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Regenerative Stem Cell Therapy in Retinal Degeneration

Irfan Ahmed Siddiqui[1] Dr. K. Babu Rao[2]

[1]Research Scholar, CMJ University, Shillong, Meghalaya

[2]Principal Donbasco College Pharmacy, Guntur District, AP

Abstract – Stem cell-based therapy has been tested for several diseases, including neurodegenerative disorders, such as Parkinson's disease, spinal cord injury, and multiple sclerosis in animal models. The replacement of lost neurons that are not physiologically replaced is pivotal for therapeutic success. In the eye, degeneration of neural cells in the retina are hallmarks of such wide-spread ocular diseases as AMD and RP. In these cases the primary cause of blindness is due to loss of photoreceptors. This can result from dysfunction in either the PRC or the underlying RPE that supports their survival.

Transplantation of RSC with the potential to generate new retinal cells provides an alternative approach to enable the replacement of lost PRC or RPE. Retinal stem cells may restore vision in patients who have degenerative retinal diseases by two possible means: 1) repopulation of the damaged retina (e.g., PRC); and/or 2) rescue of retinal neurons from further degeneration.[80] Different research groups have successfully isolated murine putative RSC from the ciliary margin (CM) and human RSC in the pars plana and pars plicata.[81,82] However, the transplantation of these cells in normal and degenerative rodent retina was only minimally successful due to the limited ability of the cells to invade and integrate into the host retina.[27] On the other hand, transplantation of immature post-mitotic rod precursors from developing retina (postnatal day 1) improves retinal integration.[83] The optimal result occurs when selected cells were biochemically committed but not yet morphologically differentiated. The capability of subretinally or intravitreously injected RSC to invade and integrate into the neural retina remains restricted to sites of retinal injury. Breakdown of physical barriers, such as the outer limiting membrane, and/or release of unknown neurotrophic factors, are most likely required to stimulate RSC integration.[84] To date only sparse data are available regarding factors that might stimulate migration, integration, and differentiation of RSC into the neural retina.

Keywords: Retinal Degeneration, Neuro Degenerative Disorders, Regenerative Stem Cells (RSCs), Bone Marrow Stem Cells (BMS), Retinal Pigment Epithelium (RPE), Transforming Growth Factor (TGF), Fibroblast Growth Factor (FGF).

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INTRODUCTION

It is assumed that neurotrophic factors, such as transforming growth factor (TGF-beta 3),[85] fibroblast growth factor (FGF),[86] or epidermal growth factor (EGF),[87,88] might play a role. Recent evidence has suggested that hepatocyte growth factor/scatter factor (HGF/SF), a pleiotrophic factor with mitogenic, and morphogenic activities, may also be involved in the development and maintenance of neurons and PRC.

The replacement of diseased RPE in AMD would be pivotal to protect or rescue the adjacent PRC. Unfortunately, no convincing animal model for AMD exists to date. Therefore, the sodium iodate ($NaIO_3$) model of RPE damage, established by G.E. Korte in 1984,[90] has been used to study at least the repopulation of bare areas of normal Bruch's membrane.[91] Briefly, the selective and patchy degeneration of the RPE monolayer after i.v. $NaIO_3$ injection is directly correlated to decreased visual function, decreased electrophysiological function and anatomical cell loss in the RPE .

The extent of the RPE damage is time and concentration-dependent. Interestingly, $NaIO_3$-damaged RPE cells express higher amounts of cytokine/growth factors involved in SC homing. After treatment with $NaIO_3$, murine RPE cells express higher levels of SDF-1, as well as other signaling factors (complement factor C3 and HGF/SF). SDF-1 is a chemokine whose receptor CXCR4 is expressed on bone marrow-derived progenitor cells and stem cells.[93] While there was no evident change in vascular endothelial growth factor and Rantes, there was increased expression of the cytokine leukocyte inhibitory factor, known to promote self-renewal in ESC.[94] Furthermore, supernatants of $NaIO_3$–

damaged RPE exert a priming effect on BMSC migration *in vitro* as they enhanced their transwell migration.[94] These results provide evidence that damage to the RPE leads to production of soluble factors that can cause specific chemotaxis of BMSC and raise the possibility of their recruitment to the site of damage. These data support the possibility of using BMSC to replace damaged cells, especially RPE, in eyes with retinal degenerations. To investigate this further, we have undertaken endogenous as well as exogenous approaches using BMSC using the above described $NaIO_3$ model. Endogenous refers to existing bone marrow cells in the host while exogenous refers to adoptively transferred cells.

Sodium iodate model of retinal pligment epithelium (RPE) degeneration A-F, Autofluorescence in flat-mount whole-eye preparations of control (D) and sodium iodate-treated mice (A-C,E, and F). The top row (A-C) compared different doses of sodium iodate at 7 days postinjection (P1): 35 mg/kg (A), 50 mg/kg(B), and 70 mg/kg (C) of body weight, E, B, and F compare different times PI at the same dose (50 mg/kg) : 3 days PI (E): 7 days PI (B)L and 21 days PI (F). Beinning on 3 days PI, a patchy loss of RPE can be detected by the decrease in autofluorescence (black areas). The total area bare of RPE (autofluorescent areas) is dose dependent and increased over time (original magnification x 1000).

REVIEW OF LITERATURE

The use of stem cells to replace degenerated RPE cells has not yet demonstrated the ability to rescue photoreceptors cells at risk of damage. If stem cell differentiation and reconstitution of the damaged RPE monolayer occurs after photoreceptor degeneration, a rescue effect will not be possible. Alternatively, if the mobilization of endogenous stem cells occurs continuously or over a prolonged period of time, photoreceptor damage and/or rescue may be possible.[96]

The regenerative capability of BMSC in the ocular system is not only restricted to RPE replacement. Chiou et al. showed that BMSC have multilineage differentiation potential *in vitro* and differentiate into retinal cells and photoreceptor lineages after co-culture with RPE cells.[97] Other groups have followed different approaches to replace diseased RPE cells. Haruta and colleagues harvested RPE-like ESC *in vitro* and achieved functional improvement after subretinal transplantation into RCS rats.[98]

Only a small percentage of total bone marrow cells are chemoattracted to supernatants from damaged RPE *in vitro*, as well as into damaged RPE *in vivo*, the properties of this subset of BM-derived cells need to be considered. Recent data indicate that the $CD45^+$ population of stem cells is committed to hematopoietic lineages, while the $CD45^-$ population is believed to remain pluripotent and thus capable of differentiation into various non-hematopoietic tissues.

Kucia et al. showed that $CD45^-$ BMSC are comprised of subsets of cells already committed to skeletal muscle, heart muscle, liver and neural tissues.[55] These so called TCSC, more recently re-named very small embryonic-like cells (VSEL)[100] express Oct-4, a stem cell marker, in addition to markers of tissue-specific progenitors. These TCSC are mobilized into PB during organ injury.[101] SDF-1-based chemotactic isolation combined with RT-PCR analysis of mRNA revealed that early TCSC: 1) reside in the normal human and murine BM; 2) express CXCR4 on their surface; and 3) can be highly enriched in humans and mice after chemotaxis to an SDF-1 gradient. These studies were performed on freshly isolated cells, ruling out the potential contribution of culture-related transdifferentiated HSC or mesenchymal cells. In our experiments we found that $Sca\text{-}1^+$ $CD45^-$ BMSC are highly enriched in mRNA for retinal/RPE progenitors (Six-3, OTX, Pax-6, MITF; data not shown) and furthermore, that this is the subset of BMSC that has migrated in response to supernatants from damaged RPE in transwell assays. Thus, it appears that RPE-committed VSEL cells (approximately 0.05% of the population) are present within the $Sca\text{-}1^+$ $CD45^-$ subset of BMSC. This is supported by data from *in vitro* experiments using a co-culture of BMSC and RPE cells to trigger SC differentiation into the RPE-lineage .

MATERIAL AND METHOD

Two types of approaches can be used to promote stem-cell-mediated regenerative repair of RPE: endogenous and exogenous. Endogenously, RPE injury combined with pharmacologically enhanced growth factor-mediated mobilization lead to migration of BM-derived cells into the subretinal space. BMSC ($c\text{-}kit^+$), macrophages (F4/80) and leukocytes such as granulocytes, monocytes (CD11b) could be identified. Thereby, the number of $c\text{-}kit^+$ BMSC in the eye after $NaIO_3$ injection and mobilization increased dramatically compared to the mobilized control mice who did not have RPE damage.[91] The migrated BMSC had incorporated in a monolayer along the RPE four weeks after transplantation and expressed the RPE markers RPE-65 and MITF (Figure 2). These findings suggest that bone marrow-derived stem cells are attracted to damaged RPE and are induced to differentiate into components of RPE. Mobilization enhances the outcome.

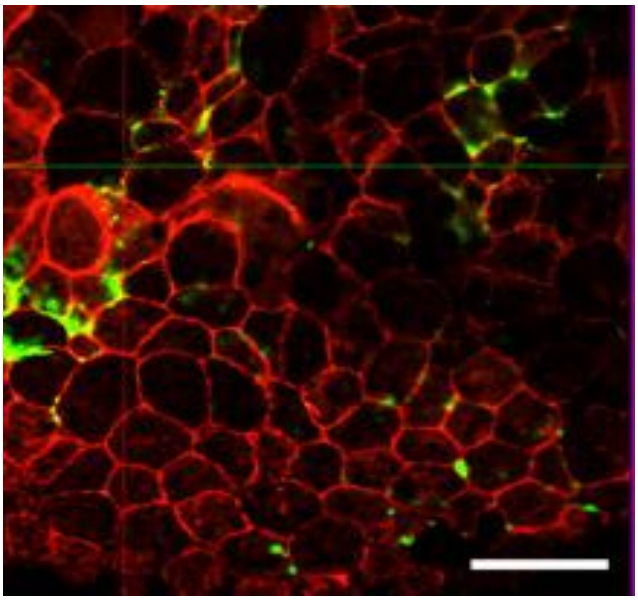

Figure 2 R??EPE-65 and MITF

EXPRESSION OF RPE MARKERS RPE-65 AND MITF

The results above demonstrated that a physiological process is in place *in vivo* to recruit stem cells to the damaged RPE and that endogenous BM-derived cells are able to integrate into the damaged RPE and express markers of RPE differentiation. Nevertheless, the significant experimental damage to the RPE could not be repaired by this endogenous approach, nor does this endogenous program appear capable of repairing or preventing the progressive damage to the RPE that occurs in AMD and retinitis pigmentosa. Thus, it appears that such recruitment of endogenous cells may not be sufficient to physiologically repair significant damage to the RPE in the same fashion that recruitment of endogenous SC cannot repair major damage to spinal cord or heart.

To optimize number and availability of circulating BMSC, we then examined an exogenous approach for regeneration of damaged RPE. Additionally, this allows us to define the precise cell types involved using cell sorting as opposed to the mixture of stem cells and other BM-derived cells mobilized into the periphery with the endogenous approach. We injected FACS-sorted BMSC with the phenotype lin^- (negative for all lineages of differentiated BM cells), stem cell antigen 1 (Sca-1)-positive intravenously (i.v.) into $NaIO_3$ treated animals. BMSC could be detected in the subretinal space on Bruch's membrane in areas of RPE loss on day four after cell injection, whereas controls without $NaIO_3$ injection showed no BMSC. The double staining for Sca-1 and green fluorescence protein (GFP) confirms the BM origin of the cells systemically transferred and confirms that HSC home to the area of damaged RPE after $NaIO_3$ injection. One and two weeks after transfer, BMSC could be identified in the subretinal space but they did not express RPE markers. Immunocytochemical staining showed the expression of RPE-65 in BMSC four and six weeks after transplantation. These results suggest that, as with the endogenous cells, BMSC injected systemically into the host home to the site of damage where they integrate and express markers of RPE differentiation in a time-dependent fashion.[95].

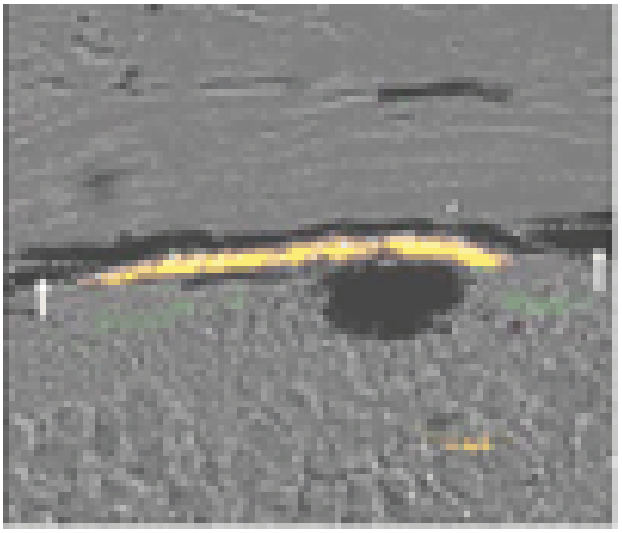

Figure 3 Immunocytochemical staining of vertical sections of a GFP chimeric

MOUSE EYE FOUR WEEKS AFTER $NAIO_3$ TREATMENT AND BMC MOBILIZATION

A third route for BMSC delivery is by direct subretinal injection. It is observed that subretinally injected BMSC integrated into the RPE and expressed markers of differentiation (e.g., RPE65). The optimal route for SC delivery remains to be determined. Concentrating the cells might provide a kinetic advantage for incorporation of the cells into the altered tissue. Thereby, the cells would not have to home to sites of damage from the circulation.

BMSC changed their morphology from round to epithelial–like and expressed the epithelial markers cytokeratin, MITF - expressed on common progenitors of retina and RPE and persisting expression following RPE differentiation (its expression diminishes in cells that progress along a retina lineage), and the RPE-specific marker RPE-65 after two weeks. The process required direct cell-cell contact between BMSC and RPE. No staining for RPE markers was detected when a membrane separated the two populations of cells. This was a specific effect, as no positive staining was detected when RPE cells were replaced with fibroblasts.[91]

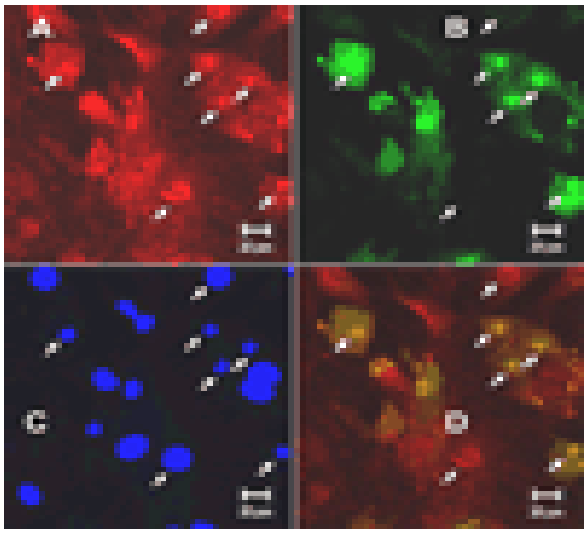

Figure 4Co-culture with RPE cells for two weeks leads to the expression of

RPE-specific markers on sorted Sca-1$^+$ BMSC

Figure 5Cross section of a mouse eye six weeks after $NaIO_3$ injection and i.v. transplantation of EGFP$^+$ BMSC

CONCLUSIONS

It is important to note that degenerations in the mammalian retina, initiated by defects in photoreceptors or RPE, often leave the neural retina deafferented. It responds to this challenge by remodelling, first by subtle changes in neuronal structure and later by large-scale reorganization and represents the invocation of mechanisms resembling developmental and CNS plasticity. This neuronal remodelling and the formation of a glial seal may abrogate many cellular and bionic rescue strategies. On the other hand, survivor neurons appear to be stable, healthy, active cells and given the evidence of their reactivity to deafferentation, it may be possible to influence their emergent rewiring and migration habits.[102]

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