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Activation of neural progenitor cells in human eyes with proliferative vitreoretinopathy☆

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ABSTRACT

In addition to the ability for self-renewal and functional differentiation, neural stem/progenitor cells (NSCs) can respond to CNS injuries by targeted migration. In lower vertebrates, retinal injury is known to activate NSCs in the ciliary marginal zone (CMZ). Cells expressing markers of NSCs are also present in the ciliary body epithelium (CE) and in Müller glia in the peripheral retina (PR) of the adult human eye. However, these cells seem to be quiescent in the adult human eye and recent reports have shown that CE cells have limited properties of NSCs. In order to further clarify whether NSCs exist in the adult human eye, we tested whether NSC-like cells could be activated in eyes with proliferative vitreoretinopathy (PVR). The PR and CE were studied for NSC-associated markers in human enucleated control eyes and eyes with confirmed PVR, as well as in a mouse model of PVR. Furthermore, cells isolated from vitreous samples obtained during vitrectomies for retinal detachment were directly fixed or cultured in a stem cell-promoting medium and compared to cells cultured from the post-mortem retina and CE. *In situ* characterization of the normal eyes revealed robust expression of markers present in NSCs (Nestin, Sox2, Pax6) only around peripheral cysts of the proximal pars plana region and the PR, the latter population also staining for the glial marker GFAP. Although there were higher numbers of dividing cells in the CE of PVR eyes than in controls, we did not detect NSC-associated markers in the CE except around the proximal pars plana cysts. In the mice PVR eyes, Nestin activation was also found in the CE. In human PVR eyes, proliferation of both non-glial and glial cells co-staining NSC-associated markers was evident around the ora serrata region. Spheres formed in 7/10 vitreous samples from patients with PVR compared to 2/15 samples from patients with no known PVR, and expressed glial − and NSC-associated markers both after direct fixation and repetitive passages. In conclusion, the adult human eye may harbor two different populations of neuroepithelial stem/progenitor cells; a non-glial population located in the proximal pars plana around peripheral cysts in addition to a population with Müller glia characteristics. Yet, we only found that the glial population was able to respond to retinal injury by targeted migration into the vitreous.

1. Introduction

During development of the human retina, neuroepithelial stem/progenitor cells (NSCs) from the inner layer of the optic cup give rise to both distinct cell types of the neuroretina that becomes an integrated part of the central nervous system (CNS), as well as two non-neural structures; the double-layered ciliary body epithelium (CE) and the iris pigmented epithelium (IPE) (Perron and Harris, 2000). In adults, the retina is considered to have limited regenerative potential, and severe injuries lead to permanent damage (Klassen et al., 2004). However, in cold-blooded vertebrates such as fish and amphibians NSCs located in a circumferential zone of cells known as the ciliary

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marginal zone (CMZ), situated between the retina and the CE, can regenerate new retinal neurons throughout life (Perron and Harris, 2000). In addition, new retinal neurons are generated at the peripheral edge of the postnatal chick retina up to one month after hatching (Fischer and Reh, 2000). There is also evidence of a similar CMZ-like region in monkeys (Fischer et al., 2001) and humans (Martinez-Navarrete et al., 2008) (Bhatia et al., 2009), although these cells seems to be in a quiescent state (Bhatia et al., 2010).

Indications of an analogous stem cell-like population in the CE of the human retina (Coles et al., 2004; Tropepe et al., 2000; Xu et al., 2007) has prompted a number of investigations of their proliferative and differentiation potential *in vitro* and *in vivo* after transplantation to the mouse eye (Inoue et al., 2010). Since both the CE and IPE are derived from the neuroepithelium during embryonic development, but are much more surgically accessible than the neuroretina, huge expectations have emerged for these locations as candidate sources for stem cell therapies. However, recent investigations have provided evidence that the mammalian CE does not have the abilities of NSCs as previously thought. We have recently shown that sphere-forming cells isolated from the adult human CE and IPE have more epithelial properties and limited expression of NSC-associated markers compared to progenitor cells isolated from the human brain (Froen et al., 2011; Moe et al., 2009). Furthermore, other groups have shown that although cells isolated from the CE could be induced to express low levels of neuronal markers, they retained their epithelial morphology and failed to differentiate into retinal neurons (Bhatia et al., 2011; Cicero et al., 2009; Gualdoni et al., 2010). In addition, Bhatia et al. has also shown that the normal adult human CE lack crucial markers of NSCs such as Nestin *in situ* (Bhatia et al., 2009).

A somatic stem cell is commonly defined as a cell with the ability to self-renew and give rise to all the functional cell types of the organ from which they originate (Gage, 2000; Moe et al., 2005; Reh, 2002). In suspension culture, NSCs have the ability to form spheres with a uniform well-defined spherical contour that are mainly formed through cellular divisions (Gage, 2000; Reynolds and Weiss, 1992; Westerlund et al., 2003). Although the sphere-forming process is not specific for stem cells, their three dimensional structure is known to consist of a hierarchical organization with both undifferentiated cells and more differentiated progeny (Louis et al., 2008; Singec et al., 2006). Another key property of NSCs is detection and targeted migration into CNS lesions (Aboody et al., 2000; Imitola et al., 2004; Olstorn et al., 2007). One relatively common CNS lesion in ophthalmology is the development of proliferative vitreoretinopathy (PVR) after retinal detachment (RD) surgery. If NSCs are present in adult mammalian eyes, they might detect retinal injury and respond upon PVR formation by activation and targeted migration into the lesion area. In order to further clarify whether NSCs exist in the adult human eye, we carefully investigated the CE and PR for NSC-associated markers in human enucleated control eyes and eyes with confirmed PVR, as well as in a mice model of PVR. Finally, we looked for signs of targeted migration of NSC-like cells in the vitreous of patients operated with vitrectomy for RD and PVR formation.

2. Materials and methods

2.1. Dissection procedure

All experiments were conducted in accordance with the Declaration of Helsinki and all tissue harvesting was approved by the Local Committees for Medical Research Ethics.

2.1.1. In situ analysis of PR and CE

Control eyes (with no known PVR) were enucleated from human cadavers 24–48 h post-mortem as previously described (Slettedal et al., 2007). Samples were fixed in 4% fresh paraformaldehyde (PFA). The anterior segment was removed and axial sections were made from the iris to the mid-peripheral retina (Fig. 1A) and the specimens were then embedded in paraffin prior to sectioning. One enucleated cadaveric eye with known chronic RD and PVR formation (Fig. 2A), as well as two eyes enucleated due to extensive PVR and phthisis development from a collection of PFA- and paraffin-embedded ophthalmic pathology specimens were also included in the study.

2.1.2. Vitreous samples

After written informed consent, vitreous samples were obtained during vitrectomies for RD with or without confirmed PVR based on evaluation of wide angle images (Optomap P200Tx, Optos, Dunfermline, UK) (Fig. 4A–C). Cases where retinotomies, retinectomies or cutting of the retinal tear was performed got excluded from the study. The vitreous samples were centrifuged at 15 000 rpm for 5 min and the resulting pellets were either fixed in 4% PFA (direct fixation) or cultivated *in vitro*.

2.1.3. Retinal and ciliary epithelial tissue

Retinal tissue was carefully isolated from cadaveric eyes. The CE was isolated as previously described (Moe et al., 2009). No attempt was made to separate the pigmented from the non-pigmented CE in the present study.

2.2. In vitro cultures

The tissues were rinsed in Leibowitz-15 medium (L15, Invitrogen, Carlsbad, CA) and incubated with trypsin-EDTA (0.05%, Invitrogen) for 5 + 5 min followed by careful trituration. The cell suspension was passed through a 70 μm strainer (BD Biosciences, San Diego, CA). The cells were cultured in DMEM/F12 containing B27 supplement (2%, Invitrogen), EGF (20 ng/ml, R&D Systems), bFGF (10 ng/ml, R&D Systems, MN), 1% fetal calf serum (FCS, Sigma–Aldrich), Heparin (2.5 μg/ml, LEO Pharma, Denmark) and Penicillin/Streptomycin (100 U/ml, Sigma–Aldrich, St. Louis, MO) at 37 °C in 5% CO_2 and 20% O_2. Cultures were supplemented with bFGF and EGF twice a week and passaged every two to three weeks by incubation in trypsin-EDTA (0.05%, Invitrogen) for 2 × 4 min.

2.3. Mouse model of proliferative vitreoretinopathy induced by dispase

In order to reproduce the pathological environment of PVR formation in a controlled animal study, we utilized a mouse model of PVR induced by intravitreal injection of the proteolytic enzyme dispase. This model is known to induce glial activation as well as both epi – and subretinal membrane formation (Canto Soler et al., 2002; Frenzel et al., 1998). All animal experiments were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Study protocols were approved by the Animal Care Committee of the University of Debrecen. Female 4–6 months old wildtype mice (C57/BL6, $n = 6$) were anesthetized with pentobarbital (90 mg kg^{-1}, i.p.), received one drop of 1% procaine hydrochloride (Novocaine) for local anesthesia and one drop of tropicamide (Mydrum) for iris dilatation. 4 μl of dispase (Sigma; 0.4 U μl^{-1}, dissolved in sterile physiological saline) was injected intravitreally in the right eyes under microscopically control using an automatic pipette fitted with 30G 1/6 needle, as previously described (Canto Soler et al., 2002). Control animals received 4 μl of sterile physiological saline solution. Stratus Optical Coherence Tomography images (OCT, Zarl Zeiss Meditec, DublinCA) were taken following injections to monitor disease progression (Fig. 3). Control and dispase treated mice were sacrificed between 7

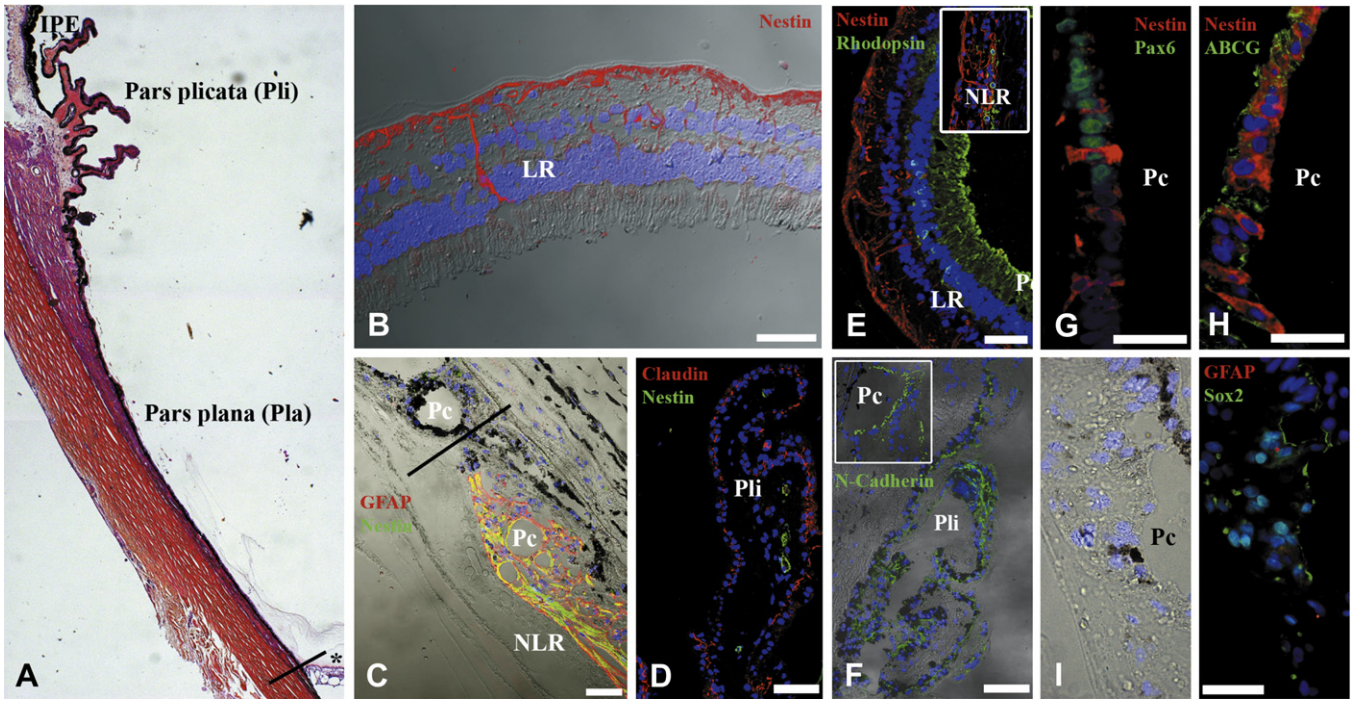

Fig. 1. *In situ* characterization of the most peripheral neural retina and ciliary body epithelium (CE) of the adult human eye. (A) Light microscopic overview from the iris pigmented epithelium (IPE), the CE and peripheral cysts (Pc, *) in the non-laminated retina. Solid line represents the ora serrata, two images are merged. (B) Nestin staining of the inner retinal surface and of cell with Müller glia morphology in the laminated retina (LR) centrally. (C) Nestin and GFAP expression in the non-laminated far peripheral retina with Pc. (D) Nestin and Claudin expression in the CE pars plicata. (E) Rhodopsin and Nestin expression in LR and non-laminated retina (NLR) peripherally (inset). (F) N-Cadherin staining in the CE pars plicata and peripheral retina (inset). (G) Nestin and Pax6 staining of cells lining the wall of Pc of the proximal pars plana. (H) Nestin and ABCG2 staining of cells lining the wall of Pc of the proximal pars plana. (I) GFAP and Sox2 staining of the proximal pars plicata with Pc. Staining for Sox2 was found both in the cytoplasm and nucleus. Nuclear staining with Hoechst (blue). Scale bars: 50 μm.

and 14 days following injections when signs of PVR formation were evident (Fig. 3 right OCT image). PVR formation was validated in cryosections by the presence of cellular hyperplasia (Fig. 3C and D), retinal folding (Fig. 3C) and GFAP$^+$ epiretinal membranes (Fig. 3G).

2.4. Immunohistochemistry

2.4.1. In situ analysis of PR and CE

For human paraffin-embedded specimens, 3–10 μm sagittal sections were cut and stained using LabVision Autostainer360 (Lab Vision Corporation, VT). Mouse eyes were fixed in neutral buffered 4% PFA, embedded in OCT (Tissue-TEK, Sakura Finetek, CA), cut into 10 μm sections on a freezing microtome, thawed onto Super Frost/Plus object glasses (Menzel-Gläser, Braunschweig, Germany) and stored at −20 °C before immunohistochemical analysis (Olstorn et al., 2007).

2.4.2. In vitro cultures

A mixture of human plasma and thrombin (Sigma–Aldrich) was used to clot spheres together before fixation in 4% PFA and embedment in paraffin. Three μm sections were cut and stained. Adherent cultivated cells were fixed with 4% PFA and stained as previously described (Olstorn et al., 2007).

2.4.3. Antibodies

The following primary antibodies and dilutions were used (rb: rabbit, ms: mouse): N-cadherin (ms, 1:50; Dako), Claudin1 (rb, 1:200; LabVision), GFAP (ms, 1:100; Santa Cruz Biotechnology), Nestin (rb, 1:200; Sigma–Aldrich), Nestin (ms, 1:80; Santa Cruz Biotechnology), β-III-tubulin (ms, 1:1000; Sigma–Aldrich), Rhodopsin (ms, 1:1500; Sigma–Aldrich), Ki-67 (rb, 1:200; Neo Markers), Sox2 (rb, 1:500, Chemicon), Pax6 (ms, 1:1000; Chemicon), RPE65 (ms, 1:2000, Millipore) and ABCG2 (ms, 1:80, Sigma–Aldrich). The secondary antibodies had the fluorescent markers Cy3 (1:1000; Sigma–Aldrich) and Alexa Fluor 488 (1:500; Invitrogen). Hoechst (1:500; Invitrogen) was used for nuclear staining. The sections were analyzed using an Olympus BV 61 FluoView confocal microscope (Olympus, Hamburg, Germany) and a ZEISS Axio Observer.Z1 fluorescence microscope (ZEISS, Oberkochen, Germany). Sections were also stained with hematoxylin & eosin (H&E) for morphological examination.

2.5. Quantitative PCR (qPCR)

Total RNA was extracted using TRIzol Reagent according to the manufacturer's instructions, RNA concentration and purity was measured using Nanodrop (Wilmington, DE). Reverse transcription (RT) was performed using the High Capacity cDNA Archive Kit (Applied Biosystems, Abingdon, U.K.) with 200 ng total RNA per 20 μl RT reaction. The qPCR was performed using the StepOnePlus RT-PCR system (Applied Biosystems) and Taqman Gene Expression assays following protocols from the manufacturer (Applied Biosystems) (Table 1). The thermo cycling conditions were 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The data were analyzed by the $2^{-\Delta\Delta Ct}$ method as the fold change in gene expression relative to CE which was arbitrarily chosen as calibrator and equals one. All samples were run in duplicates (each reaction: 2.0 μl cDNA, total volume 15 μl).

2.6. Transmission electron microscopy

The spheres were fixed for 30–60 min at room temperature by immersion in freshly prepared mixed aldehyde-fixation containing 0.1M sodium cacodylate buffer, 2% glutaraldehyde, 2% PFA and 0.025% $CaCl_2$, pH 7.4. Fixation was continued overnight at 4 °C,

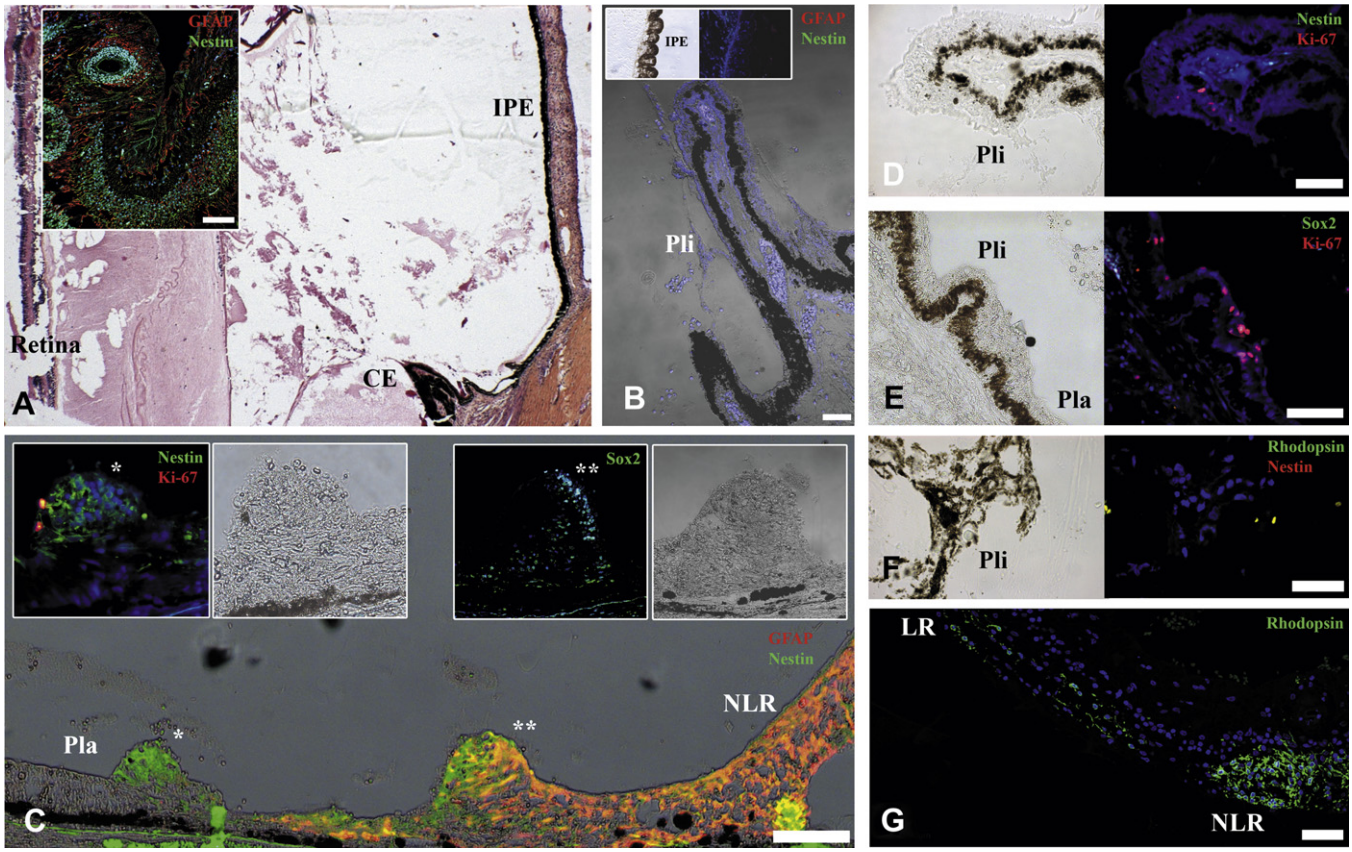

Fig. 2. *In situ* characterization of the most peripheral neural retina and ciliary body epithelium (CE) of the adult human eye with proliferative vitreoretinopathy (PVR) formation. (A) Light microscopic overview showing detached retina, exudates and adenomatous-like extension of the pars plicata CE, two images are merged. Inset: Thick central retina with PVR scar formation stained with GFAP and Nestin. (B) GFAP and Nestin staining of the pars plicata (Pli) CE and iris pigment epithelium (IPE, inset). (C) GFAP and Nestin staining of the peripheral non-laminated retina (NLR), ora serrata (line) and pars plana (Pla). The left rounded elevation of the surface (*) are also stained with Nestin and Ki-67 (left inset) and the right elevation (**) stained with Sox2 (right inset). Ki-67 and Nestin (D), Sox2 and Ki-67 (E) and Rhodopsin and Nestin (F) staining of the CE Pli, respectively. (G) Rhodopsin staining of the peripheral laminated retina (LR) and NLR. Nuclear staining with Hoechst (blue). Scale bars: 50 μm.

postfixed in 1% osmium tetroxide, and dehydrated through a graded series of ethanol up to 100%. The spheres were then immersed in propylene oxide for 20 min and embedded in Epon (Electron Microscopy Sciences, Hatfield, PA). Ultra-thin sections (60–70 nm thick) were cut on a Leica Ultracut Ultramicrotome UCT (Leica, Wetzlar, Germany), stained with uranyl acetate and lead citrate and examined using a Tecnai12 transmission electron microscope (Phillips, Amsterdam, the Netherlands).

2.7. Statistics

The percentage of cells positive for the immunofluorescent markers was calculated from counting 100 cells from 5 different sections, and the expression pattern was evaluated by two independent investigators. Results are presented as mean ± SD and differences between groups were tested by the two-tailed independent sample *t*-tests. The significance level was set to $p < 0.05$. Data were analyzed using SPSS Version 18.0.

3. Results

3.1. The ciliary epithelium and peripheral retina of the normal human eye

Nestin is an intermediate filament normally expressed in neural stem/progenitor cells (Lendahl et al., 1990), but are also upregulated in the adult CNS during pathological situations such as glial scar formation (Frisen et al., 1995). In the laminated central retina only a few cells with Müller glia morphology stained for Nestin (Fig. 1B), in addition to some Nestin staining on the inner retinal surface. In coherence with two previous reports (Bhatia et al., 2009; Martinez-Navarrete et al., 2008), a gradual increase in the density of Nestin staining inside the retina was observed towards the peripheral retina of all eyes examined ($n = 4$). In control eyes, the Nestin staining abruptly diminished at the ora serrata (Fig. 1C) and was neither found in the peripheral pars plana nor the pars plicata of the CE (Fig. 1D). Nestin staining was strongest in cells close to the wall of peripheral cysts, both in the peripheral retina (Fig. 1C) and in most proximal part of the pars plana (Fig. 1G and H). Cystic degenerations in the peripheral retina, ora serrata and the pars plana are common in the uninjured human eye and increases in frequency with age, but with no known pathological consequence (Fischer et al., 2001; O'Malley and Allen, 1967). Cells lining the wall of the cysts also stained for nuclear Pax6 (Fig. 1G) and both nuclear – and cytoplasmic Sox2 (Fig. 1I), two central transcription factors controlling eye development that are expressed in retinal progenitor cells (Bhatia et al., 2011; Davis et al., 2009; Taranova et al., 2006). Glial fibrillary acidic protein (GFAP), a marker of reactive astrocytes also expressed in Müller glia (Doetsch et al., 1999; Lawrence et al., 2007), was only found in cells around the cysts of the peripheral retina (Fig. 1C) and not around cysts in the proximal pars plana or further peripheral in the CE (Fig. 1I).

Differentiated photoreceptors in the laminated retina robustly stained with Rhodopsin (Fig. 1E). In coherence with studies in

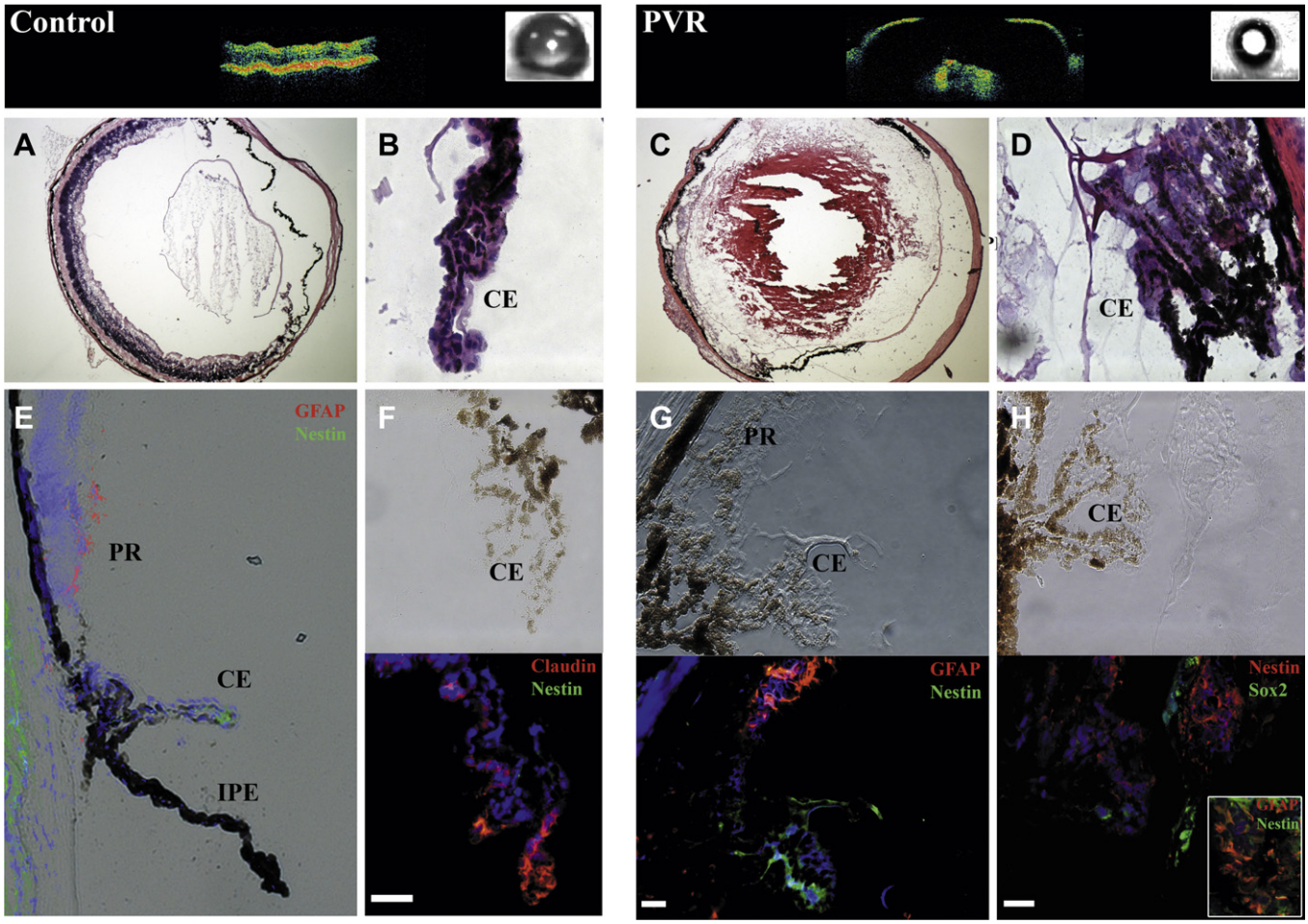

Fig. 3. *In situ* characterization of peripheral retina (PR) and ciliary body epithelium (CE) of control mice and mice with proliferative vitreoretinopathy (PVR). OCT image of control retina (left panel) and PVR retina (right panel) after intravitreal Dispase injection. (A) Light microscopic appearance of control eye with H&E staining, and (B) close-up of CE. (C) Light microscopic appearance of PVR eye with H&E staining, and (D) close-up of CE showing nuclear hyperplasia and adenomatous-like proliferations. (E) GFAP and Nestin staining of the PR and CE of control eye. (F) Claudin and Nestin staining of CE in control eye. (G) GFAP and Nestin staining of PR and CE of PVR eye. (H) Nestin and Sox2 and Nestin and GFAP (H inset) staining of CE and peripheral vitreous in PVR eye. Nuclear staining with Hoechst (blue). Scale bars: E, 100 μm; F–H, 50 μm.

monkeys (Fischer et al., 2001), some Rhodopsin$^+$ cells were also seen in the non-laminated periphery of the retina (Fig. 1E inset), but in these cells the Rhodopsin staining was more evenly distributed and the cells had a rounded cell body compared to the mature photoreceptors. We also confirmed that the adult human CE *in situ* stained for differentiated epithelial markers such as the tight-junction marker Claudin (Fig. 1D) and the adherence junction marker N-Cadherin (Fig. 1F). N-Cadherin and the ATP-binding cassette transporter G2 (ABCG2), that is expressed in both epithelial and neuroepithelial stem/progenitor cells (Ding et al., 2010; Watanabe et al., 2004), were also found in cells lining the wall of the peripheral cysts (Fig. 1F inset+H).

Table 1
Primers used for Quantitative PCR (qPCR).

Gene Symbol	Assay ID	Gene Symbol	Assay ID
GAPDH	Hs99999905_m1	Lhx2	Hs00180351_m1
KLF4	Hs00358836_m1	MITF	Hs01117294_m1
Oct4	Hs03005111_g1	Chx10	Hs01584046_m1
Nanog	Hs02387400_g1	Otx2	Hs00222238_m1
Sox2	Hs01053049_s1	Nestin	Hs00707120_s1
c-MYC	Hs00905030_m1	Tyrosinase	Hs00165976_m1
Notch1	Hs01062011_m1	GFAP	Hs00157674_m1
PAX6	Hs01088112_m1	CRALBP	Hs00165632_m1
RAX	Hs00429459_m1	Ki-67	Hs01032443_m1
Six3	Hs00193667_m1	GS	Hs00365928_g1
Six6	Hs00201310_m1	NRL	Hs00172997_m1

3.2. *The ciliary epithelium and peripheral retina in eyes with proliferative vitreoretinopathy*

In eyes with PVR (Fig. 2A) ($n = 3$), there were extensive exudates and membrane formation and the retina appeared gliotic (Fig. 2A inset). In these eyes, the Nestin staining extended from the ora serrata and into the proximal pars plana (Fig. 2C) in all samples examined. In coherence with the findings in control eyes, two types of cell clusters could be identified based on the glial marker GFAP; Nestin$^+$ cells of the retinal periphery and around ora serrata that double-stained with GFAP (Fig. 2C) and Sox2 (Fig. 2C right inset), and clusters of Nestin$^+$/GFAP$^-$ cells found in the non-pigmented proximal pars plana epithelium (Fig. 2C). In these clusters, there were also signs of active cell division indicated by Ki-67 staining (Fig. 2C, left inset), a nuclear marker for cellular proliferation (Scholzen and Gerdes, 2000) There were also signs of increased cell proliferation more peripherally in the non-pigmented CE in response to PVR formation (Fig. 2D), and especially in the transition zone between the pars plana and pars plicata (Fig. 2E). In control eyes, 1.6 ± 1.1% ($n = 5$) of the cells in this region stained for Ki-67, compared to 12.0 ± 5.7% in PVR eyes, respectively ($p < 0.05$). No cells

positive for the glial marker GFAP or the NSC-associated markers Nestin and Sox2 were found in either the peripheral pars plana, pars plicata or the IPE (Fig. 2B, D–E). In PVR eyes, clusters of Rhodopsin positive cells were found in the non-laminated retina peripheral to large areas of photoreceptor loss (Fig. 2G). However, even in these eyes with extensive retinal damage, we found no Rhodopsin$^+$ cells (Fig. 2F), in addition to no markers of NSCs, in the peripheral pars plana, pars plicata or IPE.

3.3. The ciliary epithelium and peripheral retina in a mouse model of proliferative vitreoretinopathy

In order to reproduce the pathological environment of PVR formation in a controlled animal study, we utilized a mice model for PVR generated by intravitreal dispase injection (Canto Soler et al., 2002). The pars plana is not more than 12 to 16 cells wide in mice (Nishiguchi et al., 2008), thus no attempt was made to separate the pars plana and pars plicata of the CE. Compared to control eyes (Fig. 3A–B), the CE of mice PVR eyes showed nuclear hyperplasia and adenomatous-like proliferation (Fig. 3C–D). A few scattered Nestin$^+$ cells were found in the control CE (Fig. 3E), while most of the CE cells were Nestin$^-$ (Fig. 3F), and no cells stained for Pax6 and Sox2 (not shown). In addition, there was robust staining for the epithelial marker Claudin in the CE (Fig. 3F), and a few cells on the inner retinal surface were GFAP$^+$ (Fig. 3E).

In response to PVR formation, there was a gliotic reaction on the retinal surface evident by increased GFAP staining (Fig. 3G). In contrast to human PVR eyes, there was an upregulation of Nestin in the CE of mice PVR eyes (Fig. 3G); in control eyes $12.0 \pm 4.0\%$ ($n = 5$) cells stained for Nestin, compared to $25.2 \pm 11.5\%$ in PVR eyes ($p < 0.05$). In the PVR eyes, clusters of Sox2$^+$/Nestin$^+$ (Fig. 3H) and Nestin$^+$/GFAP$^+$ (Fig. 3H inset) cells were found in the peripheral vitreous, while this was not evident in control eyes.

3.4. Characterization of the sphere-like structures isolated from human vitreous of patients with proliferative vitreoretinopathy

If NSCs exist in the peripheral retina or CE of adult humans, they should be able to detect and migrate into CNS lesions such as retinal injuries, just as NSCs in other parts of the CNS do (Aboody et al., 2000; Imitola et al., 2004; Olstorn et al., 2007). Thus, in a further attempt to establish a clinicopathological correlation between putative NSCs and PVR formation in humans, we isolated the vitreous of patients undergoing vitrectomy for RD with and without confirmed PVR development preoperatively (Fig. 4A–C). During vitrectomy for RD with PVR, one can often visualize sphere-like structures in the far periphery close to the vitreous base (Fig. 4D). When we carefully examined the vitreous samples of these patients, we could isolate such sphere-like structures (Fig. 4E) and do further immunohistochemical characterization of their content. Care was taken not to include retinal tissue in the analysis and samples from surgeries where retinotomies, retinectomies or cutting of the retinal tear were performed, was not included in the study. Most of the cells inside the isolated sphere-like structures stained for both Nestin (Fig. 4F) and GFAP (Fig. 4F inset). Even though pigment epithelial cells were diffusely distributed within the vitreous, none of the cells inside the spheres stained for RPE65 (Fig. 4F). We were also able to detect cells positive for Sox2 (Fig. 4G) and for Pax6 (Fig. 4H inset) inside these sphere-like structures. We did not detect any cells positive for the photoreceptor marker Rhodopsin, but there were a few cells positive for the immature neuronal marker β-III-tubulin (Fig. 4H).

3.5. Sphere-forming capacity and expression of markers present in NSCs by single cells isolated from the vitreous of patients with retinal detachment

To characterize the extent to which cells in the vitreous of patients with retinal injury display a retinal stem/progenitor expression profile, isolated cells obtained during vitrectomies for RD were cultivated and morphological, immunohistochemical and qPCR analysis were performed. In the culture of cells from patients with no preoperatively confirmed PVR, primary spheres (P0) formed in 2/15 samples, compared to 7/10 from patients with confirmed PVR (Table 2). These spheres could be repetitively passaged up to P2 (no attempts were made for further passages) (Fig. 5A). Transmission electron microscopy revealed light and dark

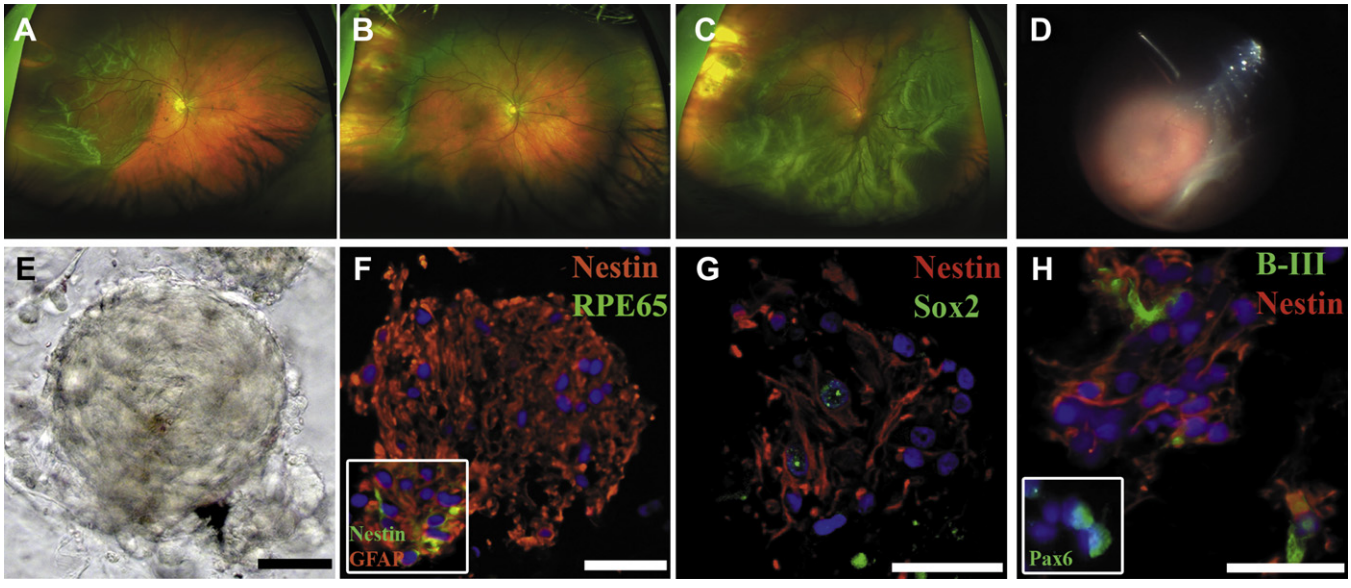

Fig. 4. Characterization of sphere-like structures isolated from the human vitreous of patients with proliferative vitreoretinopathy (PVR). (A) Primary retinal detachment with retinal tear in upper temporal quadrant. (B) Postoperative appearance after initial successful primary buckling surgery. (C) 3 months postoperatively extensive PVR formation has occurred. (D) During vitrectomy of PVR retinal detachments, sphere-like structures can be visualized close to the vitreous base. (E) Light microscopic appearance of isolated sphere-like structure of eye with PVR formation. Nestin and RPE65 (F), Nestin and GFAP (F inset), Nestin and Sox2 (G), β-III-tubulin and Nestin (H) and Pax6 (H inset) staining of sphere-like structures isolated from the vitreous of patients with PVR. Nuclear staining with Hoechst (blue). Scale bars: E–H, 50 μm.

Table 2
Clinical information and sphere-forming capacity of vitreous cells in patients with retinal detachment with/without proliferative vitreoretinopathy (PVR).

Retinal detachment	Numbers of patients	Age mean (range)	Sex female/ Male	Sphere formation
+PVR	10	56 (17–82)	1/9	7/10
−PVR	15	62 (45–87)	7/8	2/15

polymorphic cells with high nuclear/cytoplasmic ratio and occasional melanosomes in the central areas of P0 spheres (Fig. 5B inset), and peripheral more elongated and polarized cells with a smaller nuclear/cytoplasmic ratio (Fig. 5B). There was a robust staining of both Nestin (Fig. 5C–D) and GFAP (Fig. 5D) inside the spheres, while peripheral cells also stained for the immature neuronal differentiation marker β-III-tubulin (Fig. 5C).

Finally, we utilized qPCR to compare spheres at P1 formed from vitreous cells of patients with PVR to two well-characterized cell populations of the adult human eye that previously have been thought to have NSCs properties: cultures of retinal cells with a Müller glia phenotype (Bhatia et al., 2011; Lawrence et al., 2007)(Fig. 5F) and CE cells forming pigmented spheres *in vitro* (Coles et al., 2004; Moe et al., 2009)(Fig. 5E). As presumptive stem cell/early eye-field transcription factors that regulate retinal progenitors during development (Belecky-Adams et al., 1997; Lamba et al., 2010; Liu et al., 1994; Nishida et al., 2003; Rowan and Cepko, 2004), Otx2 and were found more expressed in retinal cultures compared to CE cultures, ($n = 3$, Table 3), while the apparent higher expression of Chx10 in retinal cultures and Sox2 in PVR cultures did not reach statistical difference ($p = 0.06$). In accordance with the presence of GFAP staining in the sphere-like structures of PVR eyes, the glial marker GFAP (Bhatia et al., 2009; Doetsch et al., 1999; Lawrence et al., 2007) showed a 40.4 higher expression in PVR cultures compared to CE cultures, while we did not detect any differences in glutamine synthetase (GS) expression. The microphthalmia-associated transcription factor (MITF) and tyrosinase, that are found in differentiating retinal pigment epithelial cells (Martinez-Morales et al., 2003; Nakayama et al., 1998), showed comparable expression in CE and PVR spheres, while MITF were significantly less expressed in retinal cultures (Table 3). Even though we did not detect Nestin$^+$ or Pax6$^+$ cells in the adult human CE *in situ* except around cysts in the proximal pars plana, their mRNA expression was comparable in all groups after *in vitro* cultures (Table 3). This further supports the previous findings that markers found in NSCs may be upregulated in epithelial cells of CE origin during sphere-promoting cultivation (Bhatia et al., 2011; Cicero et al., 2009; Kohno et al., 2006; Moe et al., 2009).

4. Discussion

In addition to the ability for self-renewal and functional differentiation, neural stem/progenitor cells (NSCs) should have the ability to respond to CNS injuries by targeted migration. In the adult human brain, there is evidence that NSCs in the subventrizular zone migrate in chains toward the olfactory bulb through the rostral migratory stream (Curtis et al., 2007), and we have previously shown that upon transplantation of adult human NSCs into adult rat brains with a selective injury in the hippocampus, the NSCs are able to migrate towards the injury (Olstorn et al., 2007, 2011). Since Tropepe et al. (2000) and Coles et al. (2004) first proposed that the adult human CE also contains a population of NSCs able to make new retinal cell types, a wide range of studies have been performed *in vitro* to investigate their properties as putative retinal stem cells. However, *in vitro* cultivation is known to induce expression of low levels of neuronal markers in CE cells while their epithelial phenotype is retained (Cicero et al., 2009; Moe et al., 2009). Thus, signs of targeted migration and neurogenesis of CE cells in response to retinal injury *in vivo* would significantly strengthen the hypothesis of the CE being a source of NSCs in adults.

In a previous *in situ* study of uninjured adult human retina, both the peripheral retina and the CE seemed quiescent with no

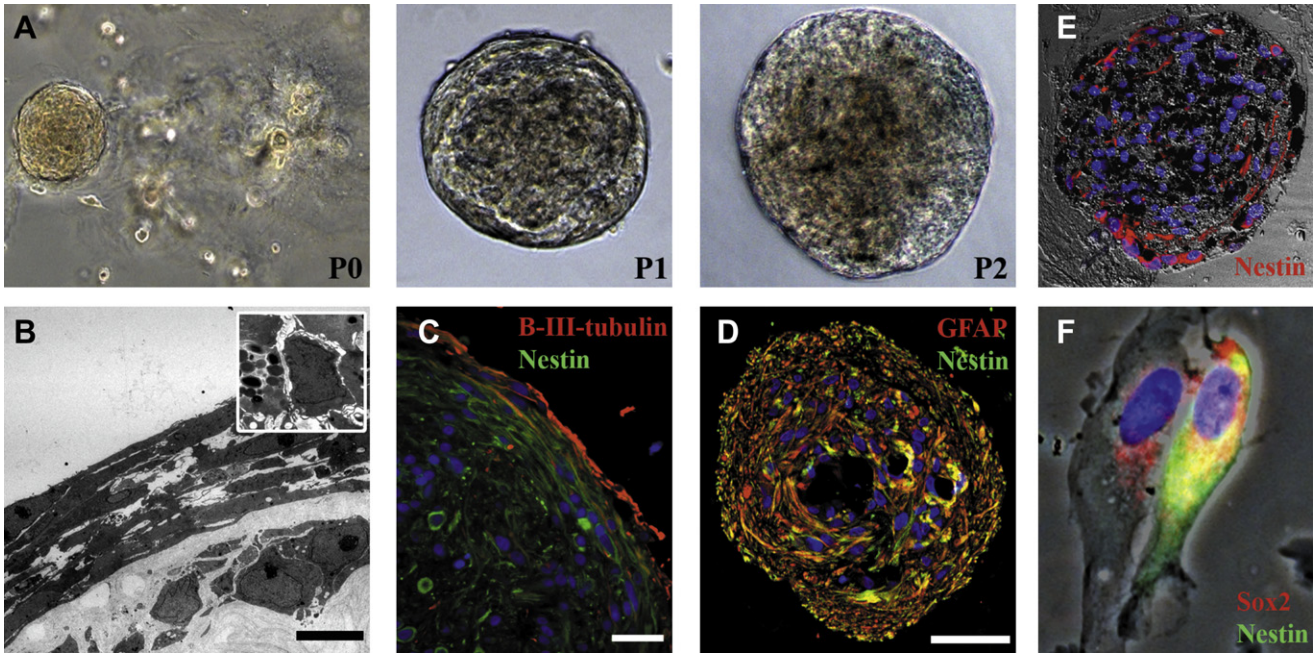

Fig. 5. Sphere-forming capacity and expression of NSC markers of isolated cells from the vitreous of patients with proliferative vitreoretinopathy (PVR). (A) Appearance of PVR spheres at P0, P1 and P2. (B) Electron microscopic appearance of peripheral and central (B inset) P0 PVR sphere. Nestin and β-III-tubulin (C) and Nestin and GFAP (D) staining of PVR sphere at P1. Pigmented ciliary body epithelium (CE) derived sphere stained with Nestin and GFAP (E) and adherent retinal cells stained with Sox2 and Nestin (F) at P1 for RT-PCR comparison analysis. Nuclear staining with Hoechst (blue). Scale bars: B. 10 μm; C, 50 μm, D, 100 μm.

Table 3
Comparative mRNA expression of genes in adherent retinal cultures and proliferative vitreoretinopathy (PVR) spheres compared to the human ciliary body epithelium (CE) spheres.

Gene symbol	Up/downregulation (fold change)	
	Retina	PVR
Pluripotency		
Oct4	2.65	−1.35
Sox2	4.25	7.28
Myc	1.39	−1.44
KLF4	−1.36	−1.62
Nanog	3.46	−1.11
Notch1	1.74	1.00
Early eye-field		
Pax6	−1.85	−1.85
RAX	1.44	1.75
SIX3	−1.16	−1.21
Six6	1.54	2.17
Lhx2	1.86	1.99
MITF	**−3.32**	1.57*
Otx2	**6.57**	2.66
Chx10	85.97	8.30
Nestin	−1.02	1.77
Differentiation		
GFAP	26.18	**40.42**
CRALBP	**31.37**	7.51
Ki-67	6.88	3.21
GS	**9.30**	2.52
NRL	**77.42**	3.54*
TYR	−11.25	1.34

The data were analyzed by $2^{-\Delta\Delta Ct}$ method as the fold change in gene expression and normalized to GAPDH as endogenous reference gene and relative to CE spheres, the mean value of which was arbitrarily chosen as calibrator and equals one. Bold values and values with * represents significant differences in the corresponding ΔΔCt value of the gene compared to CE and a significant difference between retina and PVR samples, respectively, with a significance level of $p < 0.05$.

evidence of cell division evidenced by lack of Ki-67 staining (Bhatia et al., 2009). However, these cells were able to re-enter the cell cycle following retinal explant culture. In the present study, we found that cell proliferation may be induced in the CE by retinal injury. Still, we did not find any NSC-associated markers in the peripheral pars plana and pars plicata epithelium in the eyes with PVR formation. In contrast, the mice CE contained both cellular hyperplasia and Nestin upregulation in response to PVR, suggesting that one should be careful in projecting key findings obtained in other mammals to whether NSCs exist in the CE of adult humans.

Another recent *in situ* report of 3 human eyes with PVR formation (Ducournau et al., 2011) describes nuclear hyperplasia and cell proliferation of the CE in response to injury forming "neurosphere-like" clusters, but without evidence of GFAP staining, which partly correspond with our *in situ* analysis of the CE. However, in contrast to our findings, they found a few Rhodopsin$^+$ cells in the vicinity of the pigmented CE in these eyes with extensive retinal injury. Since we did not detect markers present in NSCs such Sox2, Pax6 and Nestin in the major parts of the adult human CE, both in eyes without known retinal damage and in eyes with extensive PVR, this reduces the likehood that new photoreceptors are produced by NSCs in the CE in response to retinal injury.

However, careful immunohistochemical analysis of the most proximal pars plana epithelium and peripheral retina of adult human eyes revealed the presence of cells positive for both markers present in stem cells of neural origin (Pax6, Sox2, Nestin) and epithelial origin (ABCG2, N-Cadherin) could be detected around peripheral cysts. The cells lining the cysts in the peripheral retina were also positive for the glial marker GFAP while the cells in the proximal pars plana were not. Interestingly, both the anatomical localization close to fluid filled cavities and their immunohistochemical profile containing both neural and epithelial markers closely resembles the subventricular zone known to be a niche for NSCs in the brain (Doetsch et al., 1999; Westerlund et al., 2003). In eyes with PVR formation, we observed signs of proliferation both in the GFAP$^-$ and GFAP$^+$ population in the proximal pars plana and peripheral retina. In coherence with our findings, two morphologically distinct types of cysts have been characterized in monkey eyes (Fischer et al., 2001): one in the peripheral retina (Type I) surrounded by cells positive for Müller glia markers and early neuronal markers such as β-III tubulin and even some rounded Rhodopsin$^+$ cells, and another (Type II) in the pars plana just past the peripheral retinal edge, lined by pseudostratified epithelium of non-pigmented cells. Together, these findings support the evidence for a CMZ-like zone in the adult human eye as previously suggested (Bhatia et al., 2009; Martinez-Navarrete et al., 2008).

Even though both the present study and the study by Bhatia et al. (2009) support the presence of a glial and non-glial cell population with properties of NSCs around the ora serrata in humans, our analysis of the vitreous of patients with PVR found few GFAP$^-$ cells in the sphere-like structures, both directly fixed and *in vitro* cultivated. The qPCR also revealed a significantly higher expression of the Müller glial marker GFAP in PVR spheres compared to CE spheres, further indicating a glial origin of the PVR spheres. This was also supported by the finding that Nestin$^+$ and Sox2$^+$ cells in the vitreous of PVR mouse eyes showed co-staining with GFAP. It has been suggested that Müller glia may function in a way similar to radial glia in the brain during late development and that they may possess a latent neuroregenerative capacity also in humans (Das et al., 2006; Lawrence et al., 2007). Recently, it has been shown that by optimizing the culture conditions, Müller glia isolated from the peripheral retina during vitrectomies can be an efficient source for producing cells with properties of rod photoreceptors (Giannelli et al., 2011). Although Müller glia show neurogenic properties in the early postnatal retina of rodents and monkeys (Fischer et al., 2001; Karl et al., 2008), no evidence for their *in vivo* neurogenesis in response to injury has yet been observed in humans. In the present study, we found clusters of Rhodopsin$^+$ cells in the non-laminated retina peripheral to the large areas of photoreceptor loss and some cells positive for immature neuronal markers inside the isolated sphere-like structures of patients with PVR. Yet, we cannot conclude whether these are signs of active neurogenesis in response to injury. To do this, further analysis of more eyes with retinal damage, both using *in situ* immunohistochemical and hybridization analysis, must be performed. In addition, further characterization of clonal expansion and the differentiation potential of putative NSCs isolated from the vitreous of patients with different types of retinal damage and from the peripheral retina itself are needed.

In conclusion, the present study further supports the hypothesis that the adult human eye may harbor two different populations of neuroepithelial stem/progenitor cells: one non-glial population located close to peripheral cysts in the proximal pars plana and another population with Müller glia characteristics. So far, we only found evidence that the glial population is able to respond to retinal injury by targeted migration into the vitreous.

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References

Aboody, K.S., Brown, A., Rainov, N.G., Bower, K.A., Liu, S., Yang, W., Small, J.E., Herrlinger, U., Ourednik, V., Black, P.M., Breakefield, X.O., Snyder, E.Y., 2000. Neural stem cells display extensive tropism for pathology in adult brain:

evidence from intracranial gliomas. Proceedings of the National Academy of Sciences of the United States of America 97, 12846–12851.
Belecky-Adams, T., Tomarev, S., Li, H.S., Ploder, L., McInnes, R.R., Sundin, O., Adler, R., 1997. Pax-6, Prox 1, and Chx10 homeobox gene expression correlates with phenotypic fate of retinal precursor cells. Investigative Ophthalmology & Visual Science 38, 1293–1303.
Bhatia, B., Singhal, S., Lawrence, J.M., Khaw, P.T., Limb, G.A., 2009. Distribution of Muller stem cells within the neural retina: evidence for the existence of a ciliary margin-like zone in the adult human eye. Experimental Eye Research 89, 373–382.
Bhatia, B., Singhal, S., Jayaram, H., Khaw, P.T., Limb, G.A., 2010. Adult retinal stem cells revisited. The Open Ophthalmology Journal 4, 30–38.
Bhatia, B., Jayaram, H., Singhal, S., Jones, M.F., Limb, G.A., 2011. Differences between the neurogenic and proliferative abilities of Muller glia with stem cell characteristics and the ciliary epithelium from the adult human eye. Experimental Eye Research 93, 852–861.
Canto Soler, M.V., Gallo, J.E., Dodds, R.A., Suburo, A.M., 2002. A mouse model of proliferative vitreoretinopathy induced by dispase. Experimental Eye Research 75, 491–504.
Cicero, S.A., Johnson, D., Reyntjens, S., Frase, S., Connell, S., Chow, L.M., Baker, S.J., Sorrentino, B.P., Dyer, M.A., 2009. Cells previously identified as retinal stem cells are pigmented ciliary epithelial cells. Proceedings of the National Academy of Sciences of the United States of America 106, 6685–6690.
Coles, B.L., Angenieux, B., Inoue, T., Del Rio-Tsonis, K., Spence, J.R., McInnes, R.R., Arsenijevic, Y., van der Kooy, D., 2004. Facile isolation and the characterization of human retinal stem cells. Proceedings of the National Academy of Sciences of the United States of America 101, 15772–15777.
Curtis, M.A., Kam, M., Nannmark, U., Anderson, M.F., Axell, M.Z., Wikkelso, C., Holtas, S., van Roon-Mom, W.M., Bjork-Eriksson, T., Nordborg, C., Frisen, J., Dragunow, M., Faull, R.L., Eriksson, P.S., 2007. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science (New York, N.Y.) 315, 1243–1249.
Das, A.V., Mallya, K.B., Zhao, X., Ahmad, F., Bhattacharya, S., Thoreson, W.B., Hegde, G.V., Ahmad, I., 2006. Neural stem cell properties of Muller glia in the mammalian retina: regulation by Notch and Wnt signaling. Developmental Biology 299, 283–302.
Davis, N., Yoffe, C., Raviv, S., Antes, R., Berger, J., Holzmann, S., Stoykova, A., Overbeek, P.A., Tamm, E.R., Ashery-Padan, R., 2009. Pax6 dosage requirements in iris and ciliary body differentiation. Developmental Biology 333, 132–142.
Ding, X.W., Wu, J.H., Jiang, C.P., 2010. ABCG2: a potential marker of stem cells and novel target in stem cell and cancer therapy. Life Sciences 86, 631–637.
Doetsch, F., Caille, I., Lim, D.A., Garcia-Verdugo, J.M., Alvarez-Buylla, A., 1999. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97, 703–716.
Ducournau, Y., Boscher, C., Adelman, R.A., Guillaubey, C., Schmidt-Morand, D., Mosnier, J.F., Ducournau, D., 2011. Proliferation of the ciliary epithelium with retinal neuronal and photoreceptor cell differentiation in human eyes with retinal detachment and proliferative vitreoretinopathy. Graefe's Archive for Clinical and Experimental Ophthalmology.
Fischer, A.J., Reh, T.A., 2000. Identification of a proliferating marginal zone of retinal progenitors in postnatal chickens. Developmental Biology 220, 197–210.
Fischer, A.J., Hendrickson, A., Reh, T.A., 2001. Immunocytochemical characterization of cysts in the peripheral retina and pars plana of the adult primate. Investigative Ophthalmology & Visual Science 42, 3256–3263.
Frenzel, E.M., Neely, K.A., Walsh, A.W., Cameron, J.D., Gregerson, D.S., 1998. A new model of proliferative vitreoretinopathy. Investigative Ophthalmology & Visual Science 39, 2157–2164.
Frisen, J., Johansson, C.B., Torok, C., Risling, M., Lendahl, U., 1995. Rapid, widespread, and longlasting induction of nestin contributes to the generation of glial scar tissue after CNS injury. The Journal of Cell Biology 131, 453–464.
Froen, R.C., Johnsen, E.O., Petrovski, G., Berenyi, E., Facsko, A., Berta, A., Nicolaissen, B., Moe, M.C., 2011. Pigment epithelial cells isolated from human peripheral iridectomies have limited properties of retinal stem cells. Acta Ophthalmologica 89, e635–e644.
Gage, F.H., 2000. Mammalian neural stem cells. Science (New York, N.Y.) 287, 1433–1438.
Giannelli, S.G., Demontis, G.C., Pertile, G., Rama, P., Broccoli, V., 2011. Adult human Muller glia cells are a highly efficient source of rod photoreceptors. Stem Cells (Dayton, Ohio) 29, 344–356.
Gualdoni, S., Baron, M., Lakowski, J., Decembrini, S., Smith, A.J., Pearson, R.A., Ali, R.R., Sowden, J.C., 2010. Adult ciliary epithelial cells, previously identified as retinal stem cells with potential for retinal repair, fail to differentiate into new rod photoreceptors. Stem Cells (Dayton, Ohio) 28, 1048–1059.
Imitola, J., Raddassi, K., Park, K.I., Mueller, F.J., Nieto, M., Teng, Y.D., Frenkel, D., Li, J., Sidman, R.L., Walsh, C.A., Snyder, E.Y., Khoury, S.J., 2004. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proceedings of the National Academy of Sciences of the United States of America 101, 18117–18122.
Inoue, T., Coles, B.L., Dorval, K., Bremner, R., Bessho, Y., Kageyama, R., Hino, S., Matsuoka, M., Craft, C.M., McInnes, R.R., Tremblay, F., Prusky, G.T., van der Kooy, D., 2010. Maximizing functional photoreceptor differentiation from adult human retinal stem cells. Stem Cells (Dayton, Ohio) 28, 489–500.
Karl, M.O., Hayes, S., Nelson, B.R., Tan, K., Buckingham, B., Reh, T.A., 2008. Stimulation of neural regeneration in the mouse retina. Proceedings of the National Academy of Sciences of the United States of America 105, 19508–19513.
Klassen, H.J., Ng, T.F., Kurimoto, Y., Kirov, I., Shatos, M., Coffey, P., Young, M.J., 2004. Multipotent retinal progenitors express developmental markers, differentiate into retinal neurons, and preserve light-mediated behavior. Investigative Ophthalmology & Visual Science 45, 4167–4173.
Kohno, R., Ikeda, Y., Yonemitsu, Y., Hisatomi, T., Yamaguchi, M., Miyazaki, M., Takeshita, H., Ishibashi, T., Sueishi, K., 2006. Sphere formation of ocular epithelial cells in the ciliary body is a reprogramming system for neural differentiation. Brain Research 1093, 54–70.
Lamba, D.A., McUsic, A., Hirata, R.K., Wang, P.R., Russell, D., Reh, T.A., 2010. Generation, purification and transplantation of photoreceptors derived from human induced pluripotent stem cells. PLoS One 5, e8763.
Lawrence, J.M., Singhal, S., Bhatia, B., Keegan, D.J., Reh, T.A., Luthert, P.J., Khaw, P.T., Limb, G.A., 2007. MIO-M1 cells and similar muller glial cell lines derived from adult human retina exhibit neural stem cell characteristics. Stem Cells (Dayton, Ohio) 25, 2033–2043.
Lendahl, U., Zimmerman, L.B., McKay, R.D., 1990. CNS stem cells express a new class of intermediate filament protein. Cell 60, 585–595.
Liu, I.S., Chen, J.D., Ploder, L., Vidgen, D., van der Kooy, D., Kalnins, V.I., McInnes, R.R., 1994. Developmental expression of a novel murine homeobox gene (Chx10): evidence for roles in determination of the neuroretina and inner nuclear layer. Neuron 13, 377–393.
Louis, S.A., Rietze, R.L., Deleyrolle, L., Wagey, R.E., Thomas, T.E., Eaves, A.C., Reynolds, B.A., 2008. Enumeration of neural stem and progenitor cells in the neural colony-forming cell assay. Stem Cells (Dayton, Ohio) 26, 988–996.
Martinez-Morales, J.R., Dolez, V., Rodrigo, I., Zaccarini, R., Leconte, L., Bovolenta, P., Saule, S., 2003. OTX2 activates the molecular network underlying retina pigment epithelium differentiation. The Journal of Biological Chemistry 278, 21721–21731.
Martinez-Navarrete, G.C., Angulo, A., Martin-Nieto, J., Cuenca, N., 2008. Gradual morphogenesis of retinal neurons in the peripheral retinal margin of adult monkeys and humans. The Journal of Comparative Neurology 511, 557–580.
Moe, M.C., Varghese, M., Danilov, A.I., Westerlund, U., Ramm-Pettersen, J., Brundin, L., Svensson, M., Berg-Johnsen, J., Langmoen, I.A., 2005. Multipotent progenitor cells from the adult human brain: neurophysiological differentiation to mature neurons. Brain 128, 2189–2199.
Moe, M.C., Kolberg, R.S., Sandberg, C., Vik-Mo, E., Olstorn, H., Varghese, M., Langmoen, I.A., Nicolaissen, B., 2009. A comparison of epithelial and neural properties in progenitor cells derived from the adult human ciliary body and brain. Experimental Eye Research 88, 30–38.
Nakayama, A., Nguyen, M.T., Chen, C.C., Opdecamp, K., Hodgkinson, C.A., Arnheiter, H., 1998. Mutations in microphthalmia, the mouse homolog of the human deafness gene MITF, affect neuroepithelial and neural crest-derived melanocytes differently. Mechanisms of Development 70, 155–166.
Nishida, A., Furukawa, A., Koike, C., Tano, Y., Aizawa, S., Matsuo, I., Furukawa, T., 2003. Otx2 homeobox gene controls retinal photoreceptor cell fate and pineal gland development. Nature Neuroscience 6, 1255–1263.
Nishiguchi, K.M., Kaneko, H., Nakamura, M., Kachi, S., Terasaki, H., 2008. Identification of photoreceptor precursors in the pars plana during ocular development and after retinal injury. Investigative Ophthalmology & Visual Science 49, 422–428.
O'Malley, P.F., Allen, R.A., 1967. Peripheral cystoid degeneration of the retina. Incidence and distribution in 1,000 autopsy eyes. Archives of Ophthalmology 77, 769–776.
Olstorn, H., Moe, M.C., Roste, G.K., Bueters, T., Langmoen, I.A., 2007. Transplantation of stem cells from the adult human brain to the adult rat brain. Neurosurgery 60, 1089–1098. Discussion 1098–1089.
Olstorn, H., Varghese, M., Murrell, W., Moe, M.C., Langmoen, I.A., 2011. Predifferentiated brain-derived adult human progenitor cells migrate toward ischemia after transplantation to the adult rat brain. Neurosurgery 68, 213–222. Discussion 222.
Perron, M., Harris, W.A., 2000. Retinal stem cells in vertebrates. Bioessays 22, 685–688.
Reh, T.A., 2002. Neural stem cells: form and function. Nature Neuroscience 5, 392–394.
Reynolds, B.A., Weiss, S., 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science (New York, N.Y.) 255, 1707–1710.
Rowan, S., Cepko, C.L., 2004. Genetic analysis of the homeodomain transcription factor Chx10 in the retina using a novel multifunctional BAC transgenic mouse reporter. Developmental Biology 271, 388–402.
Scholzen, T., Gerdes, J., 2000. The Ki-67 protein: from the known and the unknown. Journal of Cellular Physiology 182, 311–322.
Singec, I., Knoth, R., Meyer, R.P., Maciaczyk, J., Volk, B., Nikkhah, G., Frotscher, M., Snyder, E.Y., 2006. Defining the actual sensitivity and specificity of the neurosphere assay in stem cell biology. Nature Methods 3, 801–806.
Slettedal, J.K., Lyberg, T., Ramstad, H., Beraki, K., Nicolaissen, B., 2007. Regeneration of the epithelium in organ-cultured donor corneas with extended post-mortem time. Acta Ophthalmologica Scandinavica 85, 371–376.
Taranova, O.V., Magness, S.T., Fagan, B.M., Wu, Y., Surzenko, N., Hutton, S.R., Pevny, L.H., 2006. SOX2 is a dose-dependent regulator of retinal neural progenitor competence. Genes and Development 20, 1187–1202.
Tropepe, V., Coles, B.L., Chiasson, B.J., Horsford, D.J., Elia, A.J., McInnes, R.R., van der Kooy, D., 2000. Retinal stem cells in the adult mammalian eye. Science (New York, N.Y.) 287, 2032–2036.
Watanabe, K., Nishida, K., Yamato, M., Umemoto, T., Sumide, T., Yamamoto, K., Maeda, N., Watanabe, H., Okano, T., Tano, Y., 2004. Human limbal epithelium contains side population cells expressing the ATP-binding cassette transporter ABCG2. FEBS Letters 565, 6–10.
Westerlund, U., Moe, M.C., Varghese, M., Berg-Johnsen, J., Ohlsson, M., Langmoen, I.A., Svensson, M., 2003. Stem cells from the adult human brain develop into functional neurons in culture. Experimental Cell Research 289, 378–383.
Xu, H., Sta Iglesia, D.D., Kielczewski, J.L., Valenta, D.F., Pease, M.E., Zack, D.J., Quigley, H.A., 2007. Characteristics of progenitor cells derived from adult ciliary body in mouse, rat, and human eyes. Investigative Ophthalmology & Visual Science 48, 1674–1682.