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ORIGINAL RESEARCH

Müller Cells Derived from Adult Chicken and Mouse Retina Neurospheres Acquire the Dopaminergic Phenotype

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Abstract

Neurospheres prepared from multipotent progenitors in the retina obtained from postnatal mice differentiate into neurons and Müller glia (De Melo Reis et al., in Cell Mol Neurobiol 31:835–846, 2011). Here, we investigated whether neurospheres prepared from adult chickens (ciliary marginal zone, CMZ) or (ciliary body) retina could also lead to differentiated neurons and glia. Neurospheres were prepared from post-hatched chickens or from adult mice after 7 days in the presence of mitogenic factors (FGFb, insulin, and EGF), generating neurons and glial cells. In addition, Müller (2M6 or glutamine synthetase positive cells) derived from post-hatch chicken CMZ neurospheres displayed the dopaminergic phenotype. Furthermore, we observed that Müller cells derived from adult chickens and mice retina neurospheres released significant amounts of dopamine as well as of its metabolites. Taken together, our data lead us to conclude that as for embryonic (chick) or newborn (mouse), the dopaminergic phenotype is a default condition of Müller glial cells obtained from neurospheres prepared from mature retina. Our data raise the possibility that Müller cells from differentiated tissue could be used to ameliorate neurodegenerative diseases involving dopaminergic dysfunction as in Parkinson's disease as shown previously (Stutz et al., in J Neurochem 128:829–840, 2014).

Keywords Retina · Müller glial cells · Retinal ganglion cells · Tyrosine hydroxylase · Dopamine · Neurospheres

Introduction

Retinal progenitors originate all types of neurons and Müller glia during the period of development (Turner and Cepko 1987). In adult vertebrates only, a few of these progenitors can be reactivated because of traumatic injuries (Alunni and Bally-Cuif 2016). In amphibians and fish, there is a mitotically active region at the border of the retina that is capable of generating new retinal neurons in response to damage throughout life (Hollyfield 1968; Raymond and Hitchcock 1997; Reh 1987). This peripheral region of the retina is known as ciliary marginal zone (CMZ) present in the chicken retina. However, chicken CMZ is fundamentally different from what is observed in lower vertebrates (Kubota et al. 2002). Proliferating cells in the CMZ of the post-hatched chicken can incorporate [^{3}H] thymidine (Morris et al. 1976) or the thymidine analogue 5-bromo-2-deoxyuridine (BrdU), later adding new neurons to the peripheral retina. However, it was shown that only bipolar and amacrine cells are generated under these conditions (Fischer and Reh 2000). The regenerating potential of the mammalian retina is limited, with no neurogenic niches prevailing after the complete maturation of the tissue. From rodents (Moshiri and Reh 2004) to primates (Fischer et al. 2001), there is no evidence of proliferating cells in the borders of the mature retina.

Müller cells, the major glial cell type in the neural retina, interact with virtually all retinal neuron, providing

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structural and synaptic support, among other functions that in the CNS is carried out by astrocytes and oligodendrocytes (Bringmann et al. 2006). In vitro studies using BrdU labeling showed that some of the pigmented cells of the ciliary body (CB) of rodents could proliferate in the presence of growth factors, as basic fibroblast growth factor (bFGF) (Ahmad et al. 2000). Also, these progenitors could give rise to self-renewing neurospheres which in turn could originate retinal cells in vitro, including neurons (Ahmad et al. 2000). Müller glia can also act as progenitor cells in the retina. These glial cells can react to retinal injury in several ways. After intraocular injection of N-methyl-D-aspartate (NMDA), Müller cells de-differentiate, proliferate, assume a progenitor-like phenotype (Fischer and Reh 2001) and can give rise to retinal neurons (Karl et al. 2008; Ooto et al. 2004). The possibility of endogenous repair identified Müller glia as candidate for cell reprogramming (Goldman 2014). Interestingly, if isolated from undifferentiated retina, Müller cells express several dopaminergic markers as the enzymes tyrosine hydroxylase (TH), L-dopa decarboxylase (L-DOPA), the D_1 dopamine receptor (Kubrusly et al. 2005), the dopamine transporter (DAT), and the nuclear receptor-related factor 1 (Nurr1) (Kubrusly et al. 2008). This shift in cell phenotype is conserved in mice and primates (Stutz et al. 2014), and occurs even though Müller cells continue to express glial markers. Müller cells in culture synthesize and release dopamine, preserving the dopaminergic phenotype even after implanted in the brains of experimental animals, where they can fully restore lost dopaminergic function (Stutz et al. 2014). Based on the finding that Müller glial cells have the capacity to acquire the synthetic machinery for dopamine production as a default, we investigated whether Müller cells obtained from adult avian or mammalian retina neurospheres can also express the dopaminergic phenotype. Here, we show that Müller glial cells obtained from adult eye progenitors maintain such characteristics.

Results

Retina Progenitor Cells Generate Neurospheres in Culture

Neurospheres were obtained from early postnatal retinas from C57/Bl6 mice isolated during late stages of retinogenesis as reported previously (De Melo Reis et al. 2011). Dissociated cells isolated from retina of P0–P2 mice were cultured in suspension with epidermal growth factor (EGF, 20 ng/ml) for 5–7 days, which allowed us to isolate neurospheres with an average diameter ranging from 70 to 150 µm (Fig. 1a). Neurospheres were collected in glass slides by cytospin centrifugation and immunostained for retinal progenitor markers. Retina progenitor cells (RPCs) were positive for Pax-6, a transcription factor expressed in neurogenic regions of the eye (Belecky-Adams et al. 1997). Pax-6-positive staining was found close to the borders of the sphere (Fig. 1b, d). Furthermore, cells located at central region of the neurospheres were positive for Nestin (Fig. 1c, d), a marker for neural progenitors that is also expressed in retina neurospheres (De Melo Reis et al. 2011).

Neurospheres plated without EGF or bFGF gave rise to clusters of round cells as well as migrating cells on coverslips (Fig. 1e). Cell cultures were immunolabeled for glial fibrillary acidic protein (GFAP) or Tuj-1, indicating the capacity of RPCs to differentiate into glial cells and neurons (Fig. 1f).

Isolation of Proliferative Retinal Cells from Adult Eye

To address whether eyes from adult animals could also give rise to RPC-derived neurospheres, mice and post-hatched chicken with a fully developed visual system were used. Chicken CMZ were isolated and dissociated as quiescent cells to reenter the cell cycle under defined culture conditions. Chicken at different ages (P1, P7, P15, P21, P28 and P35; P = days post-hatched) were used to obtain eye progenitor cells. For example, spheres with 100–120 µm grown from CMZ cells were isolated from P15 chicken (Fig. 2a). When older animals (P28) were used, fewer neurospheres were obtained (Fig. 2b). Under phase contrast microscopy, we observed that RPC-derived neurospheres from P15 chicks differentiated into glia and neurons, confirming its progenitor capacity (Fig. 2c). Retinas obtained from P35 chicken were incapable of generating spheres (data not shown).

We further investigated whether retinal progenitors of adult mice (P45) were also capable of originating neurospheres in culture. We dissected the CB region of the eyes and the isolated cells were cultured under defined medium containing the factors reported above. As shown, pigmented cells of this region proliferated and produced spheres containing both pigmented and non-pigmented progenitor cells (Fig. 2d). Differentiation of mice-derived neurospheres generated mostly cells bearing glial morphology, with pigment deposits and pigmented cells (Fig. 2e). The glial phenotype of cells derived from adult mouse neurospheres was confirmed by positive immunolabeling for both GFAP (Fig. 2f) and GS (Fig. 2g) in virtually all DAPI nuclei stained cells. These findings indicate that retinas from both postnatal chicken and mice have multipotent precursor cells with the capacity to differentiate into retinal cells, and help confirm the predominance of the glial phenotype in our cultures.

Chicken Müller Cells Derived from RPCs Express Dopaminergic-Like Phenotype

Purified Müller cells obtained from chick embryos or from newborn mice retinas can express several dopaminergic

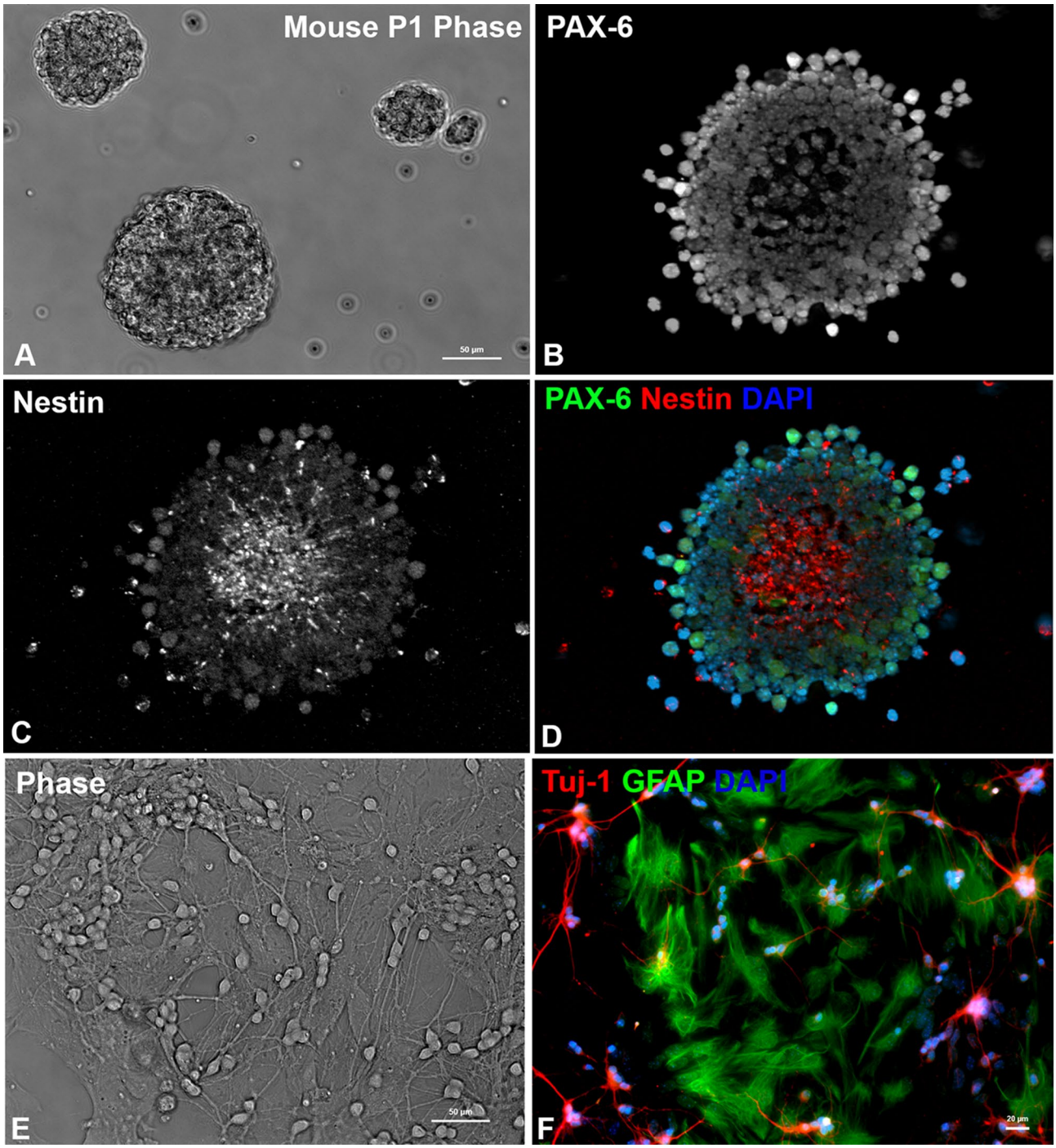


Fig. 1 Mouse newborn retina gives rise to neurospheres and neural cells in culture. **a** Photomicrograph of mouse P1 under phase contrast after 7-day incubation. **b–d** Under apotome fluorescence neurospheres can be visualized after double immunolabeling for PAX-6 (**b**) and Nestin (**c**) with nuclei counterstained with DAPI. Merged channel can be visualized in (**d**). **e, f** Round-shinning and flatted cells are observed under phase contrast after 48 h (**e**). **f** Epifluorescence apotome image obtained after double immunolabeling for Tuj-1 and GFAP. Nuclei were counterstaining with DAPI. Scale bar: **a–e** 50 µm; **f** 20 µm. $N = 5$ independent experiments

markers, which positively correlate with dopamine levels in the conditioned medium (Kubrusly et al. 2005; Stutz et al. 2014). Neurospheres obtained from P15 chicken marginal zone were maintained under differentiation conditions for 15 days. Neurons gradually disappear as culture medium was changed leading to a purified Müller glia culture (Kubrusly et al. 2005, 2008; Stutz et al. 2014). Flat semi-confluent Müller glia-like cells were

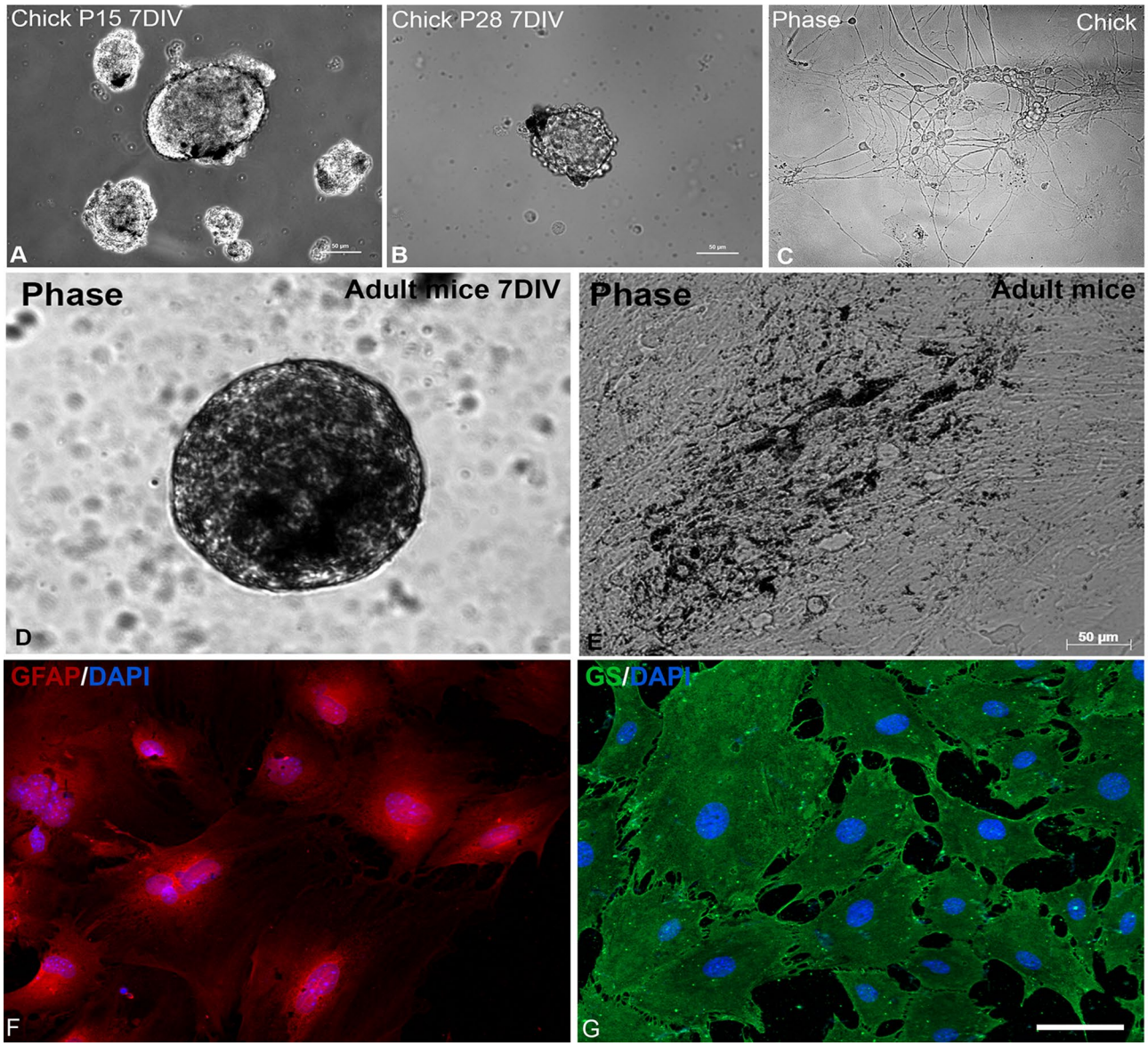


Fig. 2 Adult chickens and mice give rise to neurospheres and neural cells in culture. Phase contrast images of neurospheres derived from P15 (**a**) and P28 (**b**) chicken retinas 7 days after incubation. Round-shinning neurons and flatted glial cells are observed under the same type of microscopy (**c**). **d, e** Photomicrographs of a neurosphere derived from adult mice 7 days after incubation (**d**) and after differentiation show flatted glial cells (**e**). **f, g** Images obtained by apotome microscopy of glial cells derived from neurospheres generated from eye tissue of adult mice immunolabeled for GFAP (**f**) or glutamine synthetase (**g**). Cell nuclei were counterstained with DAPI. Scale bar: **a**–**e** 50 µm; **f, g** 100 µm. Chick neurospheres $N=4$ independent experiments; mice neurospheres. $N=5$ independent experiments

observed, but no round, shining neuronal cells were seen under phase contrast microscopy (Fig. 3a). The phenotype of Müller glia was confirmed by positive immunostaining for 2M6, a known marker for this cell type in the avian retina (Fig. 3b) in virtually all DAPI nuclei stained cells. The rate-limiting enzyme tyrosine hydroxylase (TH) for dopamine synthesis (Fig. 3c, d) and the transcription factor Nurr-1 (Fig. 3e–h) were shown in 2M6-positive Müller glial cells obtained from post-hatched chicken retina. This confirms a dopaminergic-like phenotype of Müller cells from adult avian CMZ-derived neurospheres in vitro.

Mice Müller Cells Derived from RPCs Express Dopaminergic-Like Phenotype

We asked whether neurospheres obtained from adult mice eye could also generate dopaminergic Müller glia. Müller cells (Fig. 4a, Cralbp positive) were double immunolabeled

for GS (Fig. 4b) and Nurr-1 (Fig. 4c). We also observed that GS-positive Müller cells also expressed the transcription factor Nurr-1 (Fig. 4c, e) in nearly all cells. Altogether, we conclude that cultured Müller cells derived from adult mice (Fig. 4e) also expressed Nurr-1.

Release of Dopamine and Metabolites by Müller Cells Derived from RPCs

TH is present in mouse Müller glia differentiated from neurospheres from newborn retina, as shown by western blot (Fig. 5a). TH phosphorylation at serine residues by several kinases increase its catalytic activity, increasing dopamine production (Dunkley et al. 2004). For this reason, phosphorylation at two important serine residues was evaluated, TH phospho-serine 31 and serine 40, as shown in Fig. 5b, c. The expression of dopamine transporter (DAT) (Fig. 5d) and Nurr1 (Fig. 5e) were also observed in Müller cells from newborn retina (Fig. 5a–e). Alternatively, we also confirmed the expression of TH phospho-serine 40 in Müller cells derived from neurospheres of adult mice eyes (Fig. 5f). After collecting the conditioned medium from chicken and mice Müller-derived cells, high-performance liquid chromatograph (HPLC) was performed aiming to detect dopamine and its metabolite DOPAC. Our data show that neurospheres from both chick and mice underwent differentiation into dopamine-releasing Müller cells (Fig. 5g, h, marked in blue). Moreover, 3,4-dihydroxy phenylacetic acid (DOPAC) was also detected, indicating metabolic turnover of dopamine in these cells (Fig. 5g, h, marked in pink). This set of data confirms that not only dopamine-producing markers were detected in Müller cells derived from neurospheres, but also that these cells functionally produce and release dopamine. These data confirm that progenitors of both chicken and mouse eyes can differentiate spontaneously into Müller cells with dopaminergic phenotype.

Discussion

Since the original work of Reynolds and Weiss (1992), neurospheres have been used as a model of neural differentiation and enrichment with potential uses in cell therapy (Deleyrolle and Reynolds 2009). More recently, adult progenitors have likewise become an attractive source of cells for this purpose, being particularly promising for autologous transplants (Coles et al. 2004). Here, we show that cultured and differentiated Müller glial cells from post-developmental sources acquire a unique dopaminergic phenotype, which could be harnessed for therapeutic use, in particular to degenerative diseases such as Parkinson's disease (Stutz et al. 2014).

Unlike lower vertebrates, adult mammals cannot naturally regenerate retinal neurons (Lamba et al. 2009). But while the regenerative potential found in the retinas of teleosts and some amphibians was eventually lost during the evolutionary process, some remaining niches of progenitors, or cells that may be activated to reenter the mitotic cycle, remain in the mammalian eye. In the eyes of adult mice and humans, scarce progenitor-like cells can be found, albeit mostly restricted to non-retinal areas, such as the CB and limbus (Chen et al. 2014).

Our data confirm that eyes from post-hatch chickens (P15 and P28) and adult mice (P45), can be used to generate neurospheres, expanded into a mixed culture of neurons and glia (Figs. 1, 2, 3), and later purified into Müller cell monolayers. Once in the absence of neurons, we show that Müller cultures differentiated from these progenitors and acquire the dopaminergic phenotype.

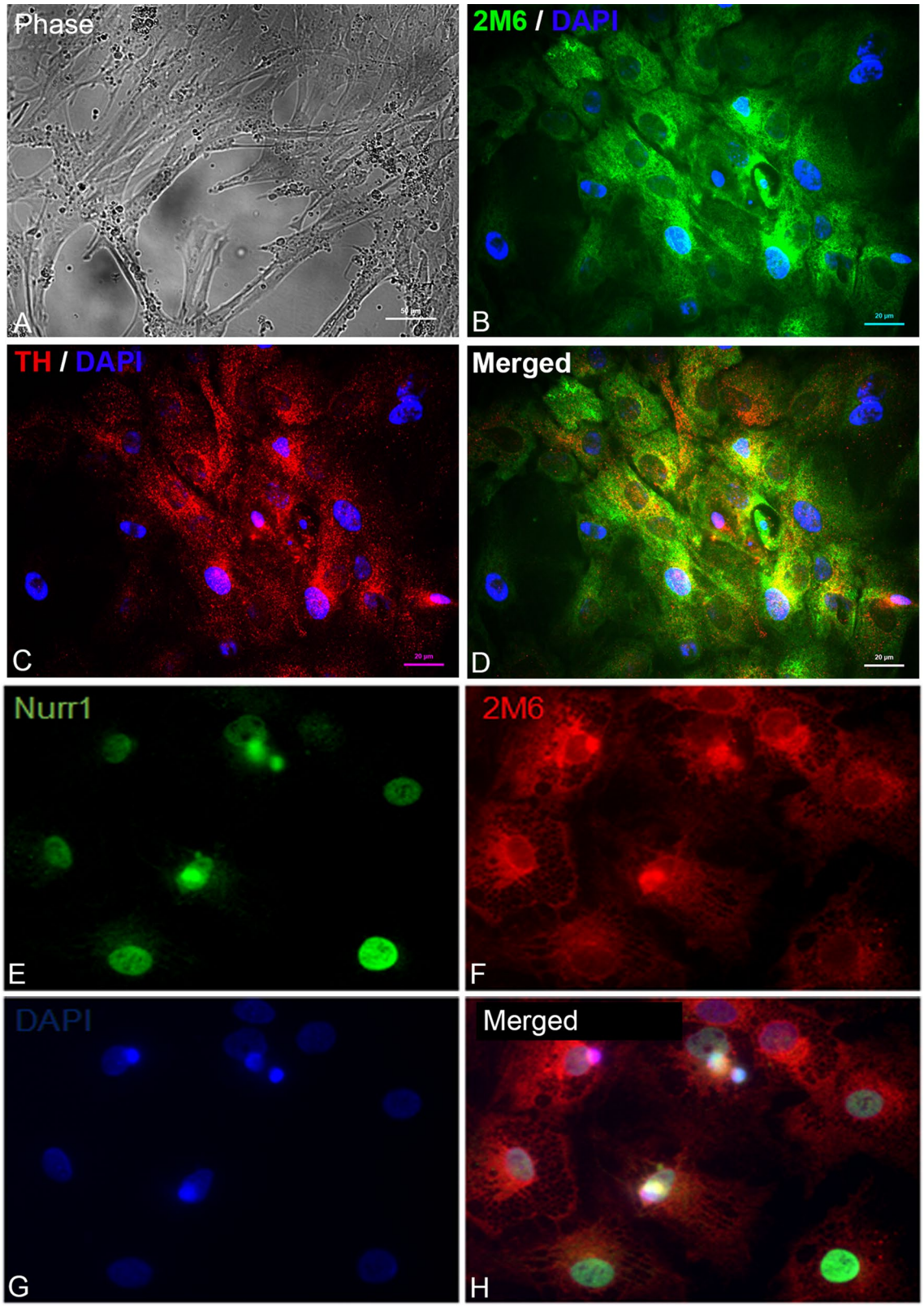
Müller glia are the latest cells to differentiate from late born progenitors. While their cell bodies are in the inner nuclear layer (INL), apical and basal processes interact with mostly every single neuron to keep retinal homeostasis and integrity. Upon retinal injury, chickens and mice Müller glia reenter the cell cycle, proliferate and under certain circumstances could generate neuronal-like cells, but are incapable to repair lesioned tissue (Goldman 2014). Our work demonstrates that progenitors obtained not only from the developing retina [in this case, from newborn mice (De Melo Reis et al. 2011; Stutz et al. 2014)], but also from other regions of the eye such as the CMZ and the CB (from post-hatched chickens and adult mice) follow the same in vitro differentiation program, resulting in TH-positive dopaminergic Müller cells. This might be linked to the fact that both tissues originate from the same neuroepithelium during embryogenesis. When cultured Müller cells obtained from neonatal mice retina are injected in the mesencephalus of Parkinsonian mice, a fully recovery of the motor deficits is observed (Stutz et al. 2014).

Our previous observation that dopaminergic Müller glia can ameliorate Parkinson's disease-like symptoms in animal models (Stutz et al. 2014) have proposed these cells as an interesting alternative approach for cell therapy in hypodopaminergic conditions. The data presented here now suggest the possibility that Müller cells derived from neurospheres isolated from adult tissue – an obtainable source even for human tissue (Coles et al. 2004) – may hold the same potential.

Materials and Methods

Animals

All animal procedures and experimental protocols were approved by the Animal Care and Use Committee of our



Institution; CEUA protocol (animal ethics committee) license #126 (for mice) and license #035 (for chicks) under supervision of Carlos Chagas Filho Biophysics Institute (IBCCF-UFRJ). C57/BL6 mice were obtained from our own facilities. Fertilized white Leghorn eggs were purchased from a local hatchery and maintained in appropriate

◂**Fig. 3** Chick neurosphere-derived Müller glial cells expressing dopaminergic markers. A-D: Neurosphere-derived Müller glial cells from retina of chicken (P15). **a** Image of Müller glial cell obtained by phase contrast microscopy. B-D: Images obtained by epifluorescence apotome microscopy of Müller glial cells double immunolabeled for 2M6 (**b**) and tyrosine hydroxylase (TH, **c**), where merged as in D. Nuclei were counterstained with DAPI. **e**–**h** Neurosphere-derived Müller glial cells from retina of adult chickens. **e**–**h** Images obtained by epifluorescence apotome microscopy of Müller glial cells double immunolabeled for Nurr-1 (**e**), 2M6 (**f**) and merged as in (**h**). Nuclei were counterstained with DAPI. Scale bars: **a** 50 µm; **b**–**h** 20 µm. $N = 3$ independent experiments

conditions, with controlled temperature (37 °C), humidity, and light. Post-hatched chicks were kept in a stainless-steel brooder at approximately 25 °C.

Isolation and Culture of Retinal Progenitor Cells (RPCs)

Suspension cultures of retinal progenitors were maintained in non-adhesive culture plates. Anti-adhesive polymer poly-HEMA (90 mg/ml) (Sigma) was used for coating tissue culture plates followed by sterilization under UV light. Individual cells were cultivated at 37 °C in a 5% CO_2 atmosphere, and spheres were allowed to form for a period of 5–7 days. Progenitor isolation, tissue digestion, and culture media differed according to the age and species used, as detailed below.

Newborn Mice

Late retinal progenitors were isolated from the retina of P0–P2 mice and cultivated in vitro. The procedures were based previous studies (Bhattacharya et al. 2004). Briefly, retinas were dissected from the mice and incubated in 0.2% papain (Worthington) at 37 °C for 10 min. The Enzymatic digestion was stopped with 5% ovomucoid (Worthington). The tissue was mechanically dissociated, centrifuged, and resuspended in Dulbecco's modified Eagle's medium's Nutrient Mixture F-12 (1:1, DMEM/F12) supplemented with 40 mg/ml gentamicin, 2% B-27 (Invitrogen), 20 ng/ml epidermal growth factor (EGF) (Invitrogen), and GlutaMAXTM.

Adult Mice

As previously described (Ahmad et al. 2000), retinal progenitors were isolated from the pigmented part of the ciliary body of adult mice. The ciliary body was removed from the eyes (P45) and subjected to digestion with 0.2% papain and collagenase/hyaluronidase (Sigma) at 37 °C for 30 min. Composition of the culture media differed only in its addition of 2 µg/ml of heparin (Stem Cell Technology) in the growth factors used (20 ng/ml EGF and 10 ng/ml bFGF).

Post-Hatched Chicken

Culture of neuropheres of chick retinal progenitors was obtained from post-hatched animals between P1 and P35 (P = days post-hatched). Removed eyes were hemisected coronally and from the anterior portion of the eye it was dissected the CMZ in Calcium and magnesium-free saline buffer. The tissue was incubated with 0.2% papain at 37 °C for 30 min and individual cells were cultivated in suspension in culture medium containing the combination of three growth factors: 20 ng/ml EGF, 10 ng/ml bFGF, and 100 ng/ml insulin-like growth factor (IGF-I).

Müller Glia Culture

All RPCs neurospheres were differentiated into Müller cell cultures according to the following procedures. The neurospheres were washed three times with phosphate-buffered saline (PBS) and resuspended in DMEM/F12 supplemented with 10% fetal calf serum (FCS). Neurospheres were then plated in culture dishes pre-treated with poly L-lysine (10 µg/ml) for adhesion and maintained at 37 °C in a 5% CO_2 atmosphere. After 15 days of differentiation, RPCs from adult mice and chicks gave rise to purified glial Müller cells. For the newborn RPCs differentiating cultures, ascorbic acid (3 mM) was used for 2–3 h to eliminate neurons, as previously described (Reis et al. 2002).

Immunocytochemistry

Retinal Neurospheres from newborn mice were cytospinned on glass slides, pre-treated with poly L-lysine (20 µg/ml) by a cytocentrifuge, for immunostaining with the neural progenitor cells markers Nestin (1:500, Chemicon), Pax-6 (1:500, Chemicon); and KI-67 (1:500, ABCAM), a marker for cell proliferation. Also, the purified Müller cells culture were immunolabeled for glial and dopaminergic markers. The cells were fixed with 4% paraformaldehyde for 10 min, at room temperature, and washed three times with PBS. Prior the antibody incubation, cells were washed with 0.3% Triton X-100 (Sigma-Aldrich) for 5 min followed by incubation with 3% bovine serum albumin (BSA) for 60 min, both diluted in PBS. Cells were incubated with primary antibodies overnight at 4 °C in a moisture chamber. Müller cells differentiated from neurospheres were labeled with the specific glial markers such as mouse monoclonal antibody (IgG) reacting with chicken retina-2M6 (Müller glia cell-specific antigen, 1:500), anti- GS (1:500, ABCAM), Anti-GFAP (1:500, DAKO), Anti-S100 (1:500, Sigma), CRALBP (1:500, Abcam). To label remaining neurons in mixed cultures it was also used the mouse monoclonal antibody against TUJ1 (1:500, Covance). The antibodies against Nurr1 (1:400, Santa Cruz) and Tyrosine Hydroxylase (1:500,

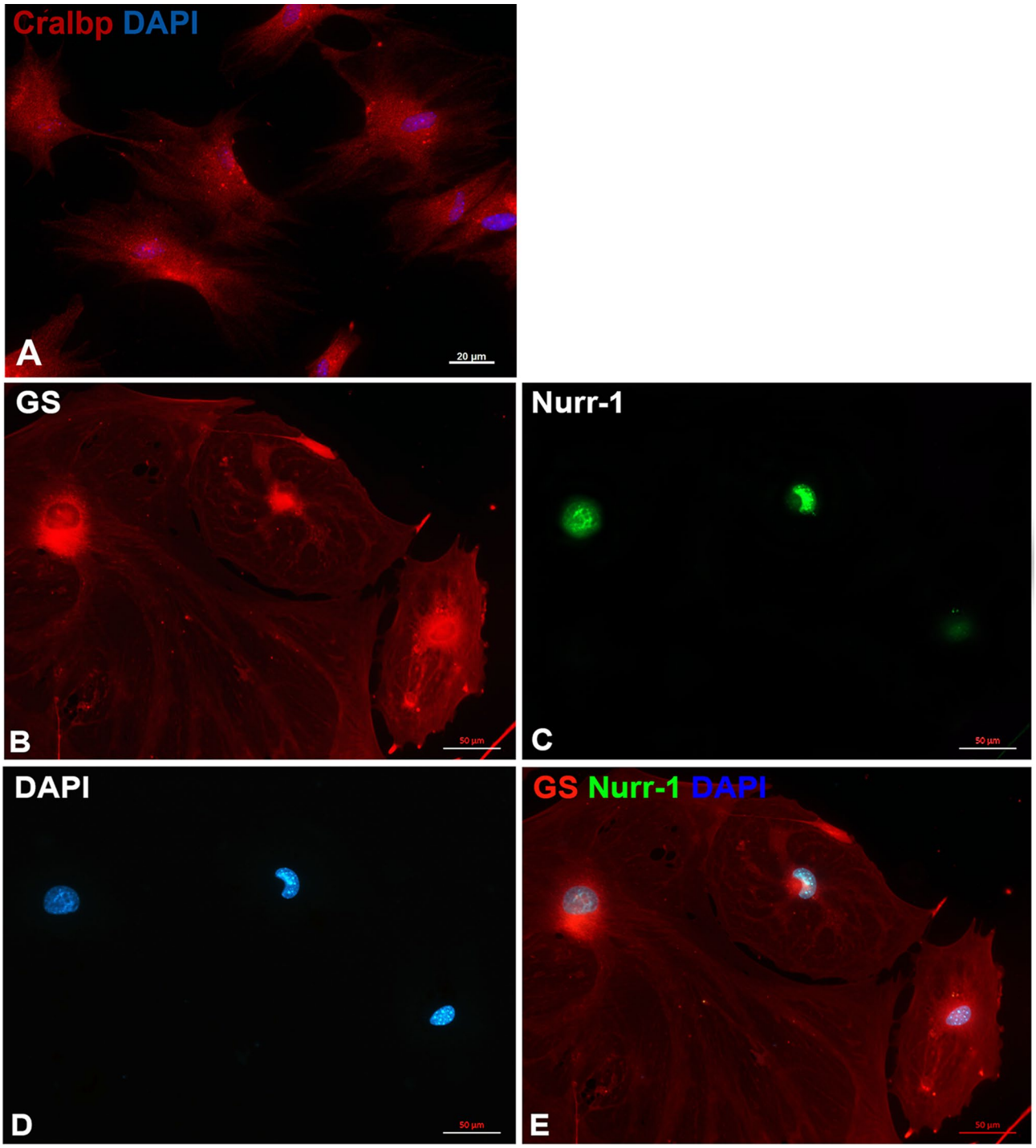


Fig. 4 Mice CB neurosphere-derived Müller glial cells expressing dopaminergic markers. A: Neurosphere-derived Müller glial cells from adult mice eye immunolabeled for Cralbp. **b**–**e** Neurosphere-derived Müller glial cells from adult mice eye double immunolabeled for GS (**a**) and Nurr-1 (**b**) after 7 days. Merged image is displayed in (**d**) with nuclei counterstained with DAPI (**g, d**). Scale bar: **a**–**d** 50 µm. $N = 3$ independent experiments

Millipore) were used as dopaminergic markers. Secondary antibodies, conjugated with Alexa 488 or 594 (1:400, Invitrogen), were incubated for 2 h in room temperature. Nuclei were stained with DAPI [4′,6-diamino-2-phenylindole (1 mg/ml, Sigma)] and slides were mounted in sodium n-propyl-gallate 0.2M pH 7.2 in glycerol. Immunofluorescent images were taken in an Imager M2 Apotome microscope (Zeiss).

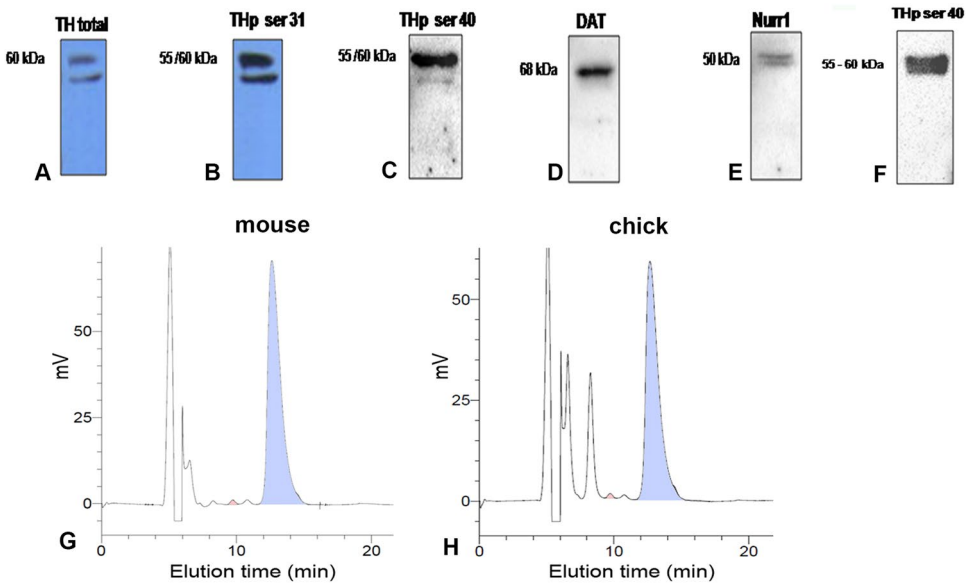


Fig. 5 Neurosphere-derived Müller glial cells express TH and release dopamine. Qualitative analysis by western blotting of samples of neurosphere-derived Müller glial cells from newborn (**a**–**e**) and adult (**f**) mice. Tyrosine hydroxylase (TH, 60 kDa, **a**), TH Serine 31 (55/60 kDa, **b**), and serine 40 residue (55/60 kDa, **c, d**), dopamine transporter (DAT, 68 kDa, **e**), Nurr-1 (50 kDa, **f**). **g, h** Chromatograph profiles from samples of adult mice (**g**) or chicken (**h**) conditioned medium identifying both dopamine (blue) and the dopamine-derived metabolite DOPAC (pink). Western blotting: $N=3$ independent experiments; HPLC: $N=3$ independent experiments

Qualitative Immunoblotting

Western blotting assessed the qualitative expression of specific dopaminergic markers. Müller glia was abundantly washed with cold PBS. The cell extracts were collected in protease inhibitor cocktail, centrifuged and the supernatants collected. The protein concentration of the samples was determined by BCA kit (Thermo Scientific) and diluted in a 2% sodium dodecyl sulfate containing solution to 2 µg/µl. Subsequently, proteins were separated by electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. The primary antibodies were incubated overnight at 4 °C followed by 2-h incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:10,000, Sigma), with gentle agitation at room temperature. For chemiluminescent detection, it was used ECL TM (GE Healthcare) or Luminata TM (Millipore), prior to image acquisition with ChemiDocTM Imager (Bio-Rad). For loading control the antibody against GAPDH (1:1000, Sigma). As primary antibodies were also used Anti-DAT (1:500, Chemicon), Anti-Nurr1 (1:400, Santa Cruz), Anti-TH (1:500, Millipore), Anti-phospho TH serine 31 (1:1000, Cell Signaling Technology) and Anti-phospho TH serine 40 (1:1000, Cell Signaling Technology).

Dopamine Release and Detection

Dopamine produced by progenitor-derived Muller cells was measured using HPLC analysis coupled with electrochemical detection (HPLC-ED), as described (Beckman et al. 2016). Briefly, differentiated Muller cells were incubated for 48 h with 10 µM Foskolin in DMEM/F12 supplemented with 2% FCS. Cultures were then washed 3 times with PBS and incubated for 2 h with 2 mM L-tyrosine diluted in Hank's solution. The resulting conditioned media was collected and perchloric acid was added to each sample to a final concentration of 0.1 M. Samples were centrifuged (10,000 *g*; 4 °C) for 10 min and supernatants were used for HPLC analysis. Isocratic separation was obtained using a reverse phase LC-18 column (4.6–250 mm; Supelco) with the following mobile phase: 20 mM sodium phosphate; 20 mM citric acid, 10% methanol, 0.12 mM Na2EDTA, and 566 mg/L of heptanesulfonic acid, pH 2.64.

Conclusions

In conclusion, our findings show that the glial dopaminergic phenotype is a default condition for Müller glia in vitro, even when cells are obtained from adult progenitors. Müller

cells have been shown to retain this phenotype when dissociated and grafted, and to functionally improve a parkinsonian mouse model (Stutz et al. 2014). Given the fact that RPCs can be isolated from early postnatal to seventh-decade human eyes using post mortem tissue (Chen et al. 2014; Coles et al. 2004), our protocol could be a viable source of dopaminergic glia to be used in cell therapy strategies against Parkinson's or degenerative retinal diseases.

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Author Contributions BSR: Performed cell culturing, fluorescent imaging, statistical analysis and manuscript writing. LES: Performed biochemistry assays of western blotting, HPLC, quantitative analysis as well as manuscript writing. RAMR: Performed cell culturing, interpretation of results, and writing of manuscript. FGM: interpretation of results, writing of manuscript and the supervision of the project. VTRR: contributed to the general administration, fluorescence imaging, interpretation of results, development, and writing of the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interests.

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