

Transplantation of NTPDase2-positive Sorted Müller Glial Cells into the Mouse Retina

Robert M Hoek^{#1}
Peter M Quinn^{#2}
Berend Hooibrink³
Jan Wijnholds^{1,2*}

¹Netherlands Institute for Neuroscience, Meibergdreef, Amsterdam, The Netherlands
²Department of Ophthalmology, Leiden University Medical Center, Leiden, The Netherlands
³Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Meibergdreef, Amsterdam, The Netherlands
[#]Contributed equally

Abstract

Tissue-specific progenitor cells form a potential population for cell-based transplantation therapy. Müller glial cells are suggested to contain, or represent the progenitor pool in the retina and can dedifferentiate and regenerate neuronal cells in fish and amphibians. However, the Müller glial cells of mammals have a more limited regenerative capacity in response to injury and disease. Transplantation experiments with Müller glial cells have shown limited success. In this study we transplant NTPDase2-positive Müller glial cells labelled with CellTracker Green or NTPDase2-positive Müller glial cells sorted from transgenic tdTomato mouse retina. We found that NTPDase2-sorted Müller glial cells do not have the intrinsic capacity to integrate into the mouse retina upon subretinal injection under the conditions used. Interestingly, we also found that CellTracker Green labelled NTPDase2-sorted Müller glial cells release CellTracker Green conjugates. NTPDase2-sorted negative cells do not. Injection of CellTracker Green alone did not lead to labelling of any retinal cell types. However, when NTPDase2-sorted cells are injected into the mouse retina these conjugates are preferentially taken up by Starburst amacrine cells. This highlights a potentially novel import transporter on the plasma membrane of Starburst amacrine cells.

Article Highlights

- NTPDase2-positive sorted Müller glial cells labelled with CellTracker Green (CTG) or sorted from transgenic tdTomato mouse retinas do not integrate into the mouse retina and remain in the subretinal space.
- Release of conjugated CellTracker Green labelled cell-material from transplanted NTPDase2-positive Müller glial cells results in cell-material migration through the retina and transfer to Starburst amacrine cells.

Keywords

Müller glial cells; Cell transplantation; NTPDase2; Retina; CellTracker Green (CTG)

Introduction

Three main types of glial cells serve to maintain retinal homeostasis: astrocytes, Müller cells and microglia, the resident macrophages [1]. Müller Glial Cell (MGC) processes span from the inner limiting membrane to the apical villi at the outer limiting membrane, guiding light through the inner retina [2,3]. MGC are generated from retinal progenitor cells between embryonic day 15 and postnatal day 10, with a peak birth rate at approximately P3 [4]. MGC ensheath all retinal neuronal cell types, providing metabolic support and control homeostasis. Furthermore, the MGC maintain retinal organization and lamination of the retina [2,5]. In the damaged teleost retina, MGC can dedifferentiate and form progenitors that generate all types of retinal neurons [6-9].

Mammalian MGC are quiescent in the adult healthy retina and have a very limited ability for self-repair throughout life [3]. Mouse MGC have relatively similar gene expression profiles as late progenitors despite their specialized glial functions [10]. In response to growth factors or damage, mammalian MGC can de-differentiate, re-enter the cell cycle, proliferate and even form new subtypes of retinal neurons but in a restricted manner [11-16]. Human MGC in culture generate both glial cells and retinal neurons such as rod photoreceptors (rods) and ganglion cells [17-22]. When transplanted, these differentiated cells can integrate into the retina providing some functional rescue [20,21]. Together, this highlights the potential regenerative ability of MGC in the repair of the mammalian retina *in vivo*. Nonetheless, this limited ability does not stop mammalian MGC in becoming reactive following injury or in disease [23-26]. Factors produced during retinal degeneration and gliosis may limit the abilities of MGC to proliferate and differentiate within the diseased mammalian retina [27].

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*Corresponding author:

Jan Wijnholds
Department of Ophthalmology
Leiden University Medical Center
2300 RC, Leiden, The Netherlands
E-mail: J.Wijnholds@lumc.nl

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Previously, intravitreal injection of MGC in the healthy rodent retina did not result in integration into the retina. However, intravitreal transplantation of lac-Z-labelled MGC into the toxin damaged mouse retina showed co-labelling with cells in the ganglion cell layer, the inner plexiform layer and the outer margin of the inner nuclear layer [28]. It remains unclear if MGC migrated within the retina or whether materials transferred from the MGC to residing cells in the retina. There are other reports on subretinal transplantation of photoreceptor precursors that resulted mainly in material transfer from the transplanted to the residing photoreceptors, with very limited migration of transplanted photoreceptors into the photoreceptor layer [29-31]. Increased material transfer has been observed in the *Crb1*^{rd8/rd8} mouse model which have a compromised outer limiting membrane when compared to healthy retina [32]. *Crb1* knockout mice (*Crb1*^{KO}) and *Crb1*^{rd8/rd8} mice show disruptions at the outer limiting membrane leading to retinal disorganisation and dystrophy limited to the inferior retina, suggesting that *Crb1* mutant mice allow for increased material transfer across the outer limiting membrane [33,34].

In this study, we find that upon subretinal injection NTPDase2-positive sorted MGC did not integrate into the mouse retina of healthy or *Crb1*^{KO} mice. NTPDase2-positive sorted MGC, dependent on injection site, resided between the photoreceptor layer and the retinal pigment epithelium, the so called sub-retinal space, or resided within the vitreous cavity. Furthermore, CellTracker-Green (CTG)-labelled NTPDase2-positive MGC transplanted either subretinally or intravitreally into the retina showed material transfer. The MGC-material migrated from the subretinal space or intravitreal cavity into the retina, and the material was transferred specifically to Starburst amacrine cells.

Materials and Methods

Animal husbandry

Animal care and use of mice was in accordance with protocols approved by the Animal Care and Use Committee at the Netherlands Institute of Neuroscience. For these studies Rosa26-lox-stop-lox-Tomato mice (B6;129S6-Gt(*ROSA*)26Sor^{tm14(CAG-tdTomato)Hze}/J, JAX stock #007908 [35]) were crossed with CamKII::cre mice (B6.Cg-Tg(Camk2a-cre)T29-1Stl/J, JAX stock #005359 [36]) that resulted in a germline recombination of the stop cassette, rendering these mice tomato-red transgenic in all cells. Germ-line recombined Rosa26-lox-Tomato mice, lacking the CamKII::cre allele, on C57BL/6J genetic background and wild type C57BL/6J mice from Harlan (Indianapolis, IN) were used as donors. Mice lacking the *Crb1* gene or wild type C57BL/6J mice were used as recipients [33]. All mice used were 8-12 weeks of age at the time of cell isolation.

Flow cytometric cell isolation from neural retina

The method for creating a cell suspension from neural retina and then using NTPDase2 for Fluorescence-Activated Cell Sorting (FACS) has been previously described [37]. In brief, murine neural retinae were isolated from enucleated eyes and a retinal cell suspension was prepared by enzyme incubation with Collagenase I (Worthington, Lakewood NJ, USA) and DNase I (Roche, Mannheim, Germany). The tissue was triturated using a pipet with tips of decreasing bore size, washed and stored in GKN/BSA for further processing. The stored cell suspension was then stained in a number of consecutive steps with anti-NTPDase2 (Enzo Life Sciences, Lausen, Switzerland) or our custom made polyclonal antibody directed against NTPDase2 from Eurogentec), biotinylated goat-anti-Rabbit (Jackson Immuno Research, West Grove PA, USA), streptavidin-APC-Cy7 conjugate (BD Biosciences, San Jose CA, USA) and finally 7-AAD. This cell suspension was then flow cytometrically sorted with a FACSaria (BD Biosciences) or InFlux (BD Biosciences). Events collected were FSC-low, SSC-low, 7-AAD-negative, and NTPDase2-positive.

Cell transplantation

Prior to MGC transplantation the cells were stained in 3 ml GKN/BSA containing 10 μ M CTG for 30 min at 4°C. After extensive washing to remove the excess CTG, the cells were resuspended in

a small volume of Earle's balanced salt solution (EBSS) medium, resulting in a density of circa $0.7\text{--}1.4 \times 10^5$ cells/ μ l, and 1.5 μ l of cell-suspension was transplanted subretinally or intravitreally. As a control 2 μ l of the 10 μ M CTG solution was injected intravitreally and eyes analysed 14 days post injection. As a further control we plated one well of a 6-well plate with sorted NTPDase2-positive cells, one well with sorted NTPDase2-negative cells and one well with no cells, in Defined Minimal Medium (DMM) with 10 μ M CTG for two days. DMM contained DMEM/F12 (Invitrogen-Gibco) supplemented with 1x N2 supplement, 2 mM glutamine, 1x MEM-nonessential amino acids, 1 mM sodium pyruvate, 0.5 mg/ml Bovine Serum Albumin (BSA) fraction V (all from Invitrogen-Gibco), and 110 mM β -mercaptoethanol (Sigma). After two days incubation with CTG the medium was taken from each well and centrifuged at 2000 rpm for 10 minutes and 1.5 μ l was taken for transplantation. The wells containing NTPDase2-positive and NTPDase2-negative cells were washed with NOSE buffer (4 g/L MgCl₂, 2.55 g/L CaCl₂, 3.73 g/L KCl, 8.95 g/L NaCl, pH 6-7) and subsequently incubated with NOSE buffer containing a collagenase/DNase mix (previously described in Hoek et al. 2018, [37] at 37°C for 10 minutes. Cells were checked under a microscope for detachment. The cells were collected in NOSE buffer and the wells washed twice, then pooled and centrifuged at 2000 rpm for 10 minutes, and resuspended in 3 ml GKN/BSA. Finally, cells were spun and resuspended in 800 μ l of EBSS, before transplantation cells were spun at 4000 rpm for 10 minutes and resuspended in 18 μ l of EBSS with 1.5 μ l taken for transplantation.

Immunohistochemistry

For immunohistochemistry the eyes were enucleated and emersion fixed in 4% PFA in PBS for 30 min, followed by an incubation of 30 min in 5% and 30% sucrose in PBS for cryoprotection. Tissue was embedded in Tissue-Tek O.C.T. cryo compound and frozen in a mold in the vapour phase of liquid nitrogen. Tissue samples were stored at -20°C until use. Cryostat sections of 8-10 μ m were collected on eighteen slides, such that each slide contained six to eight rows of two serial sections. Slides were dried over silica gel and stored at -20°C. For immunofluorescent staining sections were incubated in a dilution of primary antibody, followed by the appropriate secondary reagent conjugated to Cy3 or Alexa 488. The following primary antibodies were used: Calretinin (1:250; Chemicon), Calbindin D28 (1:400; AnaSpec), choline acetyltransferase (ChAT) (1:50; Chemicon), and Recoverin (1:500; Millipore). Sections were mounted in a DAPI-containing mounting medium to stain the nuclei. Micrographs were made on a Leica DMRE microscope fitted with a DFC360FX digital camera, or using a Leica SP5 CLSM system. Images were processed and analyzed in Image J and Photoshop.

Statistics

Six eyes were used for direct intravitreal injection of CTG in medium. Two to six eyes were used for subretinal or intravitreal transplantation studies of injected CTG-labelled or tdTomato-labelled NTPDase2-positive cells or NTPDase2-negative cells. Two eyes were used for intravitreal injection of medium from NTPDase2-positive cells cultured with CTG, medium from NTPDase2-negative cells cultured with CTG, medium without cells that had been cultured with CTG for 2 days.

Results

The fate of the subretinally transplanted NTPDase2-positive MGC was assessed by immunohistochemical analysis at different time points. The analysis showed that seven days after transplantation the deposited MGC or cell debris were still mainly in the subretinal space (Figure 1A, insert). Unexpectedly, by nine days post subretinal transplantation the deposit of MGC and debris was nearly depleted and a large number of MGC-derived-material had travelled deep into the neural retina, and took position within the inner nuclear layer (INL) (Figure 1B). At 9-days post MGC transplantation, a faint line of CTG labelled neurites were visible within the inner plexiform layer (IPL) (Figure 1B, arrowheads). At 14-days post transplantation, the pattern of CTG labeling was even more pronounced (Figure 1C). From day nine onwards, the MGC-derived material colocalised with

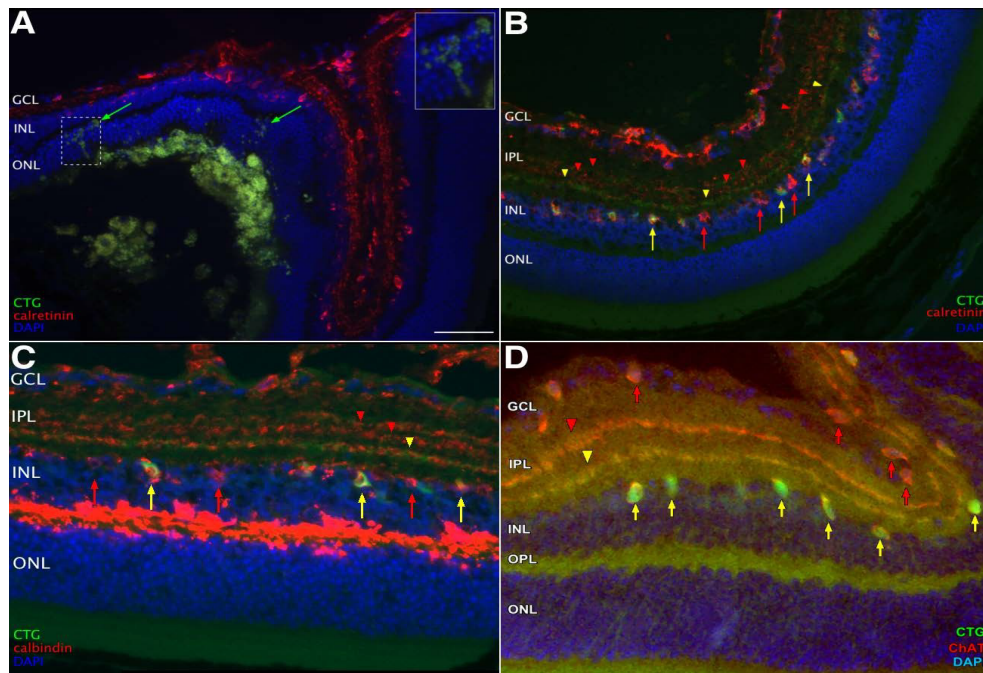


Figure 1: Transplanted MGC material migrates predominantly to the INL and develop Starburst amacrine cell (SAC) specific characteristics.

(A) Seven days post transplantation some MGC-derived material is migrated into the tissue. Inset shows boxed area at 2x magnification. (B) Nine days post transplantation calretinin⁺ transplanted cells can be seen at the inner face of the INL among the host cell derived material. Also a first indication of dendrite projections to the IPL is seen colocalizing with the outer-most calretinin positive stratum in the IPL. (C and D) Fourteen days post transplantation, donor derived cells are settled at the inner face of the INL on the border with the IPL. (C) These cells are calbindin positive, and (D) also stain for ChAT. (A-D) Yellow arrows indicate donor derived cells, red arrows indicate host derived cells or host cell derived material, yellow arrow-heads indicate colocalization of the SAC marker with donor MGC-derived CellTracker Green (CTG)-positive material in the sub-layer S1/2 of the IPL, red arrow-heads indicate host derived expression in other sub-layers of the IPL. GCL: Ganglion Cell Layer; IPL: Inner Plexiform Layer; INL: Inner Nuclear Layer; OPL: Outer Plexiform Layer; ONL: Outer Nuclear Layer. Bar indicates 50 μ m in (A and B), and 25 μ m in (C and D).

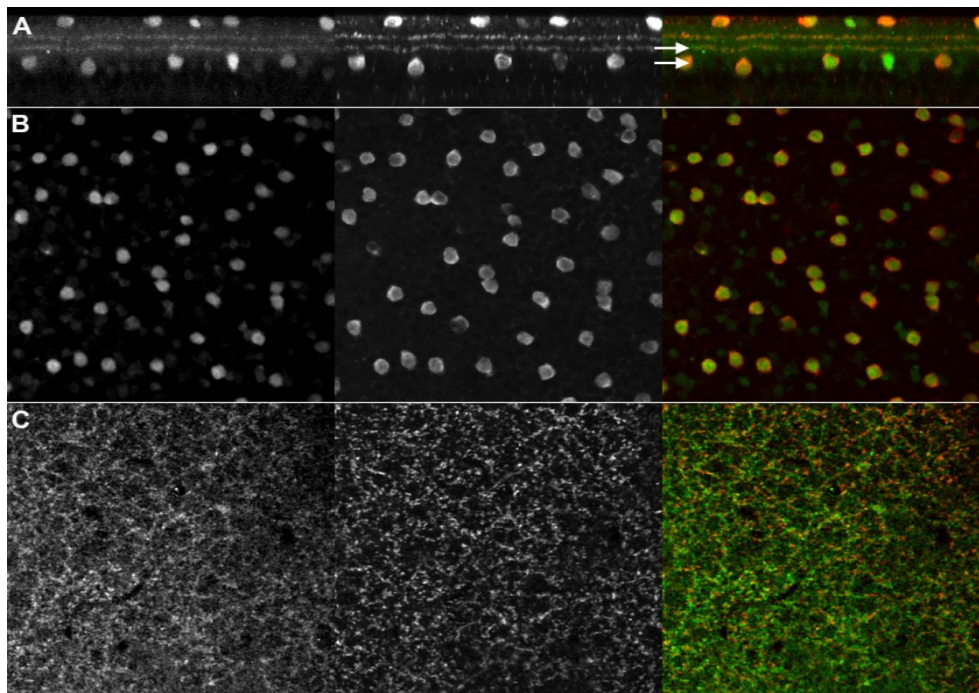


Figure 2: Transplanted cells that differentiate into SACs show typical morphology.

(A-C) CLSM images of a flat-mount preparation of CTG stained cells (left panels), a staining for ChAT (middle panels), and the merged images (right panels), (A) Orthogonal slices of the Z-stacks indicating the levels at which both panels in (B) (lower arrow) and those in (C) (upper arrow) have been taken. (B) image of INL close to IPL showing cell bodies of transplanted and ChAT⁺ cells. (C) Image at sub layer S1/2 in the IPL. Note the lattice-like structure of green projections largely overlaps with that of the ChAT⁺ cells. Bar in (C) indicates 50 μ m in (A-C).

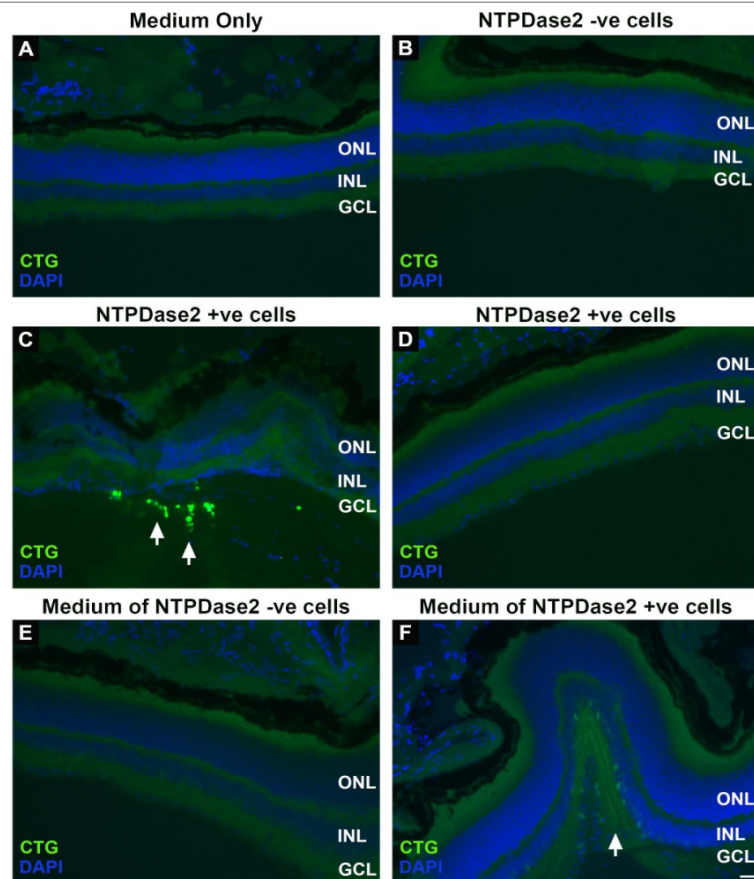


Figure 3: NTPDase2 cells release a CellTracker Green conjugate which is taken up by Starburst amacrine cells.

NTPDase2-negative and -positive cells were incubated with CTG for two days and subsequently the cells and medium were intravitreal injected into adult mouse retina. Intravitreal injections were done with culture medium without cells that had been incubated with CTG for 2 days (A), NTPDase2-negative non-MGC (B), NTPDase2-positive MGC (C, D), medium of NTPDase2-negative non-MGC (E), and medium of NTPDase2-positive MGC (F). CTG-positive staining was detected from the NTPDase2-positive injected MGC (C). Using medium from NTPDase2-positive MGC, CTG-positive staining of Starburst amacrine cells was detected in the INL, IPL and GCL suggesting the presence of CTG conjugates in the medium (F). ONL: Outer Nuclear Layer; INL: Inner Nuclear Layer; GCL: Ganglion Cell Layer. Bar indicates 20 μ m.

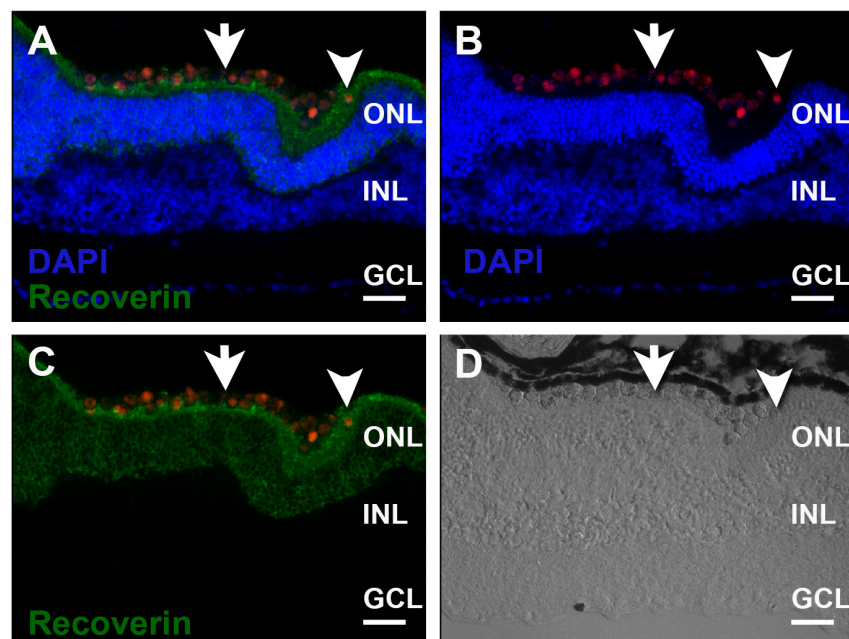


Figure 4: Transplanted tdTomato NTPDase2-positive cells do not integrate into the retina.

Subretinal transplantation of postnatal day 5 (P5) TomatoRed-positive-NTPDase2-positive MGC into *Crb1^{KO}* mouse eye. Arrows show MGC or MGC debris in the subretinal space and arrowheads show a MGC within the photoreceptor inner segment layer. Merged (A), DAPI (B), Recoverin (C), Bright Field (D). Bar indicates 100 μ m.

anti-calretinin (Figure 1B) and anti-calbindin (Figure 1C). This suggested that either the transplanted cells migrated into the retina and had differentiated into an amacrine cell type or that transplanted cell material transferred through the retina and was taken up by amacrine cell types. The position of the cell bodies, together with their projections that were shown to colocalize with the immunostaining in sub-layer S1/2 of the IPL, suggested that CTG labeling resided in Starburst amacrine cells (SACs), the only cholinergic neuron of the retina [38]. Immunohistochemical staining with anti-choline acetyltransferase (ChAT) confirmed the colocalisation with Starburst amacrine cells (Figure 1D). Although most of the CTG positive MGC-derived-material localized within the INL, a proportion ended up in displaced Starburst amacrine cells within the GCL (Figure 1D).

The dendritic arborization of SACs shows four main branches sprouting in the four cardinal directions, that further divide in sub-branches such that each occupy a quadrant and the whole dendritic tree extends into a circular area with a radius of 100-150 μm [39]. The SAC cell bodies display mosaic-like spacing of their cell bodies throughout the INL or GCL, separated from their nearest neighbours by about 15-30 μm [40,41]. Hence there is considerable overlap of dendritic arbors of nearby SACs, and viewed from the top, the entire dendritic network looks like a honeycomb structure [39]. To assess whether the potentially integrated CTG labelled cells, or CTG labelled cell material transferred to residing SACs, had adopted this phenotype, we studied their morphology using Confocal Laser Scanning Microscopy (CLSM) of flat-mount preparations. Analysis of orthogonal projections of Z-stacks showed that the CTG labelled cells displayed the same mosaicism, and that their dendritic arborization is identical to that of adjacent resident cells (Figure 2 A-C).

To validate the possible integration of the NTPDase2-positive sorted cells or of cell material labelled with CTG we performed a number of control experiments. Intravitreal injection of CTG alone in culture medium did not lead to specific labeling of any particular cell-type of the neural retina (Figure 3A). However, this experiment does not rule out the potential release of conjugated CTG from cells. Thus, we performed an experiment in which we cultured sorted NTPDase2-positive and NTPDase2-negative cells for two days in the presence of CTG and then intravitreally injected the medium and cells separately. We did not find CTG-positive NTPDase2-negative cells in the intravitreal cavity (Figure 3B). However, we did find CTG-positive NTPDase2-positive cells (Figure 3C) below the ganglion cell layer, although no CTG-positive staining was found within the retina (Figure 3D). When we analysed the retina of intravitreal injected medium from NTPDase2-negative non-MGC we did not detect any CTG within the retina (Figure 3E). Surprisingly, when we analysed the retina of intravitreal injected medium from NTPDase2-positive MGC we found the same Starburst amacrine cells staining pattern as shown in Figure 1 and 2. We thus hypothesize, that release of conjugated CellTracker Green labelled cell-material from transplanted NTPDase2-positive MGC resulted in cell-material migration through the retina and transfer to resident Starburst amacrine cells.

As an alternative approach to cell dyes we sorted NTPDase2-positive cells from tdTomato mice. Thus, sorting retinal cells from mice that were tomato-red transgenic in all cells, facilitating tracing of these cells upon transplantation. Adult and P5 mouse flow cytometric sorted NTPDase2-positive MGC were transplanted subretinally into the retina of both wild type C57BL/6J mice and *Crb1*^{KO} mice, yielding similar results. Lysed or rarely large swollen cells (auto fluorescent in all channels) were found post transplantation in the subretinal space (Figure 4 A-D), but migration of healthy MGC or MGC-derived tomato-red material into the retinal layers could not be detected. Cells resided mostly in the subretinal space (Figure 4 A-D, arrows), however some cells could be found within the photoreceptor inner segment layer (Figure 4 A-D, arrowhead).

Discussion

In this study, we have found that NTPDase2-positive cytometrically sorted MGC from mouse retinas do not integrate into the retina upon subretinal injection under the conditions used. Additionally, we show that CTG-labelled material derived from NTPDase2-positive MGC

migrated through the retina and transferred selectively to Starburst amacrine cells in the INL and GCL.

We used mouse NTPDase2-positive MGC and subretinally transplanted these cells into the retina of both wild type C57BL/6J mice and *Crb1*^{KO} mice, amounting to similar results. The isolated NTPDase2-positive MGC seem to lack the intrinsic ability to migrate directly into retinas and resided within the subretinal space. Previously, Wan et al. [28] found that intravitreal injection of MGC in healthy rodent retina led to the cells congregating in the vitreous cavity. Similarly, we found congregation of NTPDase2-positive MGC labelled with CTG upon intravitreal injection in *Crb1*^{KO} mice (Figure 3C, arrows). However, Wan et al. [28] found in *N*-methyl-*N*-nitrosourea treated retina, which leads to complete loss of photoreceptors that transplanted lac-Z-labelled MGC did integrate into the ganglion cell layer, the inner plexiform layer and the outer margin of the inner nuclear layer. It appears that the transplanted MGC require environmental cues present in toxin-damaged retinas for integration into the retina. Increased donor photoreceptor material transfer to residing photoreceptors was previously shown in *Crb1*^{rdB/rdB} compared to healthy retina [32]. We did however not observe migration of MGC into the retina of *Crb1*^{KO} or wild type mice. In these studies, we did not compare for differences in CTG-labelled MGC-derived-material transfer in *Crb1*^{KO} versus wild type retina. It would be worthwhile to test for transplantation and migration of MGC into retinas with severe disruption of the outer limiting membrane such as the *CRB1*-LCA-like mouse models [23,42,43]. However, factors present during gliosis and retinal degeneration may further hinder the potential of MGC to proliferate and differentiate in diseased retina [27].

NTPDase2-positive MGC might need some conditioning through culture systems, for instance to differentiate them into early photoreceptor precursors. Manipulation through culture systems also allows for expansion of the population, this is especially relevant for MGC as they are known to be quiescent and less plastic in mammals. MGC from adult *trp53*^{+/+} or *trp53*^{-/-} mice are able to proliferate in response to EGF stimulation compared to control mice. Highlighting the possibility of manipulating adult mammalian MGC to re-enter the cell cycle [44]. Furthermore, MGC from *trp53*^{-/-} mice can proliferate becoming progenitor-like cells. Under *in vitro* conditions the *trp53*^{-/-} progenitor-like cells could be differentiated into photoreceptors which upon transplantation migrated into the mouse retina [22]. The proliferative potency of MGC has been found to vary between mouse strains [45]. Overexpression of proneural factor Achaete-scute homolog 1 (*ASCL1*) or manipulation of its expression with miR-124-9-9* in dissociated mouse MGC leads to MGC reprogramming into retinal progenitors [11,46]. Human MGC which exhibit stem cell characteristics can be differentiated into rods and ganglion cells *in vitro*. When transplanted, these MGC can integrate into the retina providing some functional rescue [17,20,21].

Transplantation of cells from alternative cell lineages is also another promising avenue for manipulation of MGC. Recently, human umbilical tissue-derived cells have been used to attenuate MGC reactivity and preserve visual function in Royal College of Surgeons rats [47]. Similarly, olfactory ensheathing cells have previously been used to reduce gliosis of MGC through downregulation of components of the Notch signalling pathway [48,49]. Cellular reprogramming *in vivo* provides another avenue to explore the potential of MGC. Overexpression of *ASCL1* along with a histone deacetylase inhibitor enabled adult mice to generate neurons from MGC after retinal injury which responded to light stimulus [50]. Transplantation of hematopoietic stem and progenitor cells with activated Wnt functionality into degenerating retinas resulted in cell fusion with MGC. The fused cells reprogrammed into immature photoreceptors and resulted in morphological and functional retinal rescue in rd10 mice [51].

To assess the behaviour of NTPDase2-positive MGC upon subretinal transplantation we post-sort stained them with CellTracker Green (CTG) to track their localization. CTG along with a number of other vital dyes have been reported to preferentially accumulate in animal and postmortem human cadaveric donor

retina MGC [52]. This is thought to be due to the high concentrations of glutathione present in MGC [53-55]. The CTG probe is a cell impermeant dye used for long term lineage tracing. These cell tracker probes can freely diffuse through the membrane of living cells and undergo esterase hydrolysis turning the probe fluorescent but also making it mildly thiol-reactive. The cell tracker probes are believed to undergo a glutathione S-transferase-mediated reaction to produce a membrane-impermeant glutathione-fluorescent dye. In this study, we found that NTPDase2-positive MGC but not NTPDase2-negative non-MGC cytometric sorted cells, incubated with CTG, produced CTG-labelled-MGC-material that migrated through the retina and was transferred to Starburst amacrine cells. One potential route for CTG conjugate uptake is by import transporters on the plasma membrane of Starburst amacrine cells, such as the Anion Transporting Polypeptide Superfamily (OATPs). OATP1A2 and OATP2B1 have been found expressed in the soma and processes of amacrine cells, respectively [56]. Additionally, amacrine cells are enriched for two glutathione S-transferases subunits [57]. Thiol-reactive CTG released from living, lysed or dying NTPDase2-positive MGC would be able to undergo glutathione S-transferase-mediated reactions to produce membrane-impermeant glutathione-fluorescent dye in Starburst amacrine cells.

Starburst amacrine cells have been linked to congenital nystagmus a neurological disease affecting the optokinetic reflex and leading to impaired vision [58,59]. Patients with mutations in the *FRMD7* gene have congenital nystagmus [59]. The *FRMD7* protein is exclusively expressed in Starburst amacrine cells of the mouse retina and enriched in Starburst amacrine cells of non-human primate retina. A *FRMD7* hypomorphic mutant mouse model, similarly to in humans, lead to the loss of the horizontal optokinetic reflex due to the genes role in establishing spatially asymmetric inhibitory inputs from Starburst cells to direction-selective ganglion cells. Additionally, loss of Starburst amacrine cells have been implicated in the early stages of retinal neuropathy in a mouse model for diabetes [60]. Thus, further investigation into the identity of the importer that leads to the uptake of CTG-labelled-MGC-derived material by Starburst amacrine cells may yield a route for pharmacological intervention.

Conclusion

Our study provides a foundation on which NTPDase2-positive Müller glial cells transplantation approaches can be further developed. Additionally, the transfer of CTG-labelled-MGC-derived material to Starburst amacrine cells gives insight into potential novel characteristics of import transporters on the plasma membrane of Starburst cells.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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