF-β produced a decrease in the proportion of PDGFRα+ cells, while TGF-β receptor II (TβRII) inhibition also rendered a decrease in the proportion of PDGFRα+ cells, concomitantly with a decrease in Jagged1 levels. These findings demonstrate the participation of Notch signaling in TGF-β effects and illustrate the impact of TGF-β on glial cell fate decisions of adult brain SVZ NPCs, as well as on oligodendroglial progenitor cell proliferation and maturation.

KEYWORDS
cytokine, Jagged1 ligand, neural progenitor cell, oligodendrocytes

1 | INTRODUCTION

The adult rodent, nonhuman primate, and human brain contain two major neurogenic niches, that is the subgranular zone of the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles (Eriksson et al., 1998; Jin et al., 2001). Neural progenitor cells (NPCs) present in the adult mouse SVZ, also known as type B cells, give origin to transit amplifying Nestin+ and Olig2+ type C cells, which differentiate into either neurons or NG2+ oligodendroglial progenitor cells (OPCs) (Lois & Alvarez-Buylla, 1994; Menn et al., 2006). Neurons migrate to the olfactory bulb through the rostral migratory stream, whereas OPCs proliferate and migrate into the corpus callosum, striatum, or fimbria fornix and mature into myelinating oligodendrocytes (OLs) (Gonzalez-Perez & Alvarez-Buylla, 2011; Menn et al., 2006). Studies carried out in adult rats have shown NPCs to bypass the type C stage and convert directly into neuroblasts (Danilov et al., 2009). In physiological conditions, few OLs are generated from adult SVZ type B cells as compared with newly born neurons, probably due to slow OL turnover (McCarthy & Leblond, 1988). However, in demyelinating conditions, both OPCs residing in the adult brain and those derived from NPCs present in the adult SVZ have been shown to generate new OLs in response to injury (Aparicio, Mathieu, Pereira Luppi, Almeira Gubiani, & Adamo, 2013; Guo et al., 2015; Nait-Oumesmar et al., 1999; Petratos et al., 2004; Picard-Riera et al., 2002).

*Authors equally contributed to this work.

Different roles have been assigned to cytokines present in the neurogenic niches of the adult brain. In particular, transforming growth factor βs (TGF-βs) in their three mammalian isoforms—TGF-β1, -β2, and -β3—participate in the regulation of proliferation, differentiation, and survival of different cell types (Kitisin et al., 2007). TGF-β1 has been shown to exert pro-neurogenic effects both in the dentate gyrus of the adult hippocampus in an *in vivo* model of adrenalectomy characterized by enhanced neurogenesis (Battista, Ferrari, Gage, & Pitossi, 2006) and in the SVZ of adult rats injected with adenoviral vectors expressing TGF-β (Mathieu, Piantanida, & Pitossi, 2010). In this field, we have also demonstrated the involvement of the Smad2/3 pathway in TGF-β1 pro-neurogenic effects in cultured hippocampal NPCs (Graciarena, Roca, Mathieu, Depino, & Pitossi, 2013). Furthermore, we have recently shown TGF-β1 pro-neurogenic effects on NPCs in the SVZ and, most important, Fibulin-2 mediation (Radice et al., 2015). These results add up to the growing evidence supporting a pro-neurogenic role of TGF-β1 in the adult brain.

TGF-β signaling is initiated by the binding of the ligand to its serine and threonine kinase receptors on the cell membrane, that is type I (TβRI) and type II (TβRII) receptors. Ligand binding results in the formation of a receptor heterocomplex in which TβRII phosphorylates the threonine and serine residues of TβRI and thus activates it (Massague & Chen, 2000; Wrighton, Lin, & Feng, 2009). The activated TβRI recruits and phosphorylates R-Smad proteins, Smad2/3 for TGF-β, which then makes up a heterocomplex with Smad4. The Smad complexes then translocate to the nucleus to regulate target gene transcription.

Another signaling pathway involved in cell fate commitment, Notch is known to play a key role in controlling the proliferation and differentiation of NPCs. In particular, in demyelinating pathological conditions, previous work by our group using lysolecithin-induced focal demyelination in rats proved Jagged1-mediated Notch activation to trigger Hes5 expression in the SVZ, concomitant with an expansion in OPC population. During subsequent apotransferrin-induced remyelination, Notch activation seemed to be mediated by the expression of F3/contactin, inducing apotransferrin-mediated OL maturation (Aparicio et al., 2013).

Notch family is constituted by transmembrane receptors Notch1, 2, 3, and 4, which are activated by the binding of ligands Delta1, 3, and 4 and Jagged1 and 2 (Dave et al., 2011). Ligand binding induces a first cleavage, by ADAM metalloprotease, which then generates a membrane-tethered intermediate acting as a substrate for the γ-secretase complex which, in turn, releases the Notch intracellular domain (NICD) into the cytoplasm. NICD then translocates to the nucleus, where it activates the transcription of Notch target genes such as the basic helix loop helix (bHLH) transcriptional repressors known as Hairy/Enhancer of Split (Hes) genes (Kopan & Ilagan, 2009).

Both TGF-β and Notch appear to be implicated in the myelin repair process associated to demyelinating disorders such as Multiple Sclerosis (MS). The presence of reactive Jagged1-expressing astrocytes in response to the upregulation of TGF-β appears to be one of the reasons for remyelination failure. In particular, the presence of Notch1 and its effector, Hes5, in OPCs of MS lesions suggests that Jagged-activated Notch signaling might inhibit OPC maturation (John et al., 2002). Along the same line, TGF-β1 has been proven to induce the expression of Jagged1 in human astrocyte cultures (Zhang et al., 2010). Finally, Notch and TGF-β signaling have been shown to act synergistically in co-cultures of mesenchymal stromal cells and NPCs, inducing NPC differentiation into astrocytes and OLs (Robinson, Foraker, Ylostalo, & Prockop, 2011).

In this context, and with a view to unveiling possible NPC response mechanisms upon demyelination, the present work characterized cell populations arising from adult brain SVZ cultures and, most importantly, assessed changes induced by TGF-β1 (TGF-β) and the underlying interplay between TGF-β and Notch signaling.

2 | MATERIALS AND METHODS

2.1 | Animals

Adult Wistar rats (5–6 weeks old) were housed in a temperature—(22–23°C) and photoperiod—(12-hr light/dark) controlled room and provided food and water *ad libitum*. All animal procedures were performed according to the regulations of the National Institutes of Health, USA. Experimental protocols were approved before implementation by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) at Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

2.2 | NPC isolation and culture

Adult rat brain SVZ were dissected and submitted to mechanical disruption. Cells were thoroughly washed in DMEM/F12 medium, placed in 10-cm dishes and cultured in DMEM/F12 medium (Gibco-ThermoFisher, Grand Island, NY), containing 2% B27-supplement (Gibco-ThermoFisher), 20 ng/ml human basic fibroblast growth factor-2 (b-FGF, PeproTech, Rocky Hill, NJ), 20 ng/ml rat epidermal growth factor (EGF; PeproTech), 4 g/l anhydrous glucose and 1% penicillin/streptomycin (proliferation medium), and incubated in a humidified chamber at 37°C in 5% CO_2/20% O_2. Factors were added every 48 hr and, after 7 days in culture, neurospheres were plated in 10-cm dishes (for RNA extraction or Western blot analysis) or glass coverslips (for immunocytochemical assays) pre-coated with 50 μg/ml of poly-ornithine. In all cases, neurospheres were kept in proliferation medium for 48 hr after plating, after which the medium was replaced with DMEM/F12 containing 2% B27-supplement, 5 ng/ml b-FGF, 5 ng/ml EGF, 4 g/l anhydrous glucose and 1% penicillin/streptomycin (differentiation medium) supplemented according to each experimental design. For all experimental designs, fields were selected at random and cells away from the neurospheres were counted and expressed as a percentage of total cell count. A total of at least 300 cells were counted for each cell marker analyzed in each one of three independent experiments performed in triplicate.

2.3 | Cell proliferation

To study changes in cell proliferation rates, mouse anti-Ki67 (1:500, BD Pharmingen, San José, CA) was used to stain proliferating cells following the procedure described for immunocytochemical studies.

Proliferation rates were further assessed through 5-bromo-2′-deoxyuridine (BrdU) experiments. After 7 days in culture, neurospheres were plated for 48 hr in proliferation media, after which cells were pulse-labeled by BrdU (10 μM) in control or TGF-β-containing media. After a 90-min pulse, media were replaced by control or TGF-β-containing media for an additional 24 hr. Cells were then washed with phosphate buffer saline (PBS) and fixed in a solution of paraformaldehyde 4% w/v in PBS. They were then treated with 2 M HCl in PBS containing 1% Triton X-100 for 15 min at room temperature. After blockade with a solution of PBS containing 1% donkey serum (Sigma-Aldrich) and 0.1% Triton X-100, cells were treated with a monoclonal anti-BrdU antibody (1:500) and then with a Cy2-conjugated donkey anti-mouse secondary antibody (1:1000). In addition, NPC marker Nestin, astrocyte-like NPC marker glial fibrillary acidic protein (GFAP) and OPC marker platelet-derived growth factor receptor α (PDGFRα) were used to characterize proliferating cells. Taking 300 cells for each experimental condition, BrdU+ cells and Ki67+ cells were analyzed by fluorescence microscopy using ImageJ software. Three independent experiments were performed in triplicate.

2.4 | Cell differentiation and inhibition experiments

Cells were plated and submitted to the different treatments 48 hr later according to the experimental design.

- For short term experiments, media were replaced every 48 hr with differentiation medium containing either vehicle or 20 ng/ml TGF-β1. This procedure was repeated for 4 days and rendered two experimental conditions (control and +TGF-β).
- For long term experiments, media were replaced every 48 hr and rendered the following three experimental conditions: (a) cells were kept in differentiation medium for 8 days; (b) cells were kept in differentiation medium containing 20 ng/ml TGF-β1 for the first 4 days and in differentiation medium without TGF-β1 for another 4 days; and (c) cells were kept in differentiation medium containing 20 ng/ml TGF-β1 for 8 days.

Treatment time points were chosen on the basis of previous work (Battista et al., 2006; Radice et al., 2015).

In addition, TGF-β signaling inhibition experiments were performed by treating cultures with TβRII inhibitor SB431542 (Sigma-Aldrich, St. Louis, MO). After 48-hr cell plating, cultures were pretreated with dimethyl sulfoxide (DMSO), 5 μM SB431542 or 10 μM SB431542 (control + DMSO, control + 5SB, and control + 10SB, respectively) for 90 min, and then kept in control media or added 20 ng/ml TGF-β1 (+TGF-β + DMSO, +TGF-β + 5SB, and +TGF-β + 10SB, respectively) for 24 hr. Cells were then fixed for immunocytochemical analyses or harvested for Western blot analyses.

Furthermore, Notch signaling inhibition experiments were carried out using γ-secretase inhibitor N-[N-(3,5-Difluorophenalcetyl-L-alanyl)]-S-phenylglycine-t-butyl ester (DAPT, Calbiochem, San Diego, CA). After 48-hr cell plating, cells were pre-incubated for 1 hr with either DMSO or 10 μM DAPT (control + DMSO and control + DAPT, respectively) and media were then changed to control or +TGF-β media (+TGF-β + DMSO and +TGF-β + DAPT, respectively) for 24 hr. Notch inhibition was also evaluated in long term experiments. Cells were then fixed for immunocytochemical analyses.

2.5 | Immunocytochemistry

After different experimental protocols, cells were fixed with 4% paraformaldehyde in PBS for 20 min and then washed twice with PBS. Nonspecific binding was blocked by 30-min incubation at room temperature in PBS containing 1% donkey serum (Sigma-Aldrich) and 0.1% Triton X-100. Coverslips were subsequently incubated for 2 hr at room temperature with the following primary antibodies diluted in blocking solution: rabbit anti-GFAP (1:1000, Dako, Carpinteria, CA) and mouse anti-Nestin (1:1000, BD Pharmingen) to stain astrocyte or type B NPCs; rabbit anti-β III tubulin (TujI, 1:500, Sigma-Aldrich) or mouse anti-TujI (1:1000, Promega, Madison, WI) to stain early differentiated neurons, goat anti-PDGFRα (1:500, Neuromics, Edina, MN) and rabbit anti-NG2 (1:1000, Millipore, Billerica, MA) to stain OPCs, rabbit anti-Olig2 (1:1000, Millipore) to stain the oligodendroglial progeny, mouse anti-myelin basic protein (MBP, 1:750, BioLegend, San Diego, CA) to stain mature OLs, anti-TβRII (1:500, Santa Cruz, Dallas, TX) to detect cells expressing this receptor and goat anti-Jagged1 (1:500, Santa Cruz) to evaluate Notch signaling. After PBS washes, coverslips were incubated with fluorescent dye-conjugated secondary antibodies diluted in PBS for 2 hr at room temperature. Secondary antibodies used were Cy2- or Cy3-conjugated donkey anti-rabbit, Cy3-conjugated donkey anti-goat (1:1000, Jackson Immunoresearch Laboratories Inc, West Grove, PA) and Cy2-conjugated donkey anti-mouse in combination with 1 μg/ml Hoechst (Sigma-Aldrich). Once incubations were finished, coverslips were washed twice in PBS and mounted onto glass slides with a mounting medium. Microscopic observations were done using an Olympus BX50 microscope and digital images were taken with a CoolSnap digital camera. Marker-positive cells were counted using ImageJ (Media Cybernetics, Silver Spring, MD) and results were expressed as the percentage of positive cells over total cell number. A total of at least 300 cells were counted for each cell marker analyzed in each one of three independent experiments performed in triplicate. To study the colocalization between TβRII and Nestin and GFAP or PDGFRα, cells were examined by laser confocal microscopy using an Olympus FV 1000 microscope (Olympus, Japan).

2.6 | OL morphological differentiation

Mature OL diameter and process complexity, from TGF-β-treated and control cultures, were evaluated using Sholl analysis. Briefly, principal OL processes were initially outlined using NeuronJ plugin for ImageJ

(National Institutes of Health, Bethesda, MD) to exclude adjacent cells and secondary processes. Next, a minimum of 30 MBP+ cells were analyzed in terms of branching process number and length by counting the number of process intersections with each one of the concentric circles with increasing radius superimposed on OL cell bodies (starting radius, 10 μm; step size, 10 μm; end, 180 μm) using Sholl analysis plugin for ImageJ.

2.7 | Western blot

TGF-β signaling inhibition experiments results were further corroborated by evaluating the level of Notch ligand Jagged1 by Western blot. After 24-hr culture in the presence or absence of TGF-β, and in the presence or absence of TβRII inhibitor, cells were harvested and washed twice with PBS and the cellular pellet was then resuspended in buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 0.1% Triton X-100 (v/v), 1% NP40 (v/v), 2 mM ethylenediaminetetraacetic acid, 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid and protease inhibitors (Roche Cocktail, Hoffmann-La Roche, Basilea, Switzerland). Samples containing 20 μg protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (7.5% acrylamide; Laemmli, 1970) and blotted onto polyvinylidene difluoride membranes. After 45-min blockade in 5% (w/v) non-fat milk in tris-buffered saline containing 0.1% Tween 20, membranes were probed overnight at 4°C with anti-Jagged1 (1:200) or anti-β tubulin (1:1000) antibodies. Membranes were subsequently incubated for 2 hr at room temperature with the corresponding secondary horseradish peroxidase-conjugated antibody (1:10000). Bands were visualized by enhanced chemiluminescence (ECL plus, Amersham Biosciences, Piscataway, NJ) using Image Quant LAS500 equipment (GE Healthcare, Piscataway, NJ) and quantified from three independent experiments using ImageJ software (National Institutes of Health).

2.8 | Real-time polymerase chain reaction

After differentiation protocols, cells were lysed and total RNA was isolated by means of an RNAqueous-Micro Kit (Ambion, Foster City, CA) according to the manufacturer's instructions. Samples were used for reverse transcription with random hexamer primers, using a High-Capacity cDNA Reverse Transcription Kit and a TaqMan RT Kit. Real-time polymerase chain reaction (RT-PCR) was performed on an Applied Biosystems 7500 RT-PCR System as follows: 5 min at 95°C and then 40 cycles of 4 sec at 95°C, 1 min at 67°C, and 1 min at 72°C. Specificity of the amplification product was confirmed by examination of dissociation reaction plots. A distinct single peak indicated that a single DNA sequence was amplified during PCR. Each sample was tested in triplicate and samples obtained from three independent experiments were used to determine the relative expression levels of each transcript by employing the comparative C_T method ($\Delta\Delta C_T$ method). Normalized to endogenous reference β2-microglobulin and relative to a control, the amount of target is given by $2^{-\Delta\Delta CT}$ (RQ). The primers used for RT-PCR were:

Gen	Forward primer	Reverse primer
β2-migroglobulin	**TCTTTCTGGTGCTTG TCTC**	**AGTGTGAGCCAG GATGTAG**
Jagged1	GACGACTG TAACACCTGCCA	AGTGGTGAGACCT GGAGACA
Hes1	**ACACCGGACAAA CCAAAGAC**	**ATGCCGGGAGCT ATCTTTCT**
Hes5	**TCCAGAGCTC CAGGCATGG**	**CCGCAGTCGATT TTTCTCCTT**
MAG	TCCTGGCCACGGTCAT CTA	CACACCAGTACTCC CCATCGT
Ascl1	**TCCGGTTTCGTCCTA CTCCT**	**CTGCCATCCT GCTTCCAAAG**

2.9 | Statistical analysis

Results were expressed as the mean ± *SEM* and statistical differences among treatments were determined using Student's *t* test and one- or two-factor analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test.

3 | RESULTS

3.1 | Culture characterization

Adult brain SVZ cells grown as neurospheres can differentiate into both neurons and OLs *in vitro*, although this differentiation pattern may be different in the presence of growth factors. To characterize cell populations originating from SVZ NPCs *in vitro* in proliferation media, adult brain SVZ neurospheres were plated in coated coverslips and cultured for 48 hr until fixation for cell phenotype characterization. In this condition, cell characterization revealed Nestin+ (67% ± 5%), GFAP+ (70% ± 3%), Nestin+/GFAP+ (64% ± 4%), Olig2+ (83% ± 4%), PDGFRα+ (10% ± 10%), NG2+ (8% ± 4%), PDGFRα+/NG2+ (9% ± 8%), and TujI+ (1% ± 0%) cells. Nestin+/GFAP+ cells probably reflect the abundance of adult brain SVZ type B progenitor cells. In turn, the values observed for PDGFRα+ or NG2+ cells may respond to the presence of OPCs, while the even lower proportion of TujI+ cells reflects a small degree of neurogenesis in these culture conditions. It should be pointed out that the proportion of Olig2+ cells was remarkably higher than that of PDGFRα+/NG2+ cells (Figure 1).

3.2 | TGF-β effects on culture phenotype

With the aim of unveiling TGF-β effects on adult brain SVZ neurosphere cultures, cells expressing cytokine receptor TβRII were first analyzed through immunocytochemical studies using markers Nestin, GFAP, and PDGFRα. Results revealed TβRII colocalization with all GFAP+, Nestin+, and PDGFRα+ cells (Figure 2a) and no changes in TβRII expression pattern upon TGF-β addition to the culture media (Supporting Information Figure 1).

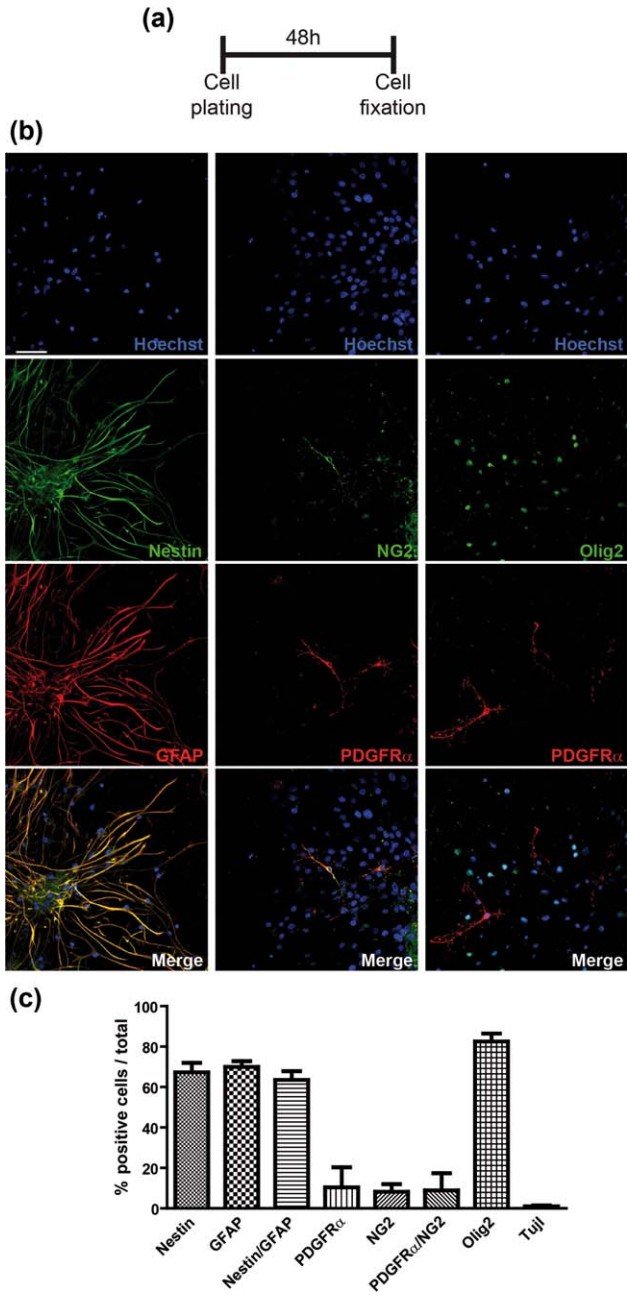

FIGURE 1 Neurosphere culture characterization. (a) Experimental timeline. (b) Fluorescence microscopy images of immunocytochemistry for Nestin, GFAP, NG2, PDGFRα, Olig2, and the corresponding merges. Scale bar, 50 μm. (c) Quantitation of relative percentages. Results are expressed as the percentage of positively stained cells over total cells visualized with Hoechst. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate [Color figure can be viewed at wileyonlinelibrary.com]

In addition, cell subpopulations identified in culture characterization assays were analyzed for changes induced by TGF-β. Four-day cultures in the presence of TGF-β exhibited a significant increase in the proportion of GFAP+ (46%) and, most important, in the proportion of PDGFRα+ (153%), NG2+ (207%), and PDGFRα+/NG2+ cells (300%) regarding controls (Figure 2c,d). However, no changes were observed in the percentage of TujI+ (data not shown), Nestin+ or Nestin+/GFAP+ cells (Figure 2d). To further establish whether the changes induced by TGF-β affect NPC fate decisions, cell proliferation, or both, proliferation rates were evaluated through Ki67 expression and BrdU incorporation assays in the total cell population and in each cell type. Ki67 expression assays rendered a significant increase in total cell proliferation after 24-hr culture (200%) and a significant increase in the proportion of PDGFRα+/Ki67+ cells (160%; Figure 3c,e,f). Accordingly, BrdU incorporation assays also revealed a significant increase in total cell proliferation (105%) and a significant increase in the proportion of PDGFRα+/BrdU+ cells (402%) 24 hr after TGF-β stimulus (Figure 3d,g,h). Furthermore, no significant differences were observed between the proportions of Ki67+ and BrdU+ cells either in control or TGF-β-treated cells (Figure 3i).

On the basis of these results showing an expanded OPC subpopulation, long term experiments were carried out to establish the effect of TGF-β treatment on OPC maturation using mature oligodendroglial marker MBP. To such end, changes in the proportion of cell populations present in the culture, that is Nestin+, GFAP+, Nestin+/GFAP+, PDGFRα+ and MBP+ cells, were analyzed in experimental conditions (a), (b), and (c), described in Section 2 (Figure 4a).

Interestingly, 8-day cultures in the presence of TGF-β exhibited no changes in the percentages of Nestin+/GFAP+ cells. However, the presence of TGF-β either during 4 or 8 days induced a significant decrease in the percentage of Nestin+ cells regarding controls (52% and 48% for conditions (b) and (c), respectively), probably at the expense of an increase in PDGFRα+ (65% and 150% for conditions (b) and (c), respectively) and GFAP+ cells (33% and 26% for conditions (b) and (c), respectively). These findings may be thought to hint at a progliogenic effect of TGF-β on type C Nestin+ cells present in this adult SVZ neurosphere cultures (Figure 4b).

In addition, and most interestingly, a significant increase was observed in the proportion of MBP+ (115%) after 8 days' culture in the presence of TGF-β. This increase may respond to the increase previously detected in the proportion of OPCs or a direct effect of TGF-β upon OL differentiation. In order to further analyze the possible effects of TGF-β on OPC maturation, cell morphological complexity was evaluated in the experimental conditions described above using Sholl analysis. Although control cells showed a larger number of intersections at low radius values, TGF-β-treated cells in both (b) and (c) conditions exhibited a significantly higher number of intersections at radii raging between 40 μm and 90 μm (Figure 5), which reveals more and longer processes and, hence, higher mature OL morphological complexity.

3.3 | Notch involvement in TGF-β effects

Given the relationship between TGF-β and Notch ligand Jagged1, the proportion of cells expressing Jagged1 was immunocytochemically evaluated after 4-day culture, with results showing a significantly higher proportion in TGF-β-treated cultures as compared with control conditions (95%; Figure 6b,c). Worth highlighting, Jagged1 was exclusively expressed by GFAP+ cells. Additional support was obtained through Western blot studies, which also rendered significantly higher

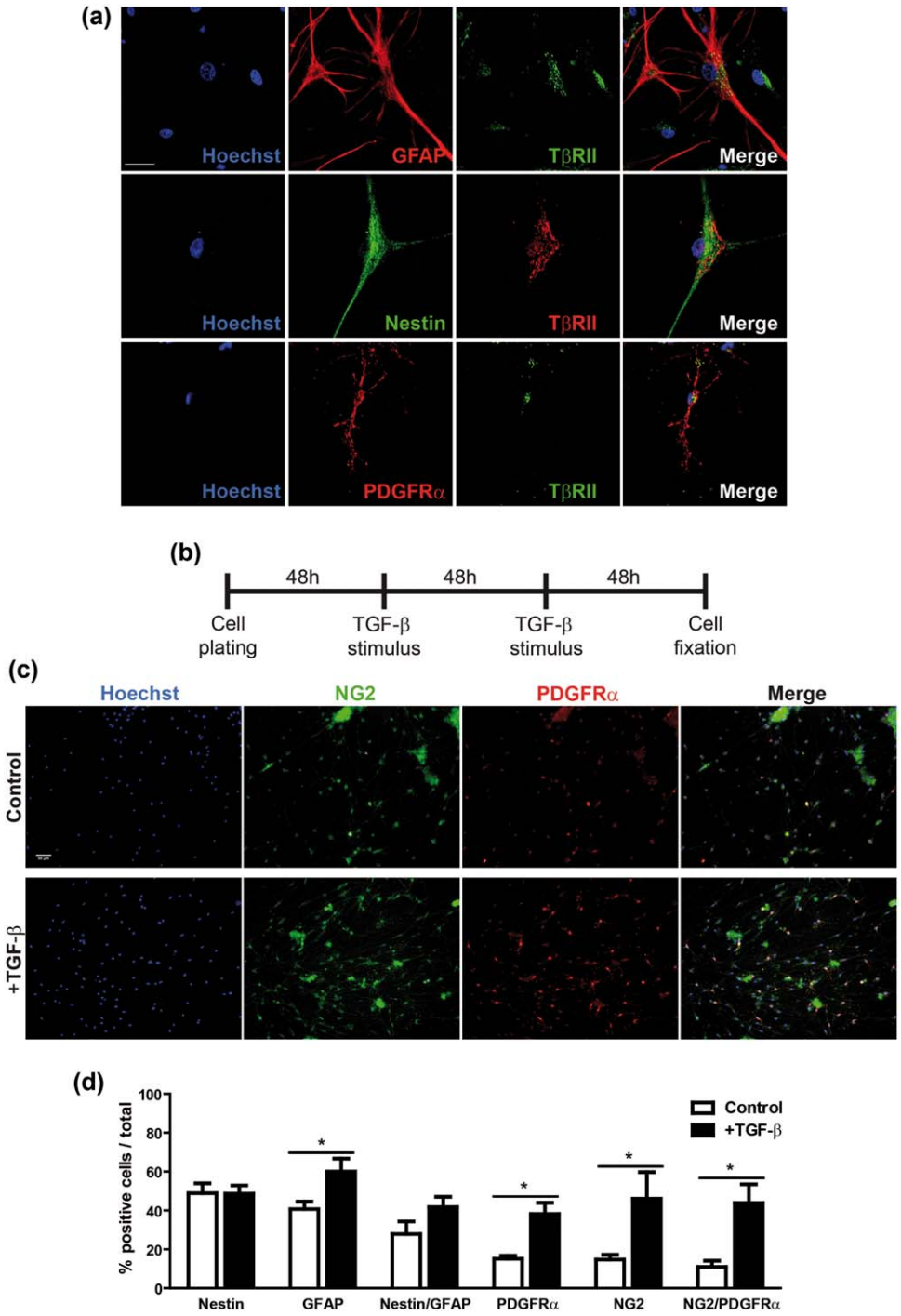

FIGURE 2 TβRII expression and TGF-β effects on cell populations in short term cultures. (a) Confocal microscopy images of immunocytochemistry for TβRII colocalization with Nestin, GFAP, and PDGFRα. Scale bar, 20 μm. (b) Experimental timeline. (c) Fluorescence microscopy images of immunocytochemistry for PDGFRα, NG2, and merge in control and TGF-β-treated cultures. Scale bar, 50 μm. (d) Quantitation of relative percentages. Results are expressed as the percentage of positively stained cells over total cells visualized with Hoechst. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate (two-way ANOVA, Bonferroni's post-test) $^{*}p < .05$ [Color figure can be viewed at wileyonlinelibrary.com]

Jagged1 levels in TGF-β-treated cultures (Figure 6d). To further corroborate the interaction between TGF-β and Notch signaling, ligand Jagged1 transcript levels were evaluated by RT-PCR, together with Notch downstream signaling genes potentially involved in OPC proliferation (Hes1 and Hes5) and maturation (MAG) and a gene involved in neuronal commitment (Ascl1). In agreement with findings reported above, TGF-β-treated cultures exhibited a 50% increase in Jagged1 transcript levels after 6-hr culture (Figure 6e), which is also in line with the 1-fold increase observed in Hes1 expression after 6-hr culture and the 2-fold increase detected after 24-hr culture (Figure 7a), without changes in Hes5 expression (Figure 7b). As expected, MAG gene expression showed no changes either after 6- or 24-hr culture (Figure

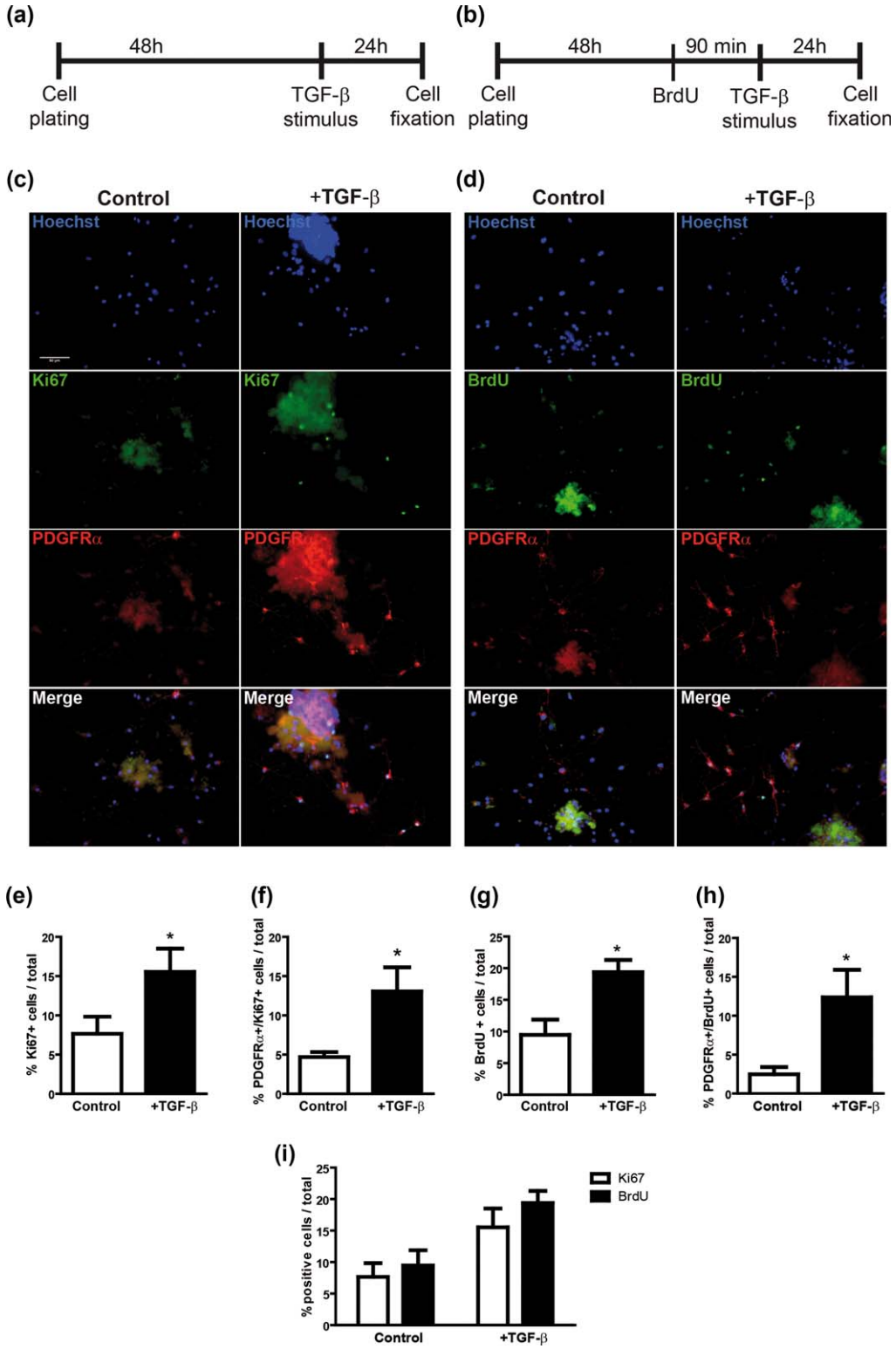

FIGURE 3 OPC proliferation after TGF-β treatment. (a,b) Experimental timelines. (c) Fluorescence microscopy images of immunocytochemistry for PDGFRα and Ki67 in control and TGF-β-treated cultures. (d) Fluorescence microscopy images of immunocytochemistry for PDGFRα and BrdU in control and TGF-β-treated cultures. Scale bar, 50 μm. (e–h) Quantitation of relative percentages. Results are expressed as the percentage of Ki67, PDGFRα/Ki67, BrdU, or PDGFRα/BrdU positive cells over total cells visualized with Hoechst. (i) Quantitation of relative percentages. Results are expressed as the proportion of Ki67+ and BrdU+ cells over total cells visualized with Hoechst. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate (Student's *t* test for independent samples) $^{*}p < .05$ [Color figure can be viewed at wileyonlinelibrary.com]

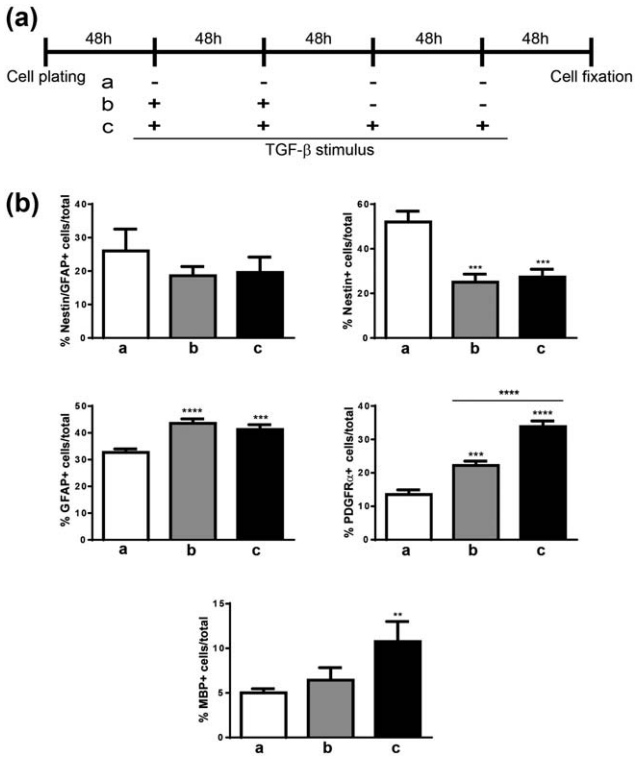

FIGURE 4 TGF-β effects on cell populations in long term cultures. (a) Experimental timeline. (b) Quantitation of relative percentages of immunocytochemistry for different cell markers in experimental conditions (**a**), (**b**), and (**c**). Results are expressed as the percentage of positively stained cells for Nestin/GFAP, Nestin, GFAP, PDGFRα, and MBP over total cells visualized with Hoechst. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate (one-way ANOVA, Bonferroni's post-test) $^{**}p < .01$, $^{***}p < .001$, $^{****}p < .0001$

7c), which reflects the absence of mature OLs at early culture time points. Finally, no changes were observed in Ascl1 levels, in parallel with the unaltered proportion of Tuj1+ cells reported above (Figure 7d).

On the basis of these findings and evidence of TGF-β pro-oligodendrogenic effects, γ-secretase inhibitor DAPT was used in differentiation assays in order to corroborate the involvement of Notch signaling. Notch pathway inhibition produced a significant decrease in the percentage of PDGFRα+ cells as compared with DMSO in TGF-β-treated cultures (78%) but no changes in control cultures. In agreement with these results, TGF-β-treated cultures exhibited a significant reduction in PDGFRα+/Ki67+ cells in the presence of Notch signaling inhibitor as compared to DMSO (85%), but no variation in total Ki67+ cells (Figure 8b).

In the same way, TGF-β pathway inhibition experiments using TβRII inhibitor SB431542 were conducted to verify that the changes observed in PDGFRα+ cell proliferation were unequivocally induced by TGF-β through Jagged1-driven Notch activation. Western blot analyses and immunocytochemical assays showed a decrease in Jagged1 levels and in the percentage of Jagged1+ cells at both inhibitor concentrations, which proves the effective inhibition of TGF-β signaling (Figure 9b,c). In addition, immunocytochemical assays showed a significant decrease in the percentage of PDGFRα+ cells in TGF-β-treated cultures at both inhibitor concentrations (9% ± 2% with 5 and 10 μM versus 18% ± 2% with DMSO), which corroborates Notch mediation in the pro-oligodendrogenic effect of TGF-β on adult brain SVZ neurosphere cultures. Worth pointing out, no changes were observed in the percentage of PDGFRα+ cells in control cultures at either inhibitor concentration (10% ± 3% with 5 μM and 9% ± 2% with 10 μM) or with DMSO (9 ± 2; Figure 9d). Finally, SB431542 produced no significant changes in the proportion of cells expressing TβRII in control or TGF-β-treated cultures (Supporting Information Figure 2).

As regards other subpopulations identified in culture characterization assays, changes induced by Notch signaling inhibition were also evaluated in Nestin+/GFAP+, Nestin+/GFAP– and Nestin–/GFAP+ cells in TGF-β-treated and control cultures. No significant changes were observed upon Notch inhibition in cell populations cultured in control conditions. In contrast, in TGF-β-treated cultures, Notch inhibition produced a significant increase in the percentage of Nestin+/GFAP+ cells (58%), no changes in Nestin+/GFAP– cells and a significant decrease in the percentage of Nestin–/GFAP+ cells (73%; Figure 10b). In the same way, analyses of Notch inhibition in long term experiments rendered a significant decrease in both PDGFRα+ (51%) and MBP+ (67%) cells in +TGF-β+DAPT cultures regarding +TGF-β+DMSO ones (Figure 10c,d).

Finally, to elucidate the contrasting effects of TGF-β, that is inducing both OPC proliferation and OL maturation, analyses were conducted on cell type distribution in the neurosphere cultures. Results revealed clear interaction between neighboring PDGFRα+ and Jagged1-expressing GFAP+ cells after 48-hr TGF-β treatment (Figure 11a). Most interestingly, and probably as a consequence of cell migration (Supporting Information Figure 3), PDGFRα+ cells appeared to be detached from Jagged1-expressing GFAP+ cells after 8-day treatment (Figure 11b,c).

These results suggest that OPC initial proliferation may take place upon Notch activation Jagged1-mediated effect of TGF-β, while later maturation may be the consequence of a direct effect of TGF-β on its own receptor TβRII.

4 | DISCUSSION

One of the most frequent demyelinating neuropathologies affecting young adults, MS is characterized by central nervous system (CNS) demyelination as a consequence of persistent inflammation in the brain and spinal cord. The development of therapeutic strategies continues to prove a challenging task and requires thorough understanding of the molecular regulation of OLs, the myelin forming cells in the CNS. The adult brain SVZ has been shown to harbor neurons, astrocytes and OLs (Menn et al., 2006), which respond to different pathological insults such as trauma, ischemia, neurodegeneration, demyelination, and inflammation (Nait-Oumesmar, Picard-Riera, Kerninon, & Baron-Van Evercooren, 2008). In addition, growth factors are known to play an important role in OL development and maintenance and are involved in

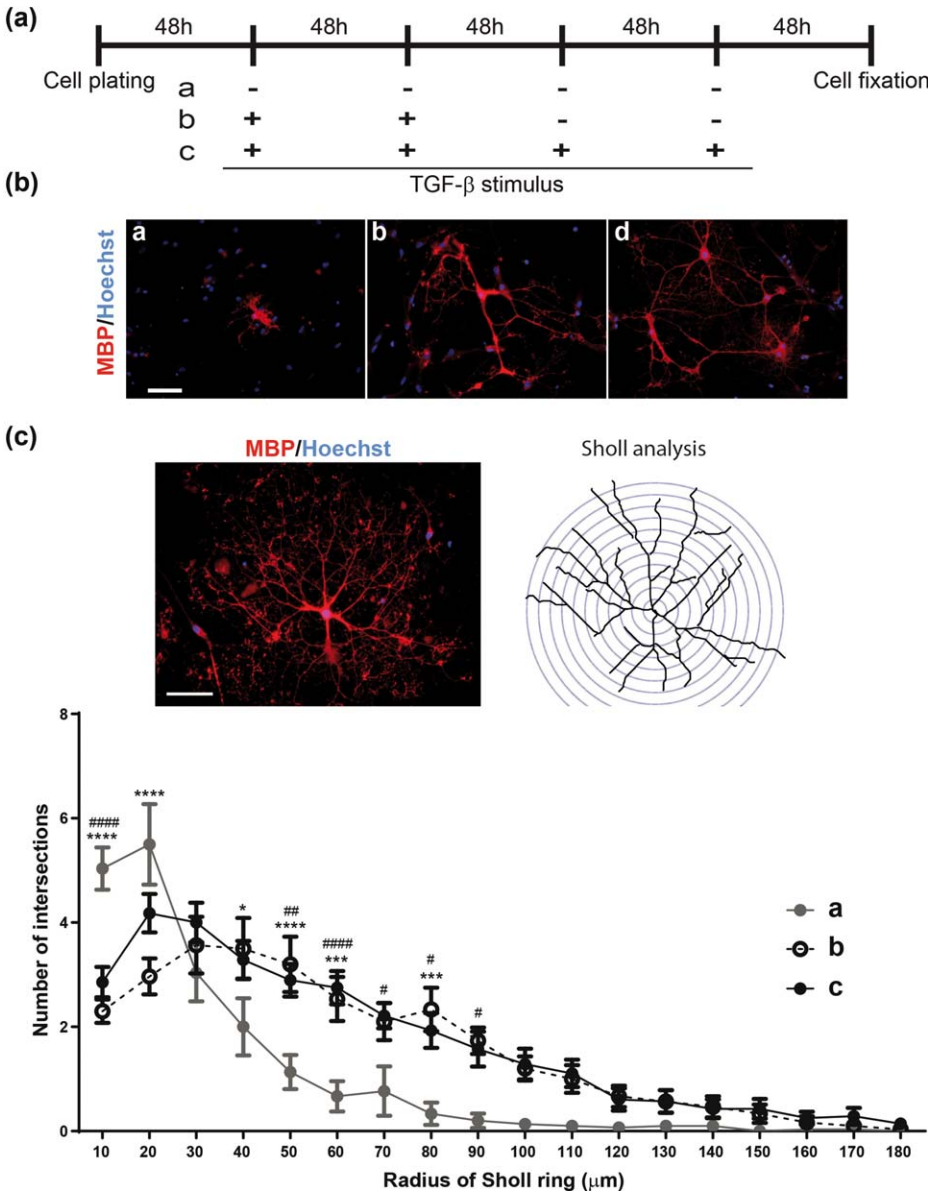

FIGURE 5 OL morphological complexity after TGF-β treatment in long term cultures. (a) Experimental timeline. (b) Fluorescence microscopy images of immunocytochemistry for MBP in experimental conditions (**a**), (**b**), and (**c**). Scale bar, 20 μm. (c) Representative image of condition (**c**) and processes outlined with superimposed Sholl rings. Scale bar, 20 μm. Quantitation of Sholl ring-OL process intersection as a function of Sholl ring radius in experimental conditions (**a**), (**b**), and (**c**). Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate (two-way ANOVA, Bonferroni's post-test) *(**a**) versus (**b**); #(**a**) versus (**c**); $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, $^{****}p < .0001$, $\#p < .05$, $\#\#p < .01$, $\#\#\#p < .001$, $\#\#\#\#p < .0001$ [Color figure can be viewed at wileyonlinelibrary.com]

the modulation of glial responses in various pathological scenarios. In particular, previous work has proven TGF-β to be upregulated mainly during maximal demyelination together with HGF, FGF-2, LIF, and IGF-I (Lassmann, Bruck, & Lucchinetti, 2007).

In this context, and considering the repair potential of multipotent NPCs in the adult SVZ, capable of migrating and differentiating into the three major CNS cell types, this work investigated the pro-oligodendrogenic effects of TGF-β on adult brain SVZ neurosphere cultures and the possible participation of Jagged1-Notch activation in these effects.

First, the characterization of cell populations originating from adult brain SVZ NPCs cultured in the presence of EGF and FGF revealed high proportions of Nestin+, GFAP+, and Nestin+/GFAP+ cells. Adult brain SVZ type B Nestin+ and GFAP+ cells can generate neurons and young migrating NG2+ OPCs, which in turn proliferate locally or mature into OLs with myelinating capacity (Menn et al., 2006). However, the predominance observed of Olig2+ cells in our studies is in agreement with results obtained by Hack, Sugimori, Lundberg, Nakafuku, & Gotz (2004) showing that Olig2 is required for the generation of neurospheres and may essentially mediate EGF and FGF signaling (Hack, Sugimori, Lundberg, Nakafuku, & Gotz, 2004). Interestingly, 10% of cells were found to express NG2 proteoglycan and PDGFRα, markers of OPC population, while <1% were

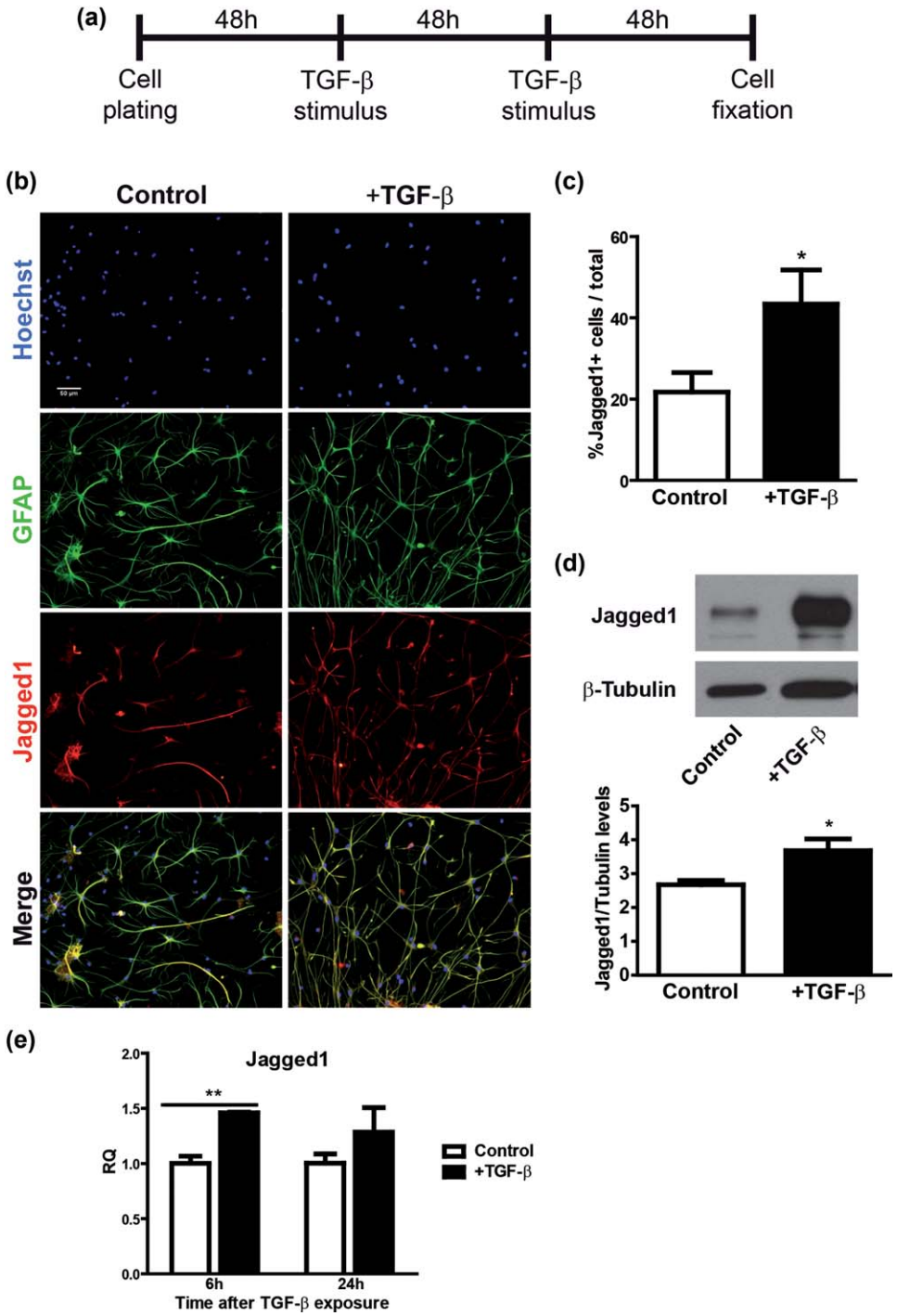

FIGURE 6 Notch ligand Jagged1 expression after TGF-β treatment. (a) Experimental timeline. (b) Fluorescence microscopy images of immunocytochemistry for Jagged1, GFAP, and merge in control and TGF-β-treated cultures. Scale bar, 50 μm. (c) Quantitation of relative percentages. Results are expressed as the percentage Jagged1+ cells over total cells visualized with Hoechst. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate (Student's *t* test for independent samples) $^{*}p < .05$. (d) Western blot analyses and relative quantification of Jagged1 levels in control and TGF-β-treated cultures. Values are expressed as the mean ± *SEM* of three independent experiments (Student's *t* test for independent samples) $^{*}p < .05$. (e) Real-time PCR analyses of Jagged1 transcript levels in control and TGF-β-treated cultures during 6 and 24 hr. All results are expressed as RQ and TGF-β results are expressed as relative to control values. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate (Student's *t* test for independent samples) $^{*}p < .05$, $^{**}p < .01$ [Color figure can be viewed at wileyonlinelibrary.com]

found to be TujI+, which suggests that relatively more NPCs are committed to the oligodendroglial fate in these culture conditions.

In agreement with findings reported by Zhang et al. (2010) and Palazuelos, Klingener, & Aguirre (2014), our immunocytochemical results showing colocalization of TβRII with Nestin, GFAP, and PDGFRα

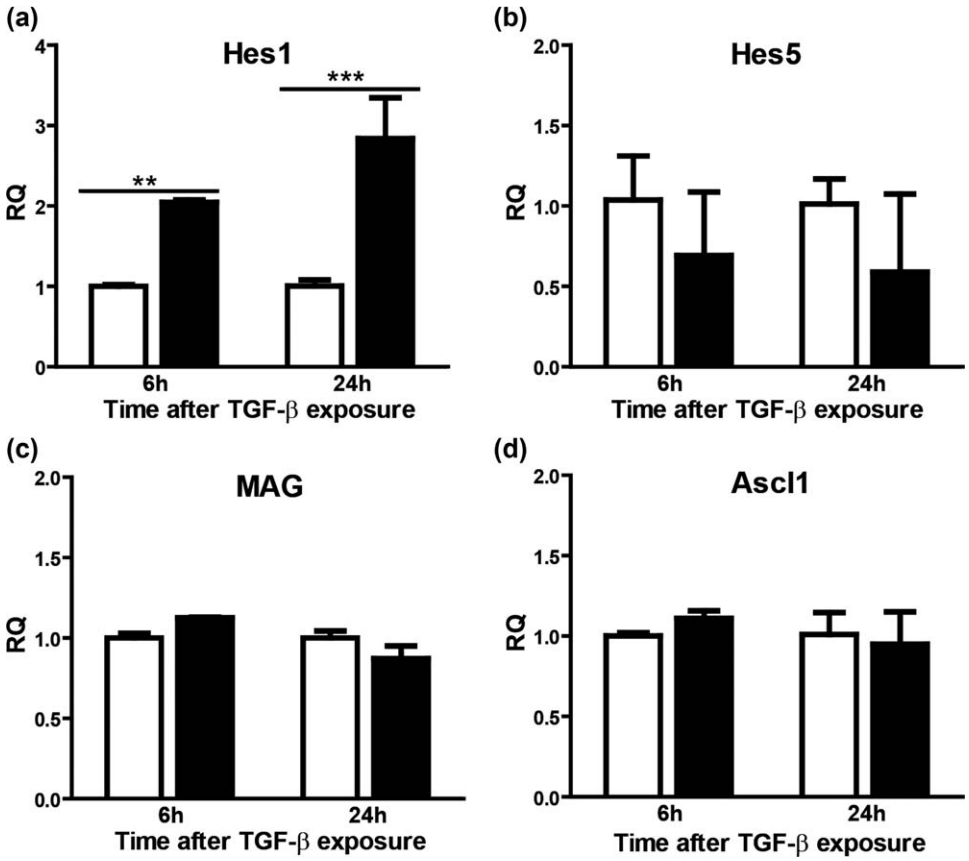

FIGURE 7 Notch signaling gene expression after TGF-β treatment. Real-time PCR analyses of (a) Hes1, (b) Hes5, (c) MAG, and (d) Ascl1 transcript levels in control and TGF-β-treated cultures during 6 and 24 hr. All results are expressed as RQ and TGF-β results are expressed as relative to control values. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate $^{**}p < .01$, $^{***}p < .001$

proved cytokine receptor to be expressed in NPCs, astrocytes and OPCs, respectively. The significant increase observed in GFAP+ and PDGFRα+/NG2+ cells in 4-day TGF-β-treated cultures may respond to an increase in total cell proliferation and, particularly, OPC proliferation, as evidenced by results obtained in Ki67 expression and BrdU incorporation assays. These most interesting results demonstrate that TGF-β induces the proliferation of OPCs generated in the adult SVZ culture. In contrast, the fact that TGF-β induced an increase in GFAP+ cells but no changes in Nestin+ or Nestin+/GFAP+ cells provides further evidence of NPC glial commitment upon TGF-β treatment. Furthermore, TGF-β did not produce changes in the population of TujI+ neuroblasts, which were nearly undetectable. Taken together, these data further suggest that TGF-β specifically targets glial cell commitment.

Remarkably, results obtained in 8-day cultures in the presence of TGF-β still showed OPC proliferation but also revealed OPC differentiation into mature OLs, as evidenced by an increase in the proportion of MBP+ cells. These findings may appear to contrast the notion that Jagged1-mediated Notch activation restricts OPC maturation. However, as different from studies conducted in co-cultures of primary astrocytes and OPCs (Zhang et al., 2010), our cultures containing Nestin+/GFAP+ type B cells may explain TGF-β effects on Jagged1-driven cell fate decisions, which render ongoing OPC expansion and still allow for some of these OPCs to differentiate into mature MBP+ OLs. Alternatively, as our studies use neurosphere-derived OPCs, the fact that OPCs migrate and lose contact with TGF-β-induced Jagged1 activation in GFAP+ cells may also explain OPC maturation in spite of Jagged1 well documented restrictive effects. Furthermore, a direct effect of TGF-β on OPC differentiation may be inferred from our results revealing higher mature OL morphological complexity. That is, TGF-β may act upon TβRII present in OPCs and hence activate Smad3/4 signaling, which in turn induces the expression of c-myc and p21, subsequent cell cycle withdrawal and final OPC maturation, recapitulating mechanisms involved in CNS myelination (Palazuelos et al., 2014). As a matter of fact, Jagged1/Notch-mediated and direct effects of TGF-β on OPCs derived from neurospheres appear to coexist and outweigh one another in a time- and space-dependent manner. In this sense, these adult brain SVZ neurosphere cultures may be thought to reproduce SVZ cell behavior *in vivo*.

In further establishing a relationship between TGF-β and Notch signaling, the addition of TGF-β to our neurosphere cultures induced a significant increase in the expression of Notch ligand Jagged1 exclusively by GFAP+ astrocytes, one of the cell subpopulations whose proportion increased upon TGF-β treatment. In addition, the increase detected in Notch effector Hes1 transcript levels is in agreement with

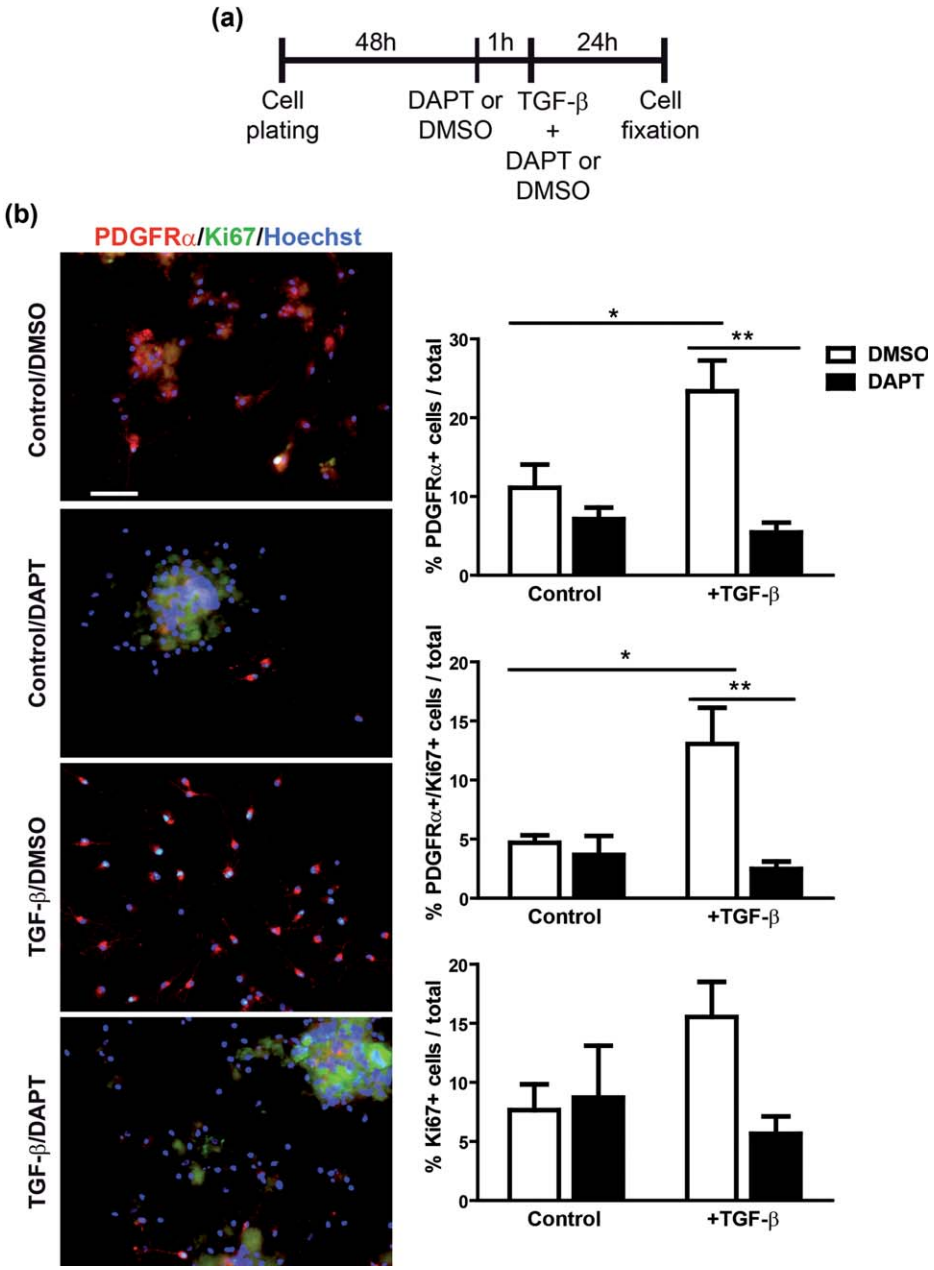

FIGURE 8 Notch signaling inhibition after TGF-β treatment in OPCs. (a) Experimental timeline. (b) Fluorescence microscopy images of immunocytochemistry for PDGFRα/Ki67 merge in control and TGF-β-treated cultures in the presence of DMSO or γ-secretase inhibitor DAPT. Scale bar, 20 μm. Quantitation of relative percentages. Results are expressed as the percentage of positively stained cells for PDGFRα, Ki67/PDGFRα, and Ki67 over total cells visualized with Hoechst in control and TGF-β-treated cultures in the presence of DMSO or γ-secretase inhibitor DAPT. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate (two-way ANOVA, Bonferroni's post-test) $^{*}p < .05$, $^{**}p < .01$ [Color figure can be viewed at wileyonlinelibrary.com]

the increase observed in Jagged1 transcript levels, Jagged1 protein levels and Jagged1+ cells. In turn, no changes were observed in MAG gene expression, which, as expected, reflects the absence of mature OLs at early culture time points. These results agree with findings mentioned above on TGF-β and Jagged1-Notch joint mediation of deficient OPC maturation in MS patients and their impact on OPC size and differentiation during remyelination (John et al., 2002; Zhang et al., 2010).

Moreover, Notch signaling inhibition reversed the effects exerted by TGF-β in cell subpopulations. That is, the fact that γ-secretase inhibitor DAPT produced a significant decrease in the population of PDGFRα+ OPCs in TGF-β-treated neurospheres—but no significant changes in control ones—is in line with the decrease observed in GFAP+ astrocytes. Also, the increase induced by Notch inhibition in the proportion of Nestin+/GFAP+ type B cells further supports a gliogenic effect of TGF-β on neurosphere cultures. Finally, and most interestingly, the comparable results obtained in TGF-β-treated cultures when inhibiting TβRII rather than Notch signaling are in agreement with findings reported by Zhang et al. (2010) in human astrocyte cultures and provide stronger evidence of interplay between TGF-β and

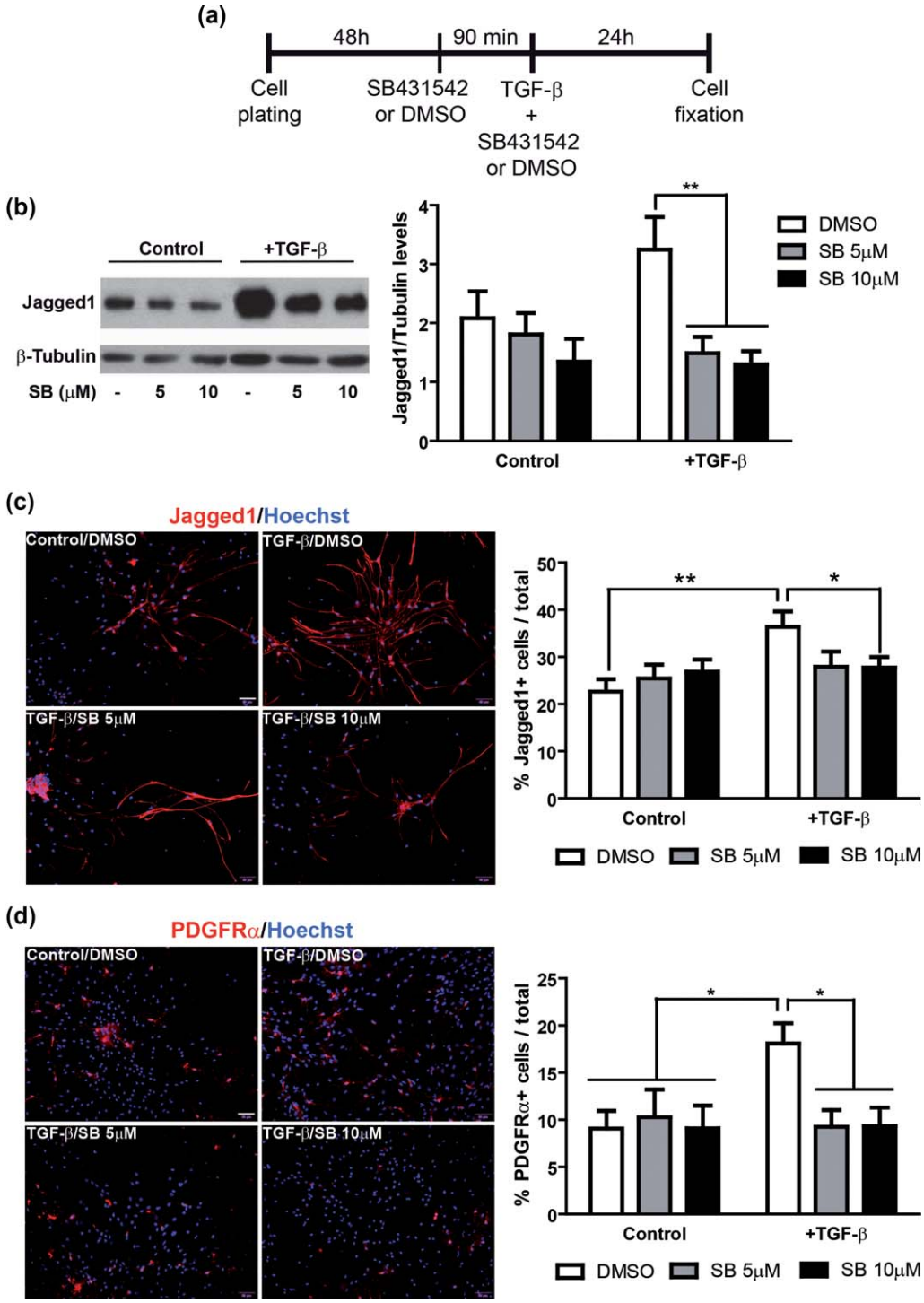

FIGURE 9 TβRII inhibition after TGF-β treatment. (a) Experimental timeline. (b) Western blot analyses and relative quantification of Jagged1 levels in control and TGF-β-treated cultures in the presence of DMSO or TβRII inhibitor SB431542. (c) Fluorescence microscopy images of immunocytochemistry for Jagged1 in control and TGF-β-treated cultures in the presence of DMSO or TβRII inhibitor SB431542. Scale bar, 50 μm. Quantitation of relative percentages. Results are expressed as the percentage of positively stained cells for Jagged1 over total cells visualized with Hoechst. (d) Fluorescence microscopy images of immunocytochemistry for PDGFRα in control and TGF-β-treated cultures in the presence of DMSO or TβRII inhibitor SB431542. Scale bar, 50 μm. Quantitation of relative percentages. Results are expressed as the percentage of positively stained cells for PDGFRα over total cells visualized with Hoechst. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate (two-way ANOVA, Bonferroni's post-test) $^{*}p < .05$, $^{**}p < .01$ [Color figure can be viewed at wileyonlinelibrary.com]

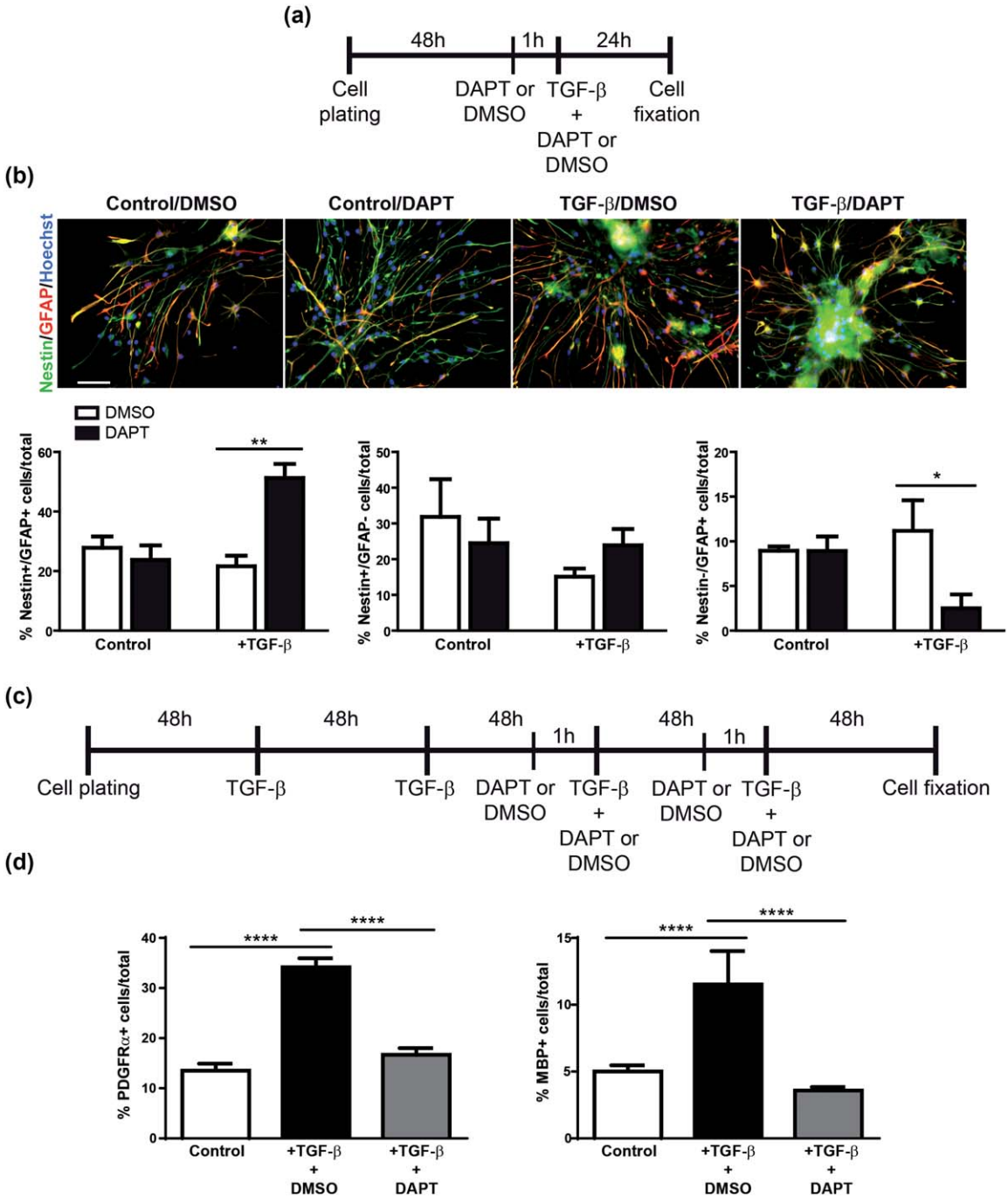

FIGURE 10 Notch signaling inhibition after TGF-β treatment in other cell subpopulations. (a) Experimental timeline. (b) Fluorescence microscopy images of immunocytochemistry for Nestin/GFAP merge in control and TGF-β-treated cultures in the presence of DMSO or γ-secretase inhibitor DAPT. Scale bar, 20 μm. Quantitation of relative percentages. Results are expressed as the percentage of Nestin+/GFAP+, Nestin+/GFAP−, and Nestin−/GFAP+ cells over total cells visualized with Hoechst in control and TGF-β-treated cultures in the presence of DMSO or γ-secretase inhibitor DAPT. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate (two-way ANOVA, Bonferroni's post-test) $^{*}p < .05$, $^{**}p < .01$. (c) Experimental timeline. (d) Quantitation of relative percentages of immunocytochemistry. Results are expressed as the percentage of PDGFRα+ and MBP+ cells over total cells visualized with Hoechst in control and TGF-β-treated cultures in the presence of DMSO or γ-secretase inhibitor DAPT. Values are expressed as the mean ± *SEM* of three independent experiments performed in triplicate (two-way ANOVA, Bonferroni's post-test) $^{****}p < .0001$ [Color figure can be viewed at wileyonlinelibrary.com]

Jagged1-driven Notch signaling in the regulation of OPC proliferation and maturation.

In conclusion, adult brain SVZ neurosphere cultures in the presence of EGF/FGF are characterized by a predominance of Nestin+/GFAP+ type B cells and by the presence of PDGFRα+/NG2+ OPCs, all of which express TβRII. At early culture stages, TGF-β favors glial cell fate decisions by inducing an increase in GFAP+ astrocytes expressing Jagged1, which in turn further promotes oligodendroglial

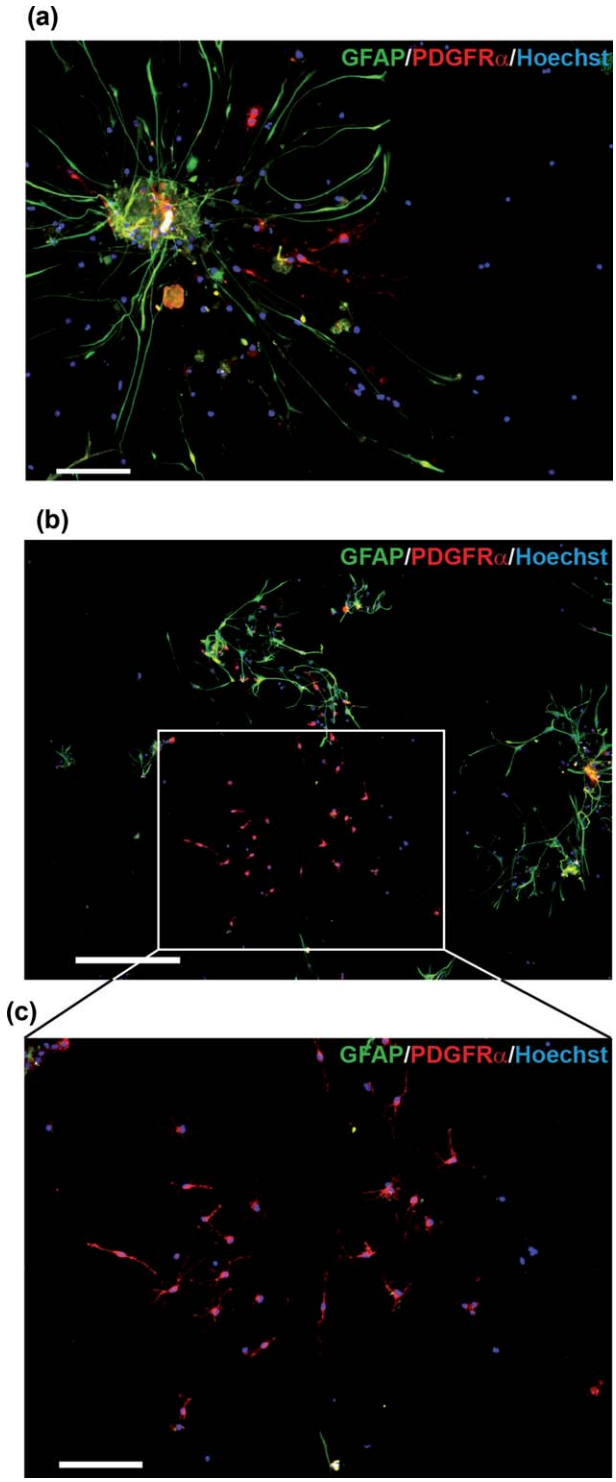

FIGURE 11 Cell type distribution in short and long term cultures. Fluorescence microscopy images of immunocytochemistry for PDGFRα/GFAP merge in TGF-β-treated cultures after (a) 48 hr. Scale bar, 20 μm (b) 10 days. Scale bar, 250 μm. (c) High magnification image of the frame in(b). Scale bar, 25 μm [Color figure can be viewed at wileyonlinelibrary.com]

cell fate decisions and induces the proliferation of PDGFRα+ OPCs. At later cultures stages, once OPCs have migrated away from GFAP+ astrocytes expressing Jagged1, TGF-β induces Notch-independent TβRII-mediated OPC differentiation into mature MBP+ OLs. Even if

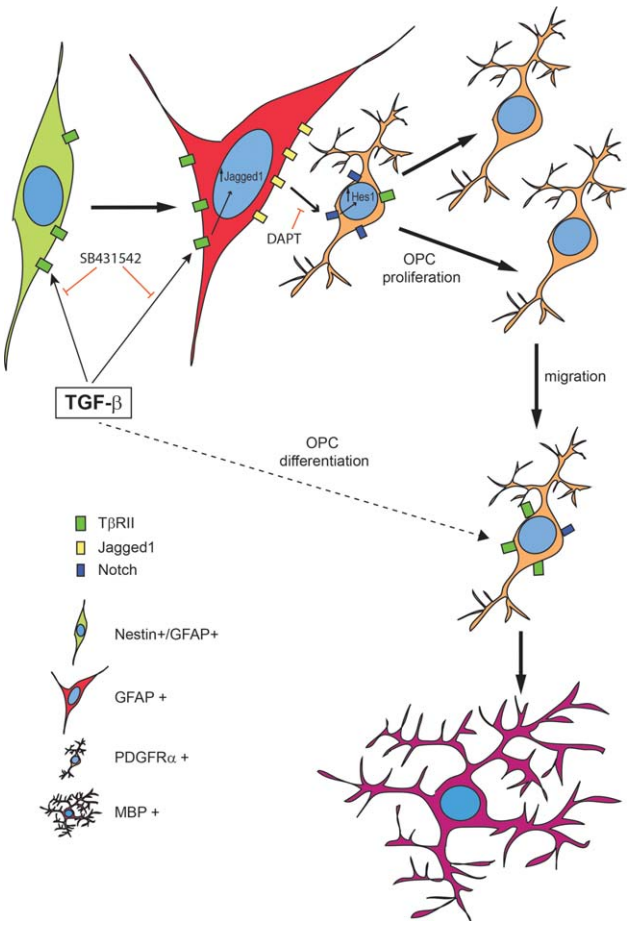

FIGURE 12 Interplay between Notch and TGF-β signaling in adult SVZ neurosphere cultures. Through its own receptor TβRII, TGF-β favors NPC glial cell fate decisions by inducing an increase in astrocytes expressing Jagged1. In turn, Jagged1 triggers Notch activation and induces Hes1 expression, thus promoting oligodendroglial cell fate decisions and OPC proliferation. Once OPCs have migrated away from astrocytes expressing Jagged1, TGF-β induces TβRII-mediated OPC differentiation into mature OLs [Color figure can be viewed at wileyonlinelibrary.com]

further experiments will be necessary to elucidate the underlying mechanisms, these results reinforce the notion of an indirect Jagged1/Notch-mediated and a direct TβRII-mediated pro-oligodendrogenic effect of TGF-β on adult brain SVZ NPC cultures (Figure 12).

ACKNOWLEDGMENT

This work was supported by grants from ANPCyT (Préstamo BID – PICT 2013 2772), Universidad de Buenos Aires (20020120100132BA) and CONICET (PIP 0567), Argentina. The authors declare no conflict of interest.

ORCID

Patricia A. Mathieu http://orcid.org/0000-0001-5373-3142

REFERENCES

Aparicio, E., Mathieu, P., Pereira Luppi, M., Almeira Gubiani, M. F., & Adamo, A. M. (2013). The Notch signaling pathway: Its role in focal

CNS demyelination and apotransferrin-induced remyelination. [Research Support, Non-U.S. Gov't]. *Journal of Neurochemistry*, *127* (6), 819–836. https://doi.org/10.1111/jnc.12440

Battista, D., Ferrari, C. C., Gage, F. H., & Pitossi, F. J. (2006). Neurogenic niche modulation by activated microglia: Transforming growth factor β increases neurogenesis in the adult dentate gyrus. [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U. S. Gov't]. *European Journal of Neuroscience*, *23*(1), 83–93. https://doi.org/10.1111/j.1460-9568.2005.04539.x

Danilov, A. I., Gomes-Leal, W., Ahlenius, H., Kokaia, Z., Carlemalm, E., & Lindvall, O. (2009). Ultrastructural and antigenic properties of neural stem cells and their progeny in adult rat subventricular zone. [Research Support, Non-U.S. Gov't]. *Glia*, *57*(2), 136–152. https://doi.org/10.1002/glia.20741

Dave, R. K., Ellis, T., Toumpas, M. C., Robson, J. P., Julian, E., Adolphe, C., … Wainwright, B. J. (2011). Sonic hedgehog and notch signaling can cooperate to regulate neurogenic divisions of neocortical progenitors. [Research Support, Non-U.S. Gov't]. *PLoS One*, *6*(2), e14680. https://doi.org/10.1371/journal.pone.0014680

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Nature Medicine*, *4*(11), 1313–1317. https://doi.org/10.1038/3305

Gonzalez-Perez, O., & Alvarez-Buylla, A. (2011). Oligodendrogenesis in the subventricular zone and the role of epidermal growth factor. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. *Brain Research Review*, *67*(1–2), 147–156. https://doi.org/10.1016/j.brainresrev.2011.01.001

Graciarena, M., Roca, V., Mathieu, P., Depino, A. M., & Pitossi, F. J. (2013). Differential vulnerability of adult neurogenesis by adult and prenatal inflammation: Role of TGF-β1. *Brain, Behavior, and Immunity*, *34*, 17–28. https://doi.org/10.1016/j.bbi.2013.05.007

Guo, F., Lang, J., Sohn, J., Hammond, E., Chang, M., & Pleasure, D. (2015). Canonical Wnt signaling in the oligodendroglial lineage–puzzles remain. [Research Support, Non-U.S. Gov't Review]. *Glia*, *63*(10), 1671–1693. https://doi.org/10.1002/glia.22813

Hack, M. A., Sugimori, M., Lundberg, C., Nakafuku, M., & Gotz, M. (2004). Regionalization and fate specification in neurospheres: The role of Olig2 and Pax6. [Research Support, Non-U.S. Gov't]. *Molecular and Cellular Neuroscience*, *25*(4), 664–678. https://doi.org/10.1016/j.mcn.2003.12.012

Jin, K., Minami, M., Lan, J. Q., Mao, X. O., Batteur, S., Simon, R. P., & Greenberg, D. A. (2001). Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. [Research Support, U.S. Gov't, P.H.S.]. *Proceedings of the National Academy of Science USA*, *98*(8), 4710–4715. https://doi.org/10.1073/pnas.081011098

John, G. R., Shankar, S. L., Shafit-Zagardo, B., Massimi, A., Lee, S. C., Raine, C. S., & Brosnan, C. F. (2002). Multiple sclerosis: Re-expression of a developmental pathway that restricts oligodendrocyte maturation. *Nature Medicine*, *8*(10), 1115–1121. https://doi.org/10.1038/nm781 nm781 [pii]

Kitisin, K., Saha, T., Blake, T., Golestaneh, N., Deng, M., Kim, C., … Mishra, L. (2007). Tgf-β signaling in development. [Review]. *Science STKE*, *2007*(399), cm1. https://doi.org/10.1126/stke.3992007cm1

Kopan, R., & Ilagan, M. X. (2009). The canonical Notch signaling pathway: Unfolding the activation mechanism. [Research Support, N.I.H., Extramural Review]. *Cell*, *137*(2), 216–233. https://doi.org/10.1016/j.cell.2009.03.045

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*(5259), 680–685.

Lassmann, H., Bruck, W., & Lucchinetti, C. F. (2007). The immunopathology of multiple sclerosis: An overview. [Research Support, Non-U.S. Gov't Review]. *Brain Pathol*, *17*(2), 210–218. https://doi.org/10.1111/j.1750-3639.2007.00064.x

Lois, C., & Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. *Science*, *264*(5162), 1145–1148.

Massague, J., & Chen, Y. G. (2000). Controlling TGF-β signaling. [Review]. *Genes and Development*, *14*(6), 627–644.

Mathieu, P., Piantanida, A. P., & Pitossi, F. (2010). Chronic expression of transforming growth factor-β enhances adult neurogenesis. *Neuroimmunomodulation*, *17*(3), 200–201. https://doi.org/10.1159/000258723

McCarthy, G. F., & Leblond, C. P. (1988). Radioautographic evidence for slow astrocyte turnover and modest oligodendrocyte production in the corpus callosum of adult mice infused with 3H-thymidine. [Research Support, Non-U.S. Gov't]. *Journal of Comparative Neurology*, *271*(4), 589–603. https://doi.org/10.1002/cne.902710409

Menn, B., Garcia-Verdugo, J. M., Yaschine, C., Gonzalez-Perez, O., Rowitch, D., & Alvarez-Buylla, A. (2006). Origin of oligodendrocytes in the subventricular zone of the adult brain. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Journal of Neuroscience*, *26*(30), 7907–7918. https://doi.org/10.1523/JNEUROSCI.1299-06.2006

Nait-Oumesmar, B., Decker, L., Lachapelle, F., Avellana-Adalid, V., Bachelin, C., & Baron-Van Evercooren, A. (1999). Progenitor cells of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. [Research Support, Non-U.S. Gov't]. *European Journal of Neuroscience*, *11*(12), 4357–4366.

Nait-Oumesmar, B., Picard-Riera, N., Kerninon, C., & Baron-Van Evercooren, A. (2008). The role of SVZ-derived neural precursors in demyelinating diseases: From animal models to multiple sclerosis. [Research Support, Non-U.S. Gov't Review]. *Journal of the Neurological Sciences*, *265*(1–2), 26–31. https://doi.org/10.1016/j.jns.2007.09.032

Palazuelos, J., Klingener, M., & Aguirre, A. (2014). TGFβ signaling regulates the timing of CNS myelination by modulating oligodendrocyte progenitor cell cycle exit through SMAD3/4/FoxO1/Sp1. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Journal of Neuroscience*, *34*(23), 7917–7930. https://doi.org/10.1523/JNEUROSCI.0363-14.2014

Petratos, S., Gonzales, M. F., Azari, M. F., Marriott, M., Minichiello, R. A., Shipham, K. A., … Kilpatrick, T. J. (2004). Expression of the low-affinity neurotrophin receptor, p75(NTR), is upregulated by oligodendroglial progenitors adjacent to the subventricular zone in response to demyelination. [Research Support, Non-U.S. Gov't]. *Glia*, *48*(1), 64–75. https://doi.org/10.1002/glia.20056

Picard-Riera, N., Decker, L., Delarasse, C., Goude, K., Nait-Oumesmar, B., Liblau, R., … Baron-Van Evercooren, A. (2002). Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. [Research Support, Non-U.S. Gov't]. *Proceedings of the National Academy of Sciences USA*, *99* (20), 13211–13216. https://doi.org/10.1073/pnas.192314199

Radice, P. D., Mathieu, P., Leal, M. C., Farias, M. I., Ferrari, C., Puntel, M., … Pitossi, F. J. (2015). Fibulin-2 is a key mediator of the proneurogenic effect of TGF-β1 on adult neural stem cells. [Research Support, Non-U.S. Gov't]. *Molecular and Cellular Neuroscience*, *67*, 75–83. https://doi.org/10.1016/j.mcn.2015.06.004

Robinson, A. P., Foraker, J. E., Ylostalo, J., & Prockop, D. J. (2011). Human stem/progenitor cells from bone marrow enhance glial differentiation of rat neural stem cells: A role for transforming growth factor β and Notch signaling. [Research Support, N.I.H., Extramural

Research Support, Non-U.S. Gov't]. *Stem Cells and Development*, *20* (2), 289–300. https://doi.org/10.1089/scd.2009.0444

Wrighton, K. H., Lin, X., & Feng, X. H. (2009). Phospho-control of TGF-β superfamily signaling. [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S. Review]. *Cell Research*, *19*(1), 8–20. https://doi.org/10.1038/cr.2008.327

Zhang, Y., Zhang, J., Navrazhina, K., Argaw, A. T., Zameer, A., Gurfein, B. T., ... John, G. R. (2010). TGFβ1 induces Jagged1 expression in astrocytes via ALK5 and Smad3 and regulates the balance between oligodendrocyte progenitor proliferation and differentiation. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Glia*, *58*(8), 964–974. https://doi.org/10.1002/glia.20978

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Gómez Pinto LI, Rodríguez D, Adamo AM, Mathieu PA. TGF-β pro-oligodendrogenic effects on adult SVZ progenitor cultures and its interaction with the Notch signaling pathway. *Glia*. 2017;00:000–000. https://doi.org/10.1002/glia.23253