	V	O	P		5	4	7	**Operator:** LinPing		**Dispatch:** 17.04.07	**PE:** Scott Yager
	Journal Name			Manuscript No.				**Proofreader:** Zhang yeping		**No. of Pages:** 9	**Copy-editor:** Jessica Deacon

Veterinary Ophthalmology (2007) **10**, 4, 000–000

Neural precursors isolated from the developing cat brain show retinal integration following transplantation to the retina of the dystrophic cat

Henry Klassen,*,§§ Philip H. Schwartz,†,¶¶ Boback Ziaeian,*,† Hubert Nethercott,*,† Michael J. Young,‡ Ragnheidur Bragadottir,§ Gregory E. Tullis,¶ Karin Warfvinge** and Kristina Narfstrom††,‡‡

**Stem Cell Research and †National Human Neural Stem Cell Resource, Children's Hospital of Orange County Research Institute, Orange, CA, USA; ‡Schepens Eye Research Institute and Department of Ophthalmology, Harvard University School of Medicine, Boston, MA, USA; §Department of Ophthalmology, Ulleval University Hospital, Oslo, Norway; ¶Department of Ophthalmology, Boston University, Boston, MA, USA; **Department of Ophthalmology, Lund University, Lund, Sweden; ††Department of Veterinary Medicine & Surgery, College of Veterinary Medicine and ‡‡Department of Ophthalmology, Mason Eye Institute, University of Missouri, Columbia, MO, USA*

Address communications to:

Henry J. Klassen

Tel.: (65) 6322-4565
Fax: (65) 6322-4599
e-mail:
henry.klassen@snec.com.sg

1 §§Present address: Singapore Eye Research Institute.

¶¶Present address: Developmental Biology Center, School of Biological Sciences, University of California, Irvine, CA, USA.

Abstract

The cat has served as an important nonrodent research model for neurophysiology and retinal degenerative disease processes, yet very little is known about feline neural precursor cells. To culture these cells and evaluate marker expression, brains were dissected from 45-day-old fetuses, enzymatically dissociated, and grown in the presence of EGF, bFGF and PDGF. Expanded cells widely expressed nestin, Sox2, Ki-67, fusin (CXCR4) and vimentin, while subpopulations expressed A2B5, GFAP, or β-III tubulin. Precursors prelabeled with BrdU and/or transduced with a recombinant lentivirus that expresses GFP were transplanted subretinally in five dystrophic Abyssinian cats. Two to 4 weeks following surgery, histology showed survival of grafted cells in three of the animals. Labeled cells were found in the neuroretina and RPE layer, as well as in the vitreous and the vicinity of Bruch's membrane. There was no evidence of an immunologic response in any of the eyes. Neural precursor cells can therefore be cultured from the developing cat brain and survive as allografts for up to 4 weeks without immune suppression. The feasibility of deriving and transplanting feline neural precursor cells, combined with the availability of the dystrophic Abyssinian cat, provide a new feline model system for the study of retinal repair.

Key Words: animal model, feline, forebrain progenitor cells, hereditary rod cone degeneration, lentivirus transduction, nestin, neural stem cells, retinal dystrophy, Sox2, transplantation

2

INTRODUCTION

The recent demonstration that multipotent neural progenitor cells can be cultured from the mammalian brain,[1,2] even in maturity,[3,4] has provided new insights into the mechanisms of neural development, as well as powerful new strategies for transplantation-based studies of neural regeneration. Forebrain-derived progenitor cells have been shown to survive following transplantation to a range of sites within the host central nervous system (CNS), including the brain, spinal cord, and retina.[5–7] Moreover, these cells tend to be less immunogenic than many other cells or tissues,[8] as might be anticipated based on a lack of detectable MHC class II expression.[5,9,10]

Cultured neural progenitor cells, or less stringently defined neural precursors, exhibit a number of characteristic features. These include high levels of proliferative activity in the presence of specific growth factors (EGF and/or bFGF) under serum-free conditions, expression of the immature markers nestin and Sox2, and the ability to differentiate into cells of both neuronal and glial lineage. Cells of this type have now been derived from the brain of a number of mammalian species, including mouse,[1] rat,[4,11] dog,[12] pig[13] and human.[11] The present investigation was undertaken to determine whether similar cultures could be derived from the domestic cat and, if so, whether these cells would survive transplantation and show evidence of integration into the retina of the dystrophic cat.

MATERIALS AND METHODS

Cell isolation

The isolation of feline neural precursor cells followed a protocol similar to that described previously for human progenitors,[14] but starting with a 45-day-timed pregnant Domestic short-haired cat (from Liberty Research, Waverly, NY, USA). The animal was placed under terminal anesthesia and the gravid uterus surgically exposed under aseptic conditions. After the embryos were removed, the heads were collected and placed in Hank's BSS, supplemented with L-glutamine (4 mM), penicillin G (200 000 units/L), streptomycin sulfate (200 000 units/L), ciprofloxacin (1 g/L, Bayer), amphotericin B (1 mg/L), and gentamicin sulfate (100 mg/L), and shipped overnight on ice from Columbia, MO, to Children's Hospital of Orange County, CA, USA. Upon arrival, the brains were removed by dissection and the forebrain removed from the cerebellum and brainstem. Neural tissue was enzymatically digested and the resulting cell suspension washed repeatedly and cultured at high density in fibronectin-coated flasks containing DMEM/F-12 high glucose; L-glutamine (2 mM); BIT9500 (10% by volume; Stem Cell Technologies, Vancouver, British Columbia, Canada); EGF (20 ng/mL); bFGF (40 ng/mL); PDGF-AB (20 ng/mL, Peprotech); and antibiotics. FBS (10% by volume, Hyclone) was included overnight to promote adherence, and the medium completely changed to serum-free medium the next day. Subsequently, cells were fed by medium exchange every 2 to 3 days and passaged at confluence using a Hank's-based cell dissociation buffer (Gibco, a subsidiary of Invitrogen Corp., Carlsbad, CA, USA) and gentle trituration with a flame-polished glass pipette.

Immunocytochemistry on cell cultures

Live cells were fixed by 10-min exposure to 4% paraformaldehyde in PBS (phosphate buffered saline solution; Irvine Scientific, Santa Ana, CA, USA). Fixed cells were washed with PBS buffer +0.05% sodium azide. A blocking solution of 0.1 M tris-buffered saline (TBS) +0.3% Triton X-100 +3% donkey serum (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) was applied for 15 min. Cells were then rinsed twice in 0.1 M TBS buffer. Primary antibodies (Table 1) were diluted in 250 μL of TBS +0.3% Trition X-100 +1% donkey serum, at concentrations determined through titration. Primary antibodies were applied to the samples and kept at 5 °C overnight. The following day the cells were rinsed once with TBS.

All secondary antibodies (Jackson ImmunoResearch Laboratories) were donkey derived and diluted 1 : 100 in 250 μL TBS +0.3% Triton X-100 +1% donkey serum. Secondaries were applied to samples and kept at 5 °C overnight. The next day, samples were rinsed with TBS for 5 min three times. Slides were mounted with Prolong® Antifade (Molecular Probes, a subsidiary of Invitrogen Corp.) and digital images obtained using an Olympus IX70 Microscope (Olympus, Center Valley, PA, USA) and Optronics Quantifire CCD camera (Optronics, Muskogee, OK, USA). Image files were managed with Image Pro Plus 4.5 software with AFA plug-in 4.5.

Table 1. Primary antibodies for immunocytochemistry. Antigen, host species, dilution, source

Human fusin, goat, 1 : 100, Santa Cruz SC-6191
Human GFAP, guinea pig, 1 : 200, Chemicon AB1540
Human Ki-67, mouse IgG, 1 : 200, BD Pharmingen 556003
Human nestin, mouse IgG, 1 : 200, Chemicon MAB5326
Human Sox2, goat IgG, 1 : 100, Santa Cruz SC-17320
Human β-III tubulin, mouse IgG, 1 : 200, Chemicon MAB1637
Pig vimentin, mouse IgG, 1 : 500, Sigma V6630

Lentivirus vector

Feline neural precursor cells were transduced using the lentivirus vector FUGW,[15] originally derived from human immunodeficiency virus type 1 (HIV-1). In this vector, the U3 region of the 5′ long-terminal repeat (LTR) has been replaced with a transcriptional enhancer from the major immediate-early promoter of cytomegalovirus (CMV). In addition, it has a 133 bp deletion in the U3 region of the 3′ LTR. This deletion significantly reduces transcription from the LTR promoter. When the vector integrates into the cellular DNA, the CMV enhancer is replaced with the deleted U3 region from the 3′ LTR.[16] Transcription of the enhanced GFP gene is driven by an internal promoter that derives from the ubiquitin-C gene. Downstream of the GFP gene is a post-transcriptional regulatory element from the woodchuck hepatitis virus that enhances gene expression.

Production of the lentivirus vector

The lentivirus vector FUGW was produced by transfecting HEK293 T17 cells (ATCC) with three plasmids: (1) pFUGW (1.6 μg/plate;[15] (2) pCMVΔR8.9 (1.3 μg/plate),[17] which expresses the HIV packaging protein; (3) pVSV-G (0.36 μg/plate), which expresses glycoprotein G from VSV. HEK293 T17 cells were seeded into plates 48 h before transfecting them using Lipofectamine PLUS (Invitrogen) in serum-free Dulbecco's modified eagle medium (DMEM). After 4 h at 37 °C in 5% CO_2, the media was aspirated and replaced with DMEM containing 5% FCS. Transfected cells were incubated at 37 °C in 5% CO_2 for another 44 h. The lentivirus was pelleted from the spent culture medium by centrifugation at 38 000 *g* in an ultracentrifuge (Sorvall, distributed by Thermo Electron Corp., Asheville, NC, USA). The virus was resuspended in DMEM without serum or other additives. To assay the FUGW virus, HEK293 T17 cells (5×10^5 cells/well) were seeded in 12-well plates of DMEM containing 5% calf serum (1 mL/well). After 24 h, the media were aspirated and 200 μL of a 10-fold serial dilution of FUGW was added. The cells were incubated for 1 h and then DMEM containing 5% FCS (1 mL/well) was added to the well. Infected cells were incubated for 48 h at 37 °C. The media was aspirated and the cells released from the plate in an isotonic buffer called Versene (136.9 mM NaCl, 4.23 mM

Na_2HPO_4, 1.47 mM KH_2PO_4, 2.68 mM KCl, 0.54 mM EDTA; pH 7.3) containing trypsin (1.25 mg/mL). Then, 1 mL of paraformaldehyde (4%) in PBS was added to fix the cells. Cells were passed through a 70-μm nylon cell strainer (BD Falcon, BD Biosciences Discovery Labware, Bedford, MA, USA) and the number of GFP-expressing cells (i.e. transduced with FUGW) was determined using a flow cytometer. Data were analyzed using Cell Quest software (version 3.3; BD Biosciences, San Jose, CA, USA). The titer of the lentivirus was calculated as follows:

$$T = [(E/Vave) * Vt] * [(G * Vinoc)/Vdil]$$

where T = titer in transducing units per ml (TU/mL), E = total events counted (e.g. total number of cells counted), Vave = average volume used to count cells, and Vt = total volume per well. G = ratio of GFP-positive cells/total cells, Vinoc = volume of inoculum, Vdil = volume of dilution.

Propagation of cells, transduction with fugw, and labeling with bromodeoxyuridine

Feline neural precursor cells were infected 3–4 days prior to transplantation with FUGW at a multiplicity of infection of one transducing unit per cell in the presence of polybrene (10 μg/mL; Chemicon, Temecula, CA, USA). Neural precursor cells (NPCs) were also labeled by adding bromodeoxyuridine (BrdU, 10 μg/mL) to the culture medium for 2–3 days prior to transplant.

Preparation of neural precursor cells for transplantation

On the day of transplantation, the feline precursor cells were rinsed three times with PBS without $CaCl_2$ or $MgCl_2$. The PBS was replaced with cell dissociation buffer (Invitrogen) containing trypsin (0.8 mg/mL). Cells were dislodged from the flasks by gentle tapping. Trypsin digestion should inactivate any lentivirus remaining on the outside of the cells. The trypsin was then neutralized using soybean trypsin inhibitor (Invitrogen). Cells were pelleted by centrifugation at 3000 r.p.m. for 3 min. Cells were resuspended in PBS and the number of cells present was estimated using a hemocytometer. The cells were primarily a suspension of individual cells at the time of transplant. An aliquot of cells was fixed in paraformaldehyde (2% in PBS) and the percentage of GFP-expressing cells was determined using a flow cytometer as described above. Cells used on day 3 post transduction were 29.2% positive for GFP (defined as at least 10-fold greater intensity than uninfected control cells). Approximately 60.2% of the cells used on day 4 post transduction scored positive for GFP-expression. Because all cells were infected with FUGW at the same time and under identical conditions, it is likely that most cells were transduced by the lentivirus, and that with the additional 24 h before transplant more GFP was being expressed in the cells and therefore more cells scored positive in the assay.

Transplantation

The recipients were five 2–5-year-old male and female Abyssinian cats (Table 2) affected with hereditary rod-cone degeneration at the moderate to moderately advanced stage.[18,19] The animals were anesthetized using medetomidine (0.1 mg/kg IM; Domitor, Vetpharma, Lund, Sweden) and ketamine (5 mg/kg IM) (Ketalar, Park-Davis, Morris Plains, NJ, USA). Aseptic vitreoretinal surgery was performed on the right eye, using a two-port pars plana vitrectomy as previously described,[20] including removal of approximately 30% of the vitreous, with particular attention to that overlying the superior temporal quadrant of the fundus. In this area a subretinal bleb was then raised, encompassing approximately 20% of the fundus area, using a hand-drawn glass micropipette and balanced salt solution (BSS, Alcon Surgical, Fort Worth, TX, USA). Prelabeled donor cells were carefully injected into the subretinal space as 10–75 μL of cell suspension containing approximately 60 000–750 000 cells, using another glass micropipette. The two sclerotomies were sutured with 7/0 vicryl and the conjunctival and skin incisions closed. Postoperatively, a single subconjunctival injection of dexamethasone (0.15 mL) was given followed by 1 week of daily topical antibiotics and mydriatics.

Postoperative examinations

Ophthalmic examinations were performed daily during the first week after surgery and included ophthalmoscopy, biomicroscopy, tonometry and fundus photography. Thereafter the eyes were examined weekly until euthanasia at 2–4 weeks post-transplantation.

Histology and immunocytochemistry on tissue sections

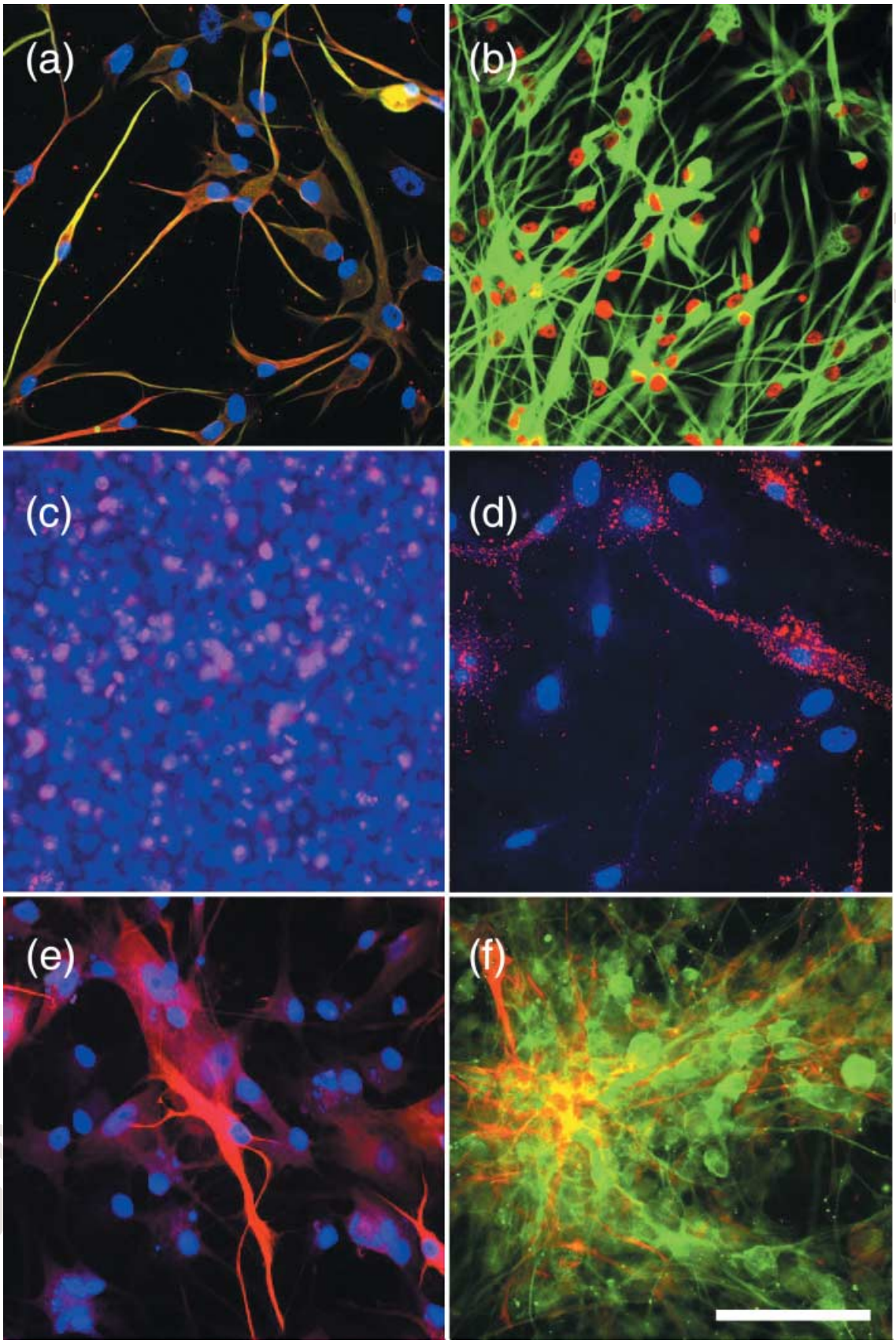
Following euthanasia, both eyes were enucleated and placed in 4% paraformaldehyde (PFA) for 10–20 min. The anterior

Table 2. Results of subretinal transplantation of feline brain precursors, by recipient

Recipient	Degeneration stage*	Donor cell labeling	Survival times	Donor cell survival†	Donor cell location
Kt	3	BrdU	4 weeks	++	Bruch's membrane, choroid
Aa	3+	BrdU, GFP	2 weeks	+	Neuroretina, RPE
Fa	2–3	BrdU, GFP	2 weeks	+	Neuroretina, subretinal space, vitreous
Ja	2+	BrdU, GFP	4 weeks	–	–
Js	2	BrdU, GFP	4 weeks	–	–

*Degeneration stage estimated by funduscopy.[19] †Surviving cells estimated in a three-grade scale. + few cells; ++, moderate number of cells; +++, many cells; –, no cells.

Color image



Color image

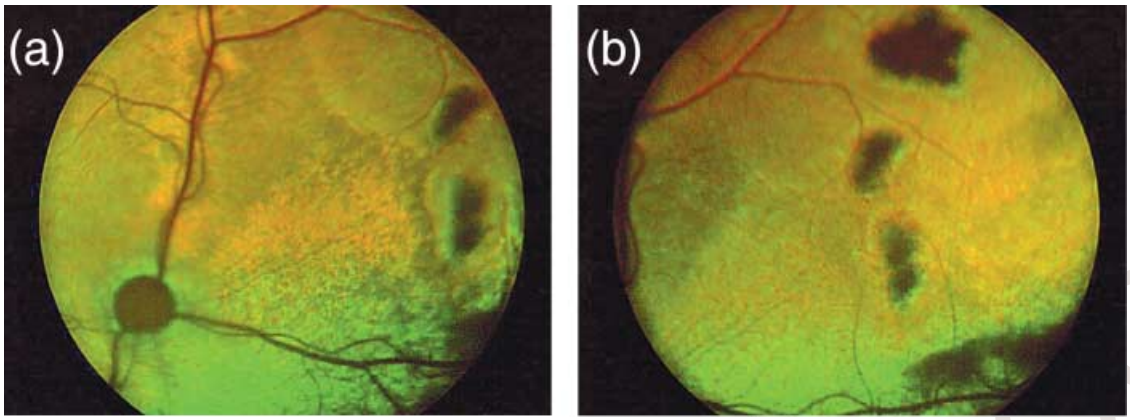


Figure 2. Fundus photographs taken at 4 weeks after surgery. (a) Image including optic nerve head. (b) Image centered on pigment changes. Both images show regions of hyperpigmentation deep into the retina in the nasal superior tapetal quadrant of the fundus, corresponding to the subretinal injection site. Note the grayish discoloration of the tapetal area and generalized mild vascular attenuation in the fundus of this affected cat at the moderately advanced stage of retinal degeneration (recipient = Kt, see Table 2).

segment and lens were then removed and the posterior segment postfixed for 2 h in 4% PFA, with subsequent rinsing in rising concentrations of sucrose containing Sörensen's phosphate buffer. A vertical slice was cut comprising the superior ciliary margin and the optic disk. The tissues were embedded in a gelatin medium and serially sectioned at 12 μm on a cryostat. Hematoxylin and eosin staining was performed on every tenth slide.

For immunohistochemistry, the retinal sections were exposed to primary mouse antisera (BrdU, 1 : 50, DAKO, or GFAP, 1 : 200, Chemicon) in a moist chamber for 16–18 h at 4 °C, followed by rinsing in 0.1 M phosphate buffered saline (PBS) with 0.25% Triton-X-100. Sections were then incubated with secondary FITC-conjugated antibodies (1 : 200, Jackson Immunoresearch) for 1–2 h at room temperature in the dark. The untreated eye, processed in parallel, served as a control. In addition, negative controls with omission of the primary antisera were performed. The specimens were examined using epifluorescence and confocal microscopy.

RESULTS

Proliferating cultures were derived from both forebrain and brainstem/cerebellum specimens and continued to expand *in vivo* for the duration of the study (4 weeks). The cells in these cultures exhibited morphologic features consistent with primitive neuroepithelial cells throughout this period. In addition, immunocytochemical analysis showed the expression of a range of markers associated with neural precursor cells (Fig. 1). These included the intermediate filament nestin, the nuclear transcription factor Sox2, the proliferation marker Ki-67, the intermediate filament vimentin, the cytoskeletal protein β-III tubulin, the surface markers A2B5 and fusin (CXCR4, CD184), as well as the intermediate filament GFAP.

Examination of the operated eyes on the day after surgery revealed them to be quiet, without signs of irritation. The pupils were widely dilated as a result of treatment with mydriatic agents. In each case, the neuroretinal bleb was identified. In some cases there was evidence of focal hemorrhage in the area of surgery. On the second day there was, in one case, three small, darkly pigmented areas (0.5–1 optic disk diameter each) in the former location of the neuroretinal bleb. Five days after surgery, the pigmented areas in this animal had increased to approximately twice their initial size. The focal hyperpigmented areas were documented photographically at 4 weeks postgrafting (Fig. 2). No inflammatory reactions or other adverse reactions were seen in any of the treated eyes throughout the follow-up period.

Histologic analysis of the animal with hyperpigmentation at 4 weeks post-transplantation showed focal thinning of the retina at the injection site (Fig. 3). This focal atrophy was associated with diffuse up-regulation of GFAP localized to the area of retina, raised by the subretinal injection at the time of surgery. In addition, there was decreased thickness of the tapetum adjacent to this area. Surviving BrdU-labeled donor cells were found deep into the RPE, dispersed along the choroidal side of Bruch's membrane and atrophied tapetum or adjacent anterior choroidal structures. In this animal, no labeled cells were identified within the neuroretina,

10

Figure 1. Feline forebrain precursor cultures. Cells shown are passage 3, maintained for 41 days in culture. (a) Cytoplasmic labeling for nestin (red) and labeling for fusin (green), with coexpression seen as yellow. Nuclei stained by DAPI (blue). (b) Cytoplasmic labeling for vimentin (green), costained with DAPI (red). (c) Widespread nuclear and nucleolar staining for Ki-67 (red), with background density of cells revealed by DAPI (blue). (d) Punctate surface staining for the glial precursor marker A2B5, costained with DAPI (blue). (e) Sox2 labeling shows nuclear localization (blue), whereas GFAP (red) is cytoplasmic. (f) Labeling for the neuronal marker β-III tubulin (green) and the glial marker GFAP (red) corresponds to different subpopulations. All images taken at ×400 magnification, scale bar: 100 μm.

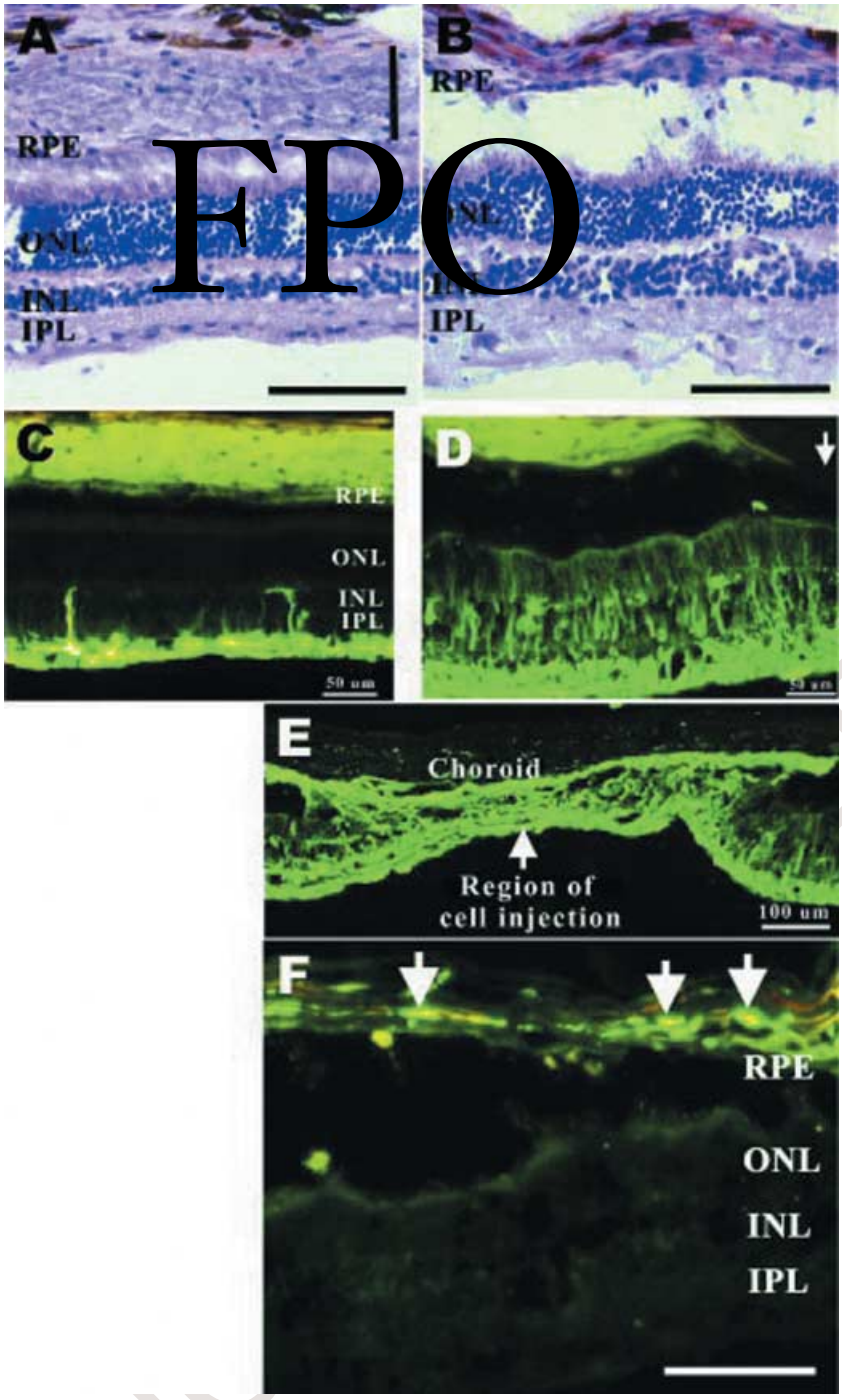


Figure 3. Histologic findings in recipient, as shown in Fig. 2. Hematoxylin staining showed an intact tapetum (vertical black bar, 60 µm) in the untreated eye (a) and loss of this structure (b) in the area of injection. Immunostaining for GFAP (green in c–e) showed inner retinal expression in the untreated eye (c) and up-regulation (d) in the treated eye. Also, autofluorescence from the tapetum confirmed local thinning of this structure (arrow). The region of cell injection (e) showed up-regulation of GFAP and focal thinning of the retina. (f) Labeling for BrdU (green) revealed positively stained donor cells (arrows) in the inner choroid, deep into Bruch's membrane. No BrdU-labeled cells were found in the retina (all images from recipient Kt, stage 3, see Table 2). Scale bars: 100 µm (a,b), 50 µm (c,d), 100 µm (e).

11

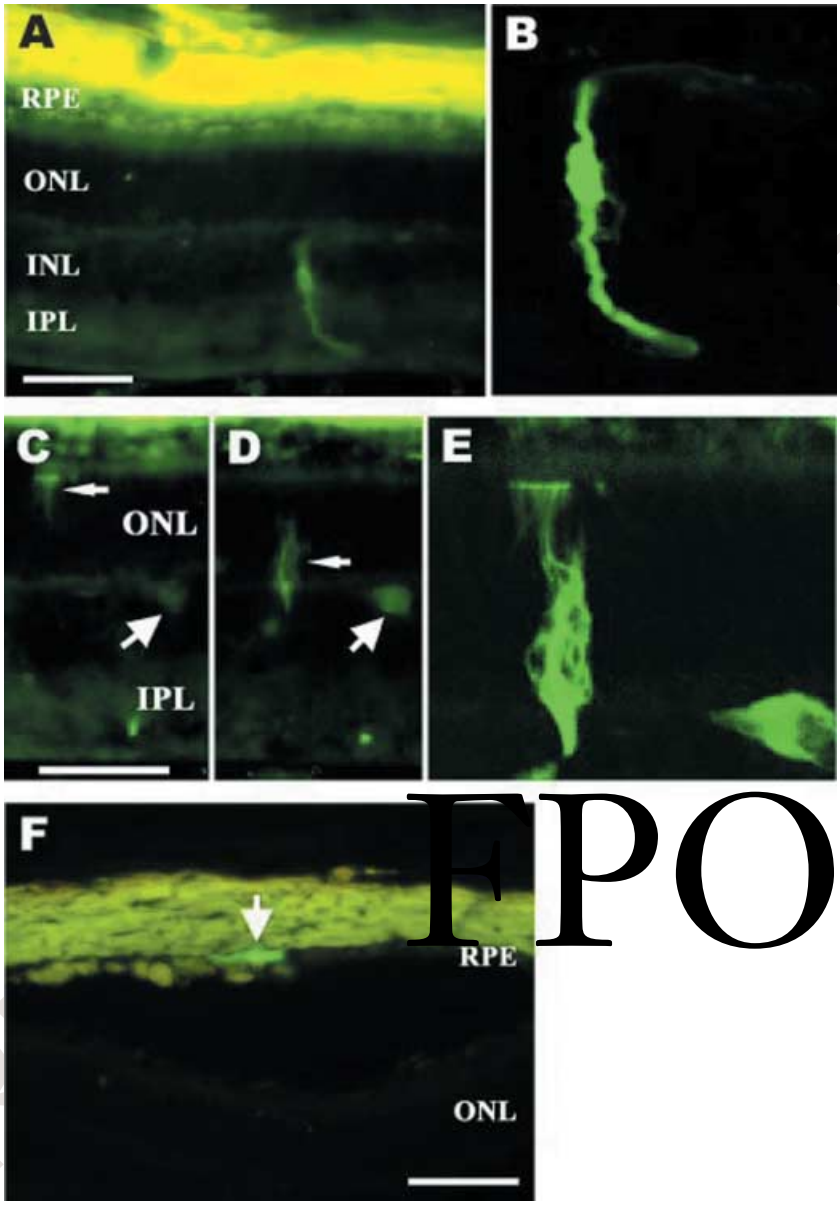
12

suggesting initial placement of the graft deep into the RPE layer.

In contrast to the first animal, data from two additional animals at 2 weeks post-transplantation showed GFP+ donor cells integrated into the neuroretina and RPE layer (Fig. 4), as well as unintegrated cells in the subretinal space and vitreous. Some integrated cells exhibited morphologic characteristics suggestive of late-generated retinal cell types such as bipolar and Müller cells; however, no co-expression of GFP and mature retinal markers was found. In the remaining two animals, examined at 4 weeks post-transplantation, no surviving cells were found (Table 2).

DISCUSSION AND CONCLUSIONS

The findings of the present study increase the number of progenitor cell types that have been identified in the cat,[21,22] as well as increasing the number of species from which neural precursor cell cultures have been successfully derived. A cardinal feature of such cells is proliferation in response to



Color image

Figure 4. Integration of GFP+ donor cells into the neuroretina and RPE layer. Immunofluorescence microscopy at 2 weeks post-transplantation of GFP-labeled feline neural precursor cells. (a) Radially oriented GFP+ cell of bipolar morphology in the inner nuclear layer (INL), extending one process to the outer plexiform layer and the other into the inner plexiform layer (IPL). (b) Confocal reconstruction of the cell in (a). (c–e) Images of GFP+ cellular profiles from the same area of two consecutive sections. One profile (small arrow) is radially oriented and appears to contribute cytoplasmic extensions to the outer limiting membrane. Another profile (large arrow) lies in the vicinity of the outer plexiform layer and appears to be horizontally oriented. Confocal reconstruction (e) shows that the cytoplasmic extensions at the outer limiting membrane seen in (c) derive from the radially oriented profile seen in (d). (f) GFP+ profile (arrow) that appears to be in the RPE layer (images shown in a–e from recipient Fa; image shown in f from recipient Aa, see Table 2). Scale bars: 50 μm.

defined exogenous growth factors, both *in vitro*[1,2] and *in vivo*.[23] This inducible mitogenic activity allows multipotent progenitor cells to be selectively enriched in comparison to postmitotic precursors and differentiated cells. It also allows relatively large numbers of the cells to be obtained from small tissue specimens, as was the case here.

Another characteristic of these cells is that they give rise to both neurons and glia. Formal demonstration of phenotypic multipotency requires clonal analysis; however, the cells in the present study did not expand at low density. Nevertheless, the initial marker data obtained here are consistent with the generation of both neuronal and glial phenotypes by these cultures. In this context it is worth noting that multipotency is now known to be a common feature of proliferative CNS progenitors up until the final cell division.[24] Although predominantly gliogenic cultures have been described, particularly in large mammals at later donor ages or high passages,[25] the theoretical possibility of neuron-only cultures remains to be substantiated. Indeed, at least some multipotent progenitor cells express GFAP, a marker classical associated with mature astroglia. Thus, the expression pattern of both neuronal and glial markers seen here is consistent with the presence of multipotent neural progenitors within feline CNS cultures, although this remains to be demonstrated.

The markers identified here include those associated with primitive neuroepithelial phenotype as well as immature, and mature, neurons and glia. Nestin is an intermediate filament protein that is highly expressed by cells of the developing neural tube. Another intermediate filament protein that is highly expressed by these cells, albeit with less specificity, is vimentin. A third intermediate filament protein, GFAP, is discussed above. Sox2 is also known to be expressed by

multipotent CNS progenitor cells,[26] as well as other primitive cells including embryonic stem cells. Ki-67 is a cell cycle marker indicative of proliferative activity, a nonspecific hallmark of progenitor cells. β-III tubulin and A2B5 are expressed by developing neurons and glia, respectively. The surface marker fusin (CXCR4, CD184) is a receptor for SDF-1 and has been implicated in stem-cell homing behavior.[27] These results are similar to those seen in similar cultures from other species, including mouse, pig and human.[14,28]

By culturing neural precursor cells from the brain of the cat, the number of mammalian species from which such cells have been derived is increased, particularly among non-rodent mammalian species. This demonstrates the general utility of the methods used, and also implies a significant degree of cross-reactivity of the human recombinant growth factors employed as mitogens. These findings help generalize the known biology of neural precursors to multiple mammalian species across vertebrate phylogeny. This commonality in turn provides the tools for discerning the differences that are likely to exist between species, particularly in terms of quantitative gene expression and functional activity. For example, despite their obvious commonality, neural precursor cultures exhibit subtle, yet discernible differences in morphologic appearance between species, although the reason for this is difficult to delineate.

The potential advantages of cultured feline neural precursor cells include the numerous experimental applications that can be envisioned for these cells. This is particularly true in terms of functional transplantation studies, given the extensive literature on retinal and cortical electrophysiology in the cat, as well as the identified feline models of retinal dystrophy. The type of Abyssinian cat used in the present study has a recessively inherited retinal rod-cone degeneration that is slowly progressive and exhibits many similarities to human retinitis pigmentosa (RP). Affected cats are ophthalmoscopically normal until the age of 1.5–2 years, at which time retinal changes appear that progress to complete atrophy and blindness over the following 3–4 years.

The results of this initial transplantation study showed that feline neural precursor cells survive transplantation within the eye for at least 4 weeks. Survival of the allogeneic cells did not depend on either exogenous immune suppression, as none was used, or engraftment inside the blood–retinal barrier, as in one case the cells were found deep into the RPE layer. In no case was there evidence of proliferation suggestive of tumor formation. The relatively poor donor cell survival at 4 weeks transplantation could be the result of a number of factors, including the relatively small number of cells transplanted, delivery as a single cell suspension, undetected immunologic responses, or possibly transfection-associated issues. Additional work is needed to optimize graft survival in this and other nonrodent species. Further areas of interest include the isolation of precursor cells from the developing feline retina, as well as evaluation of the fate of such cells following transplantation to the retina of the dystrophic cat using the methods established here.

ACKNOWLEDGMENTS

We wish to thank Drs Mark Kirk and Jason Meyer for assistance with this project and Dr Shrikesh Sachdev for assistance in the production of FUGW. The work was supported by the CHOC Foundation, Guilds, and Padrinos (HK, PHS), the Hoag Foundation (HK), the United Mitochondrial Research Foundation (PHS), Richard D and Gail Siegal (MJY), the Minda de Gunzburg Center for Retinal Transplantation (MJY), the NINDS (NS044060, HK), NEI (EY09595, MJY), the University of Missouri Research Council (KN) and the National Cancer Institute (KN).

REFERENCES

1. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 1992; **255**: 1707–1710.
2. Richards LJ, Kilpatrick TJ, Bartlett PF. De novo generation of neuronal cells from the adult mouse brain. *Proceedings of the National Academy of Sciences of the United States of America* 1992; **89**: 8591–8595.
3. Eriksson PS, Perfilieva E, Bjork-Eriksson T *et al*. Neurogenesis in the adult human hippocampus. *Nature Medicine* 1998; **4**: 1313–1317.
4. Sah DW, Ray J, Gage FH. Regulation of voltage- and ligand-gated currents in rat hippocampal progenitor cells in vitro. *Journal of Neurobiology* 1997; **32**: 95–110.
5. Klassen H, Ziaeian B, Kirov I *et al*. Isolation of retinal progenitor cells from postmortem human tissue with comparison to autologous brain progenitors. *Journal of Neuroscience Research* 2004; **77**: 334–343.
6. Young MJ, Ray J, Whiteley SJ *et al*. Neuronal differentiation and morphological integration of hippocampal progenitor cells transplanted to the retina of immature and mature dystrophic rats. *Molecular and Cellular Neuroscience* 2000; **16**: 197–205.
7. Takahashi M, Palmer TD, Takahashi J *et al*. Widespread integration and survival of adult-derived neural progenitor cells in the developing optic retina. *Molecular and Cellular Neuroscience* 1998; **12**: 340–348.
8. Hori J, Ng TF, Shatos M *et al*. Neural progenitor cells lack immunogenicity and resist destruction as allografts. *Stem Cells* 2003; **21**: 405–416.
9. Klassen H, Imfeld KL, Ray J *et al*. The immunological properties of adult hippocampal progenitor cells. *Vision Research* 2003; **43**: 947–956.
10. Klassen H, Schwartz MR, Bailey AH *et al*. Surface markers expressed by multipotent human and mouse neural progenitor cells include tetraspanins and non-protein epitopes. *Neuroscience Letters* 2001; **312**: 180–182.
11. Svendsen CN, Clarke DJ, Rosser AE *et al*. Survival and differentiation of rat and human epidermal growth factor-responsive precursor cells following grafting into the lesioned adult central nervous system. *Experimental Neurology* 1996; **137**: 376–388.
12. Milward EA, Lundberg CG, Ge B *et al*. Isolation and transplantation of multipotential populations of epidermal growth factor-responsive, neural progenitor cells from the canine brain. *Journal of Neuroscience Research* 1997; **50**: 862–871.
13. Smith PM, Blakemore WF. Porcine neural progenitors require commitment to the oligodendrocyte lineage prior to transplantation in order to achieve significant remyelination of demyelinated lesions in the adult CNS. *European Journal of Neuroscience* 2000; **12**: 2414–2424.

14. Schwartz PH, Bryant P, Fuja T *et al*. Isolation and characterization of neural progenitor cells from post-mortem human cortex. *Journal of Neuroscience Research* 2003; **74**: 838–851.
15. Lois C, Hong EJ, Pease S *et al*. Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors. *Science* 2002; **295**: 868–872.
16. Miyoshi H, Blomer U, Takahashi M *et al*. Development of a self-inactivating lentivirus vector. *Journal of Virology* 1998; **72**: 8150–8157.
17. Naldini L, Blomer U, Gage FH *et al*. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proceedings of the National Academy of Sciences USA* 1996; **93**: 11382–11388.
18. Narfström K, Nilsson SE. Morphological findings during retinal development and maturation in hereditary rod-cone degeneration in Abyssinian cats. *Experimental Eye Research* 1989; **49**: 611–628.
19. Narfström K. Progressive retinal atrophy in the Abyssinian cat: clinical characteristics. *Investigative Ophthalmology and Visual Science* 1985; **26**: 193–200.
20. Bragadottir R, Narfström K. Lens sparing pars plana vitrectomy and retinal transplantation in cats. *Veterinary Ophthalmology* 2002; **6**: 135–139.
21. Gengozian N. Development of monoclonal antibodies to erythroid progenitors in feline bone marrow. *Veterinary Immunology and Immunopathology* 1998; **64**: 299–312.
22. Martin DR, Cox NR, Hathcock TL *et al*. Isolation and characterization of multipotential mesenchymal stem cells from feline bone marrow. *Experimental Hematology* 2002; **30**: 879–886.
23. Fallon J, Reid S, Kinyamu R *et al*. In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. *Proceedings of the National Academy of Sciences of the United States of America* 2000; **97**: 14686–14691. Erratum in: *Proceedings of the National Academy of Sciences of the United States of America* 2001; **98**: 8157.
24. Turner DL, Cepko CL. A common progenitor for neurons and glia persists in rat retina late in development. *Nature* 1987; **328**: 131–136.
25. Palmer TD, Schwartz PH, Taupin P *et al*. Cell culture. Progenitor cells from human brain after death. *Nature* 2001; **411**: 42–43.
26. Cai J, Wu Y, Mirua T *et al*. Properties of a fetal multipotent neural stem cell (NEP cell). *Developmental Biology* 2002; **251**: 221–240.
27. Juarez J, Bendall L. SDF-1 and CXCR4 in normal and malignant hematopoiesis. *Histology and Histopathology* 2004; **19**: 299–309.
28. Schwartz PH, Nethercott H, Kirov II *et al*. Expression of neurodevelopmental markers by cultured porcine neural precursor cells. *Stem Cells* 2005; **23**: 1286–1294.

Author Query Form

Journal: Veterinary Ophthalmology

Article: vop_547.fm

Dear Author,

During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication.

Many thanks for your assistance.

No.	Query	Remarks
1	**Singapore Eye Research Institute** Please provide more complete address	
2	Please choose 6 keywords only (according to journal style)	
3	Please supply company details/address of **Peprotech**	
4	Please supply company details/address of **Hyclone**	
5	Please supply company details/address of **Image Pro Plus 4.5 software**	
6	**15** has been changed to superscript – is it correct that this is a reference citation?	
7	**12-well** hyphen added for sense – accurate now?	
8	**3000 r.p.m.** Please express as *g*	
9	**were** has been deleted here for sense	
10	**to** Change to 'into' here and four lines down – OK? See also fig. 2 legend	
11	Figures 3 and 4 are in poor quality, please resupply. For more information about supplying electronic artwork, please see the journal webpage or Blackwell's electronic artwork guidelines at http://www.blackwellpublishing.com/bauthor/illustration.asp	

No.	Query	Remarks
12	Do abbreviations in Figures (ONL etc.) need explanation in the figure legend?	
13	**to** has been changed to **into**	