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Review article

Gene-modified bone marrow-derived stem cells: an attractive gene delivery system in inherited retinal disorders

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Background: In spite of the therapeutic potential in several experimental models of inherited retinal degeneration, gene replacement therapy remains limited by technical/surgical difficulties of therapeutic gene delivery, and it seems to be effective only in young/neonatal animals. Bone marrow-derived stem cell (BMSC)-based therapy has been proposed as an alternative for treatment of retinal disorders. BMSC is a rich, inexhaustible source of potentially autologous adult stem cells. Despite numerous experimental evidences of their plasticity and therapeutic potential, the possibility that BMSC can populate the retina and differentiate into functionally retinal neurons and/or glia remains controversial.
Objective: The issues of BMSC plasticity in the ocular system are reviewed. The therapeutic benefit of BMSC *per se* and gene-modified BMSC (as a vehicle for gene therapy) in inherited retinal disorders is discussed.
Result: Recently, it was convincingly demonstrated that subpopulation of BMSC could restore the retinal function and structure by promoting/preserving the retinal vascularization rather than differentiating to retinal neurons/glia. In animal models of brain disorders, such as Parkinson s disease, BMSCs has been demonstrated as a promising vehicle for the delivery of therapeutic genes. Although little is known about the therapeutic potential of gene-modified BMSC in the ocular system, long-term engraftment and stable gene expression of gene-modified BMSCs have been shown in rodent retinas.
Conclusion: The experimental evidences published over the past decade imply a possibility to use BMSC as a gene delivery system which can be simply transplanted and provide a stable long-term gene expression in the retina.

Inherited retinal degenerations are a major cause of untreatable vision loss and blindness worldwide. Such inherited retinal degenerations result, for instance, from the mutation of genes in photoreceptor cells (*rd, RPGRIP, RS-1*) or in retinal pigment epithelium (RPE) (*MerTK, RPE65, OA1*). To date, inherited retinal disorders remain incurable. Gene-based therapy has been demonstrated as a promising therapy for a wide range of inherited retinal disorders. Gene-based therapy aims 1) to correct gene defects by gene replacement or silencing, or 2) to create a stable gene reservoir expressing therapeutic molecules (such as neurotrophic factors) in non-monogenetic disorders, or 3) to treat malignant neoplasias by transferring genes that code for apoptotic molecules, immunomodulators, suicide genes or neovascularization antagonists. Numerous experimental studies over the past decade have demonstrated proof-of-principle that gene-based therapies can mediate quantifiable improvements in retinal morphology and visual function. However, such therapies remain limited by the technical/surgical difficulties of *in vivo* gene delivery. Moreover, the regenerative potential of gene therapy seems to depend critically on the age of the animal and the site of implantation. Therefore, it is of interest to develop an alternative gene delivery system, which can circumvent the technical/surgical difficulties and, in addition, provide therapeutic efficacy both in young and adult animals. Bone marrow-derived stem cells (BMSCs) have been presented as one of the most effective candidates for such a gene delivery system, since they are capable of producing various trophic factors and can rescue retinal cells, as well as the retinal vascular system [1-9]. Although the plasticity

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of BMSC remains controversial, various experimental studies in rodents have demonstrated that systemically administrated BMSC can migrate into the retina, and differentiate to microglia/ macrophage. Furthermore, the engraftment of gene-marked BMSC was found to be enhanced by pathological conditions in both brain [10] and the ocular system [11], and BMSC were specifically attracted to the sites of degeneration. Although BMSC-based gene therapy for the ocular system has not yet been well explored, BMSC have been successfully demonstrated as vehicles for gene therapy in various rodent models of brain disorders (*e.g.* Parkinson s disease and stroke). Herein, the evidences of the plasticity of rodent BMSC *in vivo*, especially in the ocular system, as well as the proposed therapeutic potential of these cells to regenerate the lesioned retina will be discussed.

Gene-based therapies in inherited retinal disorders

The eye is an ideal target organ for gene therapy. It has a highly compartmentalised structure, facilitating accurate delivery of the therapeutic gene under direct visualisation using microsurgical techniques. Moreover, the eye has been readily accessible to phenotypic examination and investigation of therapeutic effects *in vivo* by fundus imaging and electrophysiological techniques. To date, there are approximately 150 retinal disease loci with 90 disease-causing genes identified, and a wide range of appropriate animal models available for the development of experimental therapies. Although inherited retinal disorders remain incurable, the therapeutic potential of the recombinant adeno-associated virus (rAAV) and lentivirus (LV)-based vectors have been successfully demonstrated to recover retinal morphology and function in animal models of inherited retinal disorders [12-17].

rAAV vectors are increasingly utilised for their ability to mediate efficient transduction of retinal pigment epithelium (RPE), photoreceptor cells and ganglion cells. rAAV vectors were shown to mediate long term expression of the therapeutic gene/protein in the retina of animal models of retinal disorder. Long term expression of therapeutic gene is a highly attractive feature offering the means to target many life-long retinal disorders following a single administration of a vector. Tissue specificity and expression kinetics of AAV depend upon the vector serotypes and the anatomical compartment of delivery within the globe. Whereas AAV-2/2 and AAV-2/5 transduce both photoreceptors and RPE cells, AAV-2/4 and AAV4/4 mediate expression that is restricted to the RPE [18]. rAAV-mediated gene replacement of peripherin, a membrane glycoprotein essential for the formation and stability of photoreceptor outer segments, restores retinal ultrastructure and function for as long as 14 weeks in the mouse model of retinal degeneration. In this study, short term gene replacement may relate to the timing of intervention or the regulation of peripherin expression [19]. In the mouse model of X-linked juvenile retinoschisis, rAAV-mediated expression of retinoschisin by photoreceptors and ganglion cells in adult mice results in an improvement in retinal function (demonstrating by ERG) but no morphological regeneration. However, rAAV-based retinoschisin replacement in young mice results in both long term functional and structural improvement [20, 21].

LV vectors are able to stably transduce non-dividing cell populations. They are thus attractive candidates for retinal gene therapy. Human immunodeficiency virus-1 (HIV-1) and feline immunodeficiency virus (FIV)-based lentiviral vectors were shown to stably transduce cells of the RPE for at least 2 years following delivery to the subretinal space in rodents [13-15]. The therapeutic effects of both HIV-1-based and simian lentiviral vectors were demonstrated in RPE-based retinal degenerations [12, 15]. Unlike transduction of RPE, lentivirus-mediated transduction of photoreceptor cells appears to occur under certain circumstances depending on retinal maturity, the promoter used and anatomical barriers. While the efficiency of transduction in adults is low, photoreceptor cells of neonatal rodents can be successfully transduced by the subretinal delivery of lentiviral vector that was driven by a rhodopsin promoter [22]. It has been, however, demonstrated that the transduction efficiency in mature photoreceptor cells can be improved by local retinal trauma and by enzymatic disruption of the inter-photoreceptor matrix.

Proof-of-principle for gene-based replacement strategy has been numerously demonstrated over the past decade in animal models of inherited retinal disorders owing to loss-of-function mutations in the particular genes encoding proteins that play a critical role in the ocular system rAAV-mediated gene replacement of RPGRIP, a protein anchored in the photoreceptor connecting cilia, rescues photoreceptor cells and retinal function in a murine model of Leber's

congenital amaurosis (LCA) by restoration of RPGR localization [23]. Another form of LCA is caused by a mutation in *RPE65* gene, encoding an RPE-specific visual cycle isomerase essential for synthesis of 11-*cis*-retinal. *In utero* intraocular delivery of rAAV-based vector expressing *RPE65* in *RPE65*$^{-/-}$ knockout mice results in efficient RPE transduction and rescue of photoreceptor degeneration [24]. rAAV-based gene replacement of RPE65 in the Swedish Briard dog, a model which is homozygous for a null mutation in *RPE65*, significantly improves visual function as demonstrated by both electrophysiology and behavioural assessments. The improvement of visual function has been maintained for longer than 3 years [25-27]. In the Royal College of Surgeons (RCS) rat, rAAV or lentiviral vector-mediated gene replacement of Mertk - an RPE receptor tyrosine kinase responsible for phagocytosis of shed photoreceptor outer segments - results in restoration of phagocytic function and slowing of photoreceptor degeneration [15,28]. In a murine model of ocular albinism, gene replacement of OA1, which encodes a protein responsible for organization of RPE melanosomes, increases melanosome numbers and improves electroretinographic abnormalities [29].

Taken together, gene-based therapies have been convincingly demonstrated to mediate significant improvements in retinal morphology and function in experimental animal models of inherited retinal degenerations by gene replacement strategy. However, these therapies are yet limited by the technical difficulties of *in vivo* gene delivery. The regenerative potential of these therapies seems to depend critically on the maturity of the retina and the site of implantation. Moreover, the cell-specificity of gene expression is highly related to the viral vector serotypes.

Can stem cells truly become retina?

Stem cells (SCs) are a type of unspecialized cells characterized by their capacity to self-renew and generate progeny capable of differentiating into multiple yet distinct cell lineages. Due to their putative ability to self-renew and provide neurotrophic factors that promote survival, migration and differentiation of endogenous precursor cells, they are believed to be able to retard or even reverse degeneration of the retina. Totipotent embryonic stem cells (ESCs) are an ideal source for stem cell-based therapy, since they have the potential to form all types of cells in the fetus or adult. However, the experimental/clinical application utilizing ESCs has raised serious ethical and legal concerns, thus limiting the use of these cells at present. SCs derived from the adult central nervous system (CNS) or bone marrow (BM), are an alternative source for treatment of retinal degenerations. Since accessibility limits use of CNS-derived SCs, adult bone marrow is the most promising source of autologous adult stem cells. Although numerous experimental evidences have shown that systemic or intraocular implantation of BMSCs can promote the functional and/or structural regeneration in the retina of animal models, a plasticity of BMSCs remains to date controversial. BMSCs comprise at least 2 populations of stem cells, haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). In addition to the ability to replenish the haematopoietic system by generating cells of the myeloid and lymphoid lineages and to reconstitute the whole immune system after allogeneic transplantation, BMSCs have been shown to contribute to nonhematopoietic cell types, including cardiac myoblast [30,31], skeletal muscle [32-35], hepatocytes [36-38] and neuron cells [39-42]. The cellular events or mechanisms leading to such cross-lineage transformations remain a subject of debate and intense research [43-48]. However, it has been widely demonstrated that systemically transplanted-BMSCs can migrate into the brain and subsequently contribute, albeit rarely, to the particular cells expressing neuronal morphology and antigens [39-43, 45, 46, 48]. This engraftment and contribution in the brain was suggested to be enhanced by injury [10]. Among these reports, many researchers transplanted donor, unfractionated bone marrow rather than the fractionated population into the recipient rodent. Therefore, the cell types responsible for demonstrated effects can hardly be determined. Besides HSCs and MSCs, it was conceivable that stem cells from other tissue, such as liver, nervous system and muscle, may circulate at low levels and be found in the bone marrow [49]. It therefore remains unclear whether BMSCs are truly pluripotent and capable of differentiating to neurons and neuroglia or the above-mentioned evidences have just occurred through the action of other circulated, organ/tissue-restricted stem cells.

Haematopoietic stem cells (HSCs) represent a rare population of 0.01-0.05% of whole bone marrow. They are capable of long-term, multilineage reconstitution and prompt the recovery of a lethally

irradiated host after transplantation. HSCs are the best characterized and understood among all adult stem cell. HSCs express stem cell antigen-1 (Sca-1), c-kit receptor (c-kit) and low level of CD90 (Thy-1), but they are negative for lineage markers (CD2, CD3, CD4, CD5, CD8, NK1.1, B220, Ter119, GR-1 and Mac-1), thus Thy-1^{low}Sca-1^{+}c-$kit^{+}$$Lin^{-}$ [11]. In addition, HSCs express also the haematopoietic markers, such as CD45, CD34, CD133 and CD 117 (c-kit). Recently, the so-called "SLAM family" - receptors that were expressed on the cell surface of BM-derived HSCs - was successfully demonstrated to be a tool for distinguishing HSCs from non-self-renewing multipotent hematopoietic progenitors (MPPs) and other restricted progenitors [50, 51]. The SLAM family contains CD150, CD244 and CD48. Highly purified HSCs showed $CD150^{+}CD244^{-}CD48^{-}$, whereas MPPs were $CD244^{+}CD150^{-}CD48^{-}$ and most restricted progenitors were $CD48^{+}CD244^{+}$ $CD150^{-}$. BM-derived HSCs have been proposed *in vivo* to acquire neuron and/or neuroglia cell fates [39-42, 48, 52, 53] and *vice versa* [54, 55]. This transdifferentiation crossing the classical embryonic germ cell layers of ectoderm, mesoderm and endoderm remains a debatable issue [56-61]. To date, the most widely accepted hypothesis is that HSCs are capable of infiltrating the brain and generating Purkinje cell and microglia, but not neuroglia [10, 41, 43, 45, 46, 48, 52, 56-61]. Based on the results investigated by Priller *et al.*, the majority of BM-derived HSCs (>50 %) are able to cross the blood brain barrier (BBB) into the brain as early as 2 weeks post-bone marrow transplantation (p-BMT) to irradiated recipients. These cells are located in the perivascular spaces between the basal laminas of endothelia and astrocytes [41, 62, 63]. These early engrafted cells differentiate into perivascular cells and parenchymal microglial cells that express a microglia/macrophage marker, Iba1. More abundant cells have been detected at least 4 months p-BMT. At 12-15 months p-BMT of donor green fluorescent protein (GFP) transgenic HSCs, ~ 40 % of the parenchymal microglial cells are found to be HSCs derived and up to 0.1 % of fully developed cerebellar neurons are shown to express GFP in the perikaryon, axon, and dendritic tree. According to the morphologic characteristics and the expressions of calbindin-D28K, these GFP^{+} cells have been identified as Purkinje cells. HSCs-derived Purkinje cells are also shown to express the glutamic acid decarboxylase, the - aminobutyric acid (GABA)-synthesizing enzyme, and to possess multiple synaptic contacts. These BM-derived cells are therefore classified as functional Purkinje cells. In the ocular system, adult BM-derived myeloid progenitor cells have been demonstrated to migrate to an avascular zone of the retina, differentiate into microglia, and facilitate normalization of the vasculature [8]. Tomita *et al.* [64] demonstrated that stem cell-enriched BM cells, that were intravitreally implanted into injured rat retina, could migrate to the outer nuclear layer (ONL) around the lesioned site and differentiate to the retinal neuron/glia expressing GFAP, calbindin, rhodopsin, and vimentin. Systemically transplanted HSCs into a mouse model of RPE damage could target the damaged site and differentiate into an RPE phenotype, demonstrated by expression of markers of RPE lineage, RPE65 [11], or by expression of melanosomes and RPE morphology [7]. A subpopulation of HSC (*i.e.* Lin^{-} HSCs) that contain endothelial precursor cells (EPCs), has been shown to be capable of forming blood vessels *in vivo* by selectively targeting retinal astrocytes [65].

Mesenchymal stem cells (marrow stromal cells, MSCs) are a heterogeneous population of BMSC that grow in the cultures as adherent cells. They are able to differentiate into non-hematopoietic tissues, *i.e.* osteoblasts, chondroblasts, adipocytes, tenocytes, and hematopoietic-supporting stroma [66]. It is generally impossible to distinguish a particular subpopulation of MSCs from another in terms of its differentiative, or trans-differentiative potential, due to the lack of specific stem cell markers for MSCs. In general, MSCs express Stro-1, HLA class 1 molecules, CD13, CD28, CD29 (β1-integrin), CD33, CD44, CD54 (ICAM-1), CD90 (Thy-1), CD105, and CD106 (vascular cell adhesion molecule-1), but not CD45 and CD34. In addition, MSCs are shown to express cytokines, such as interleukins (IL) 6 and 7, leukaemia inhibitory factor, stem cell factor, granulocyte and macrophage colony-stimulating factors, thrombopoietin, tumour necrosis factors (TNF) β1, β2, and α, and interferon [67-70]. These cytokines are known to play roles in survival, proliferation and differentiation of HSCs *in vivo*. Unlike HSCs, an application of MSCs has been preceded by driving its differentiation towards neurons/neuronal phenotypes *in vitro* prior to transplantation *in vivo*. *In vitro* pre-differentiation of MSCs is supposed to enhance their anatomical and functional integration in ameliorating lesions that are peculiar to specific brain disorders. In the retina, MSCs pretreated

with BrdU and transforming growth factor-β3, express a photoreceptor marker after intravitreal transplantation [71]. Subretinally transplanted MSCs were demonstrated to integrate into RPE layer, ONL, outer plexiform layer (OPL), inner nuclear layer (INL) and ganglion cell layer (GCL). These integrated MSCs differentiated to the retinal cells that expressed neuronal nuclei (NeuN), neuron specific enolase (NSE), GFAP and cytokeratin (CK) [72]. Two weeks after subretinal injection into the adult RCS rat, $CD90^+$ MSCs integrated into the host retina, forming photoreceptor layer-liked structures and expressed a photoreceptor-specific marker. Additionally, these $CD90^+$ MSCs were shown to attract synaptic vesicles and hence may be capable of signal transduction [2]. A study comparing the differentiation capacity of retinal progenitor cells (RPC) and MSCs showed that both types of cells migrated into retina and expressed neurofilament 200, GFAP, protein kinase C-α, and recoverin. Interestingly, RPCs (not MSCs) expressed a photoreceptor marker, rhodopsin. A vast majority of MSCs differentiated into microglial-like cells, rather than retinal neurons [73].

BMSC-based therapy in retinal disorders

The therapeutic potential of BMSCs has been proposed for retinal disorders. Despite the uncertainties of their plasticity, reports over the past decade in animal models of retinal degenerations have challenged the possibility of using BMSC to restore and preserve the function and/or the structure, as well as the vescular system, of the retina [1-9, 11, 64, 65, 72-75]. In a rat model of glaucoma, the intravitreal implantation of MSCs was shown to rescue the retinal ganglion cells (RGCs) by expressing various trophic factors [9]. Intraocular implantation of mouse MSCs into the mouse model of RP (*rho*$^{-/-}$) resulted in significant rescue effects, demonstrated by the occurrence of preserved photoreceptor cells [74]. Implantation of MSCs into the subretinal space of RCS rats, a model of retinal degeneration, could retard the retinal degeneration and, furthermore, preserve the retinal function [75]. Since a loss of retinal vasculature is a presumed metabolic consequence of photoreceptor degeneration, preservation of the vascular system may provide an advantage in treatment of a heritable group of blinding diseases resulting from loss of photoreceptors, such as RP. A particular population of BMSCs (*i.e.* Lin^- HSCs) has been shown to be capable of stabilization and rescue of the retinal vasculature after intravitreal transplantation. Moreover, a dramatic neurotrophic rescue effect of these cells was also observed in mouse models of retinal degeneration (*rd1* and *rd10*) [5]. These therapeutic effects may be due to the upregulation of various anti-apoptotic genes. On the other hand, non-purified adult HSCs showed hemangioblast activity resulting in retinal revascularization in an adult mouse model of retinal ischemia [1]. Although the capacity of BMSCs to rescue the retinal cells/vasculature has been shown in numerous studies, insufficient evidence was provided in support of the notion that the preserved retinal cells/vasculature could lead to a sufficient restoration of retinal neurons, especially photoreceptor cells, and to further improvement of retinal function. In addition, recent experimental evidence showed the proposal that BMSCs can populate and differentiate into various functional neurons (including retinal neurons) has not been universally accepted. It therefore remains controversial whether or not BMSCs *per se* can promote restoration of both structure and function of neonatal and adult mouse retinas.

BMSC-based gene therapies

BMSCs are an attractive candidate for gene transfer, since they are easily accessible for harvest, and readily delivered back to the patient by systemically autologous transplant methods.

In the brain, genetically modified HSCs were first demonstrated by Priller et al. [10] to enter the CNS and differentiate into microglia after systemic bone-marrow transplantation. This engraftment was shown to be enhanced by neuropathology, and gene-marked BMSCs were specifically attracted to the sites of neuronal damage. It was the first study that suggested the possibility to use BMSCs as a vehicle for gene delivery to the nervous system. In a mouse model of multiple sclerosis (MS), the systemic transplantation of BMSCs, transduced with retroviral vector encoding full-length phospholipids protein (PLP) into a model for relapsing, remitting experimental autoimmune encephalomyelitis (EAE), resulted in reduction of T cell proliferation in response to PLP p139-151 and abolishment of EAE. In addition, EAE could be prevented by administering PLP-transduced BMSCs on day 12 after immunization [76]. In the same mouse model, intravenously applied bone marrow-derived and TREM2-lentiviral-transduced myeloid precursor

cells, facilitate repair and resolution of inflammation within the CNS by clearance of cellular debris during EAE [77]. Schwarz *et al.* demonstrated that rat and human MSCs (r- and hMSCs) transduced with retroviruses encoded tyrosine hydroxylase (TH) and GTP cyclohydrolase I (GC) were capable of producing L-DOPA, while they remained multipotent [78]. On one hand, engrafted cells could survive up to 87 days in the lesioned hemisphere; on the other hand, by 9 days post-implantation, the production of L-DOPA *in vivo* was no longer detected according to the ceasing of the transgene expression. Similarly, a 6-hydroxydopamine (6-OHDA) rat model of Parkinson s disease showed behavioural improvement by reducing apomorphine-induced rotation only up to 7 days after intrastriatial injection of genetically modified MSCs. Interestingly, this improvement however was not detected when the donor MSCs were transfected with TH-encoded virus alone. In contrast, it was demonstrated recently that 6-OHDA rats showed behavioural improvement in apomorphine-induced asymmetric rotation throughout the 13-week observation period following the intrastriatial injection of recombinant adeno-associated virus (rAAV)-TH-transfected rat MSCs [79]. Furthermore, at 84 days after transplantation, *TH* gene was determined immunocytochemically to be expressed around the site of transplantation, and dispersed in the lesioned striatum of 6-OHD rats. At this point in time, the dopamine level was analysed to be greater in the lesioned striatum of 6-OHDA rats injected with TH-MSCs than in those treated with LacZ-MSCs (a control group). Retroviral-transfected BMSCs were demonstrated to be able to serve as a delivery system for the IFN-beta cDNA into the mouse CNS. This result suggested a therapeutic potential of gene-modified BMSCs in neurologic disorders in which IFN-beta is involved (*e.g.* MS, viral encephalitis, and brain tumors) [80].

In the murine retina, little is known about the potential efficacy of BMSCs as a vehicle for delivery of the therapeutic gene. However, several experimental studies have demonstrated that the gene-modified BMSCs can target the retina and express particular gene/protein. Green fluorescence protein (GFP)-expressing BMSCs which were applied systemically into the lethally irradiated recipient, could target the retina and differentiate microglia as early as 8 weeks after bone marrow transplantation. At this time point, the vast majority of the BMSC-derived and GFP-positive microglia were found around the ON, and in the GCL and IPL of the retina (**Fig. 1**). By 6 months, almost all retinal myeloid cells were GFP-positive. These cells were demonstrated around the ON, and within the GCL, IPL, OPL and photoreceptor layer. They were, moreover, shown to coexpress CD11b, a marker for microglia (**Fig. 2**). Since these BMSCs showed long-term engraftment as well as gene expression, in the retina, they may serve as a cellular gene transferring system that provides a stable expression. Non-viral transfected Lin^- HSCs expressing an anti-angiogenic peptide were observed to incorporate into the developing vasculature and markedly inhibited vessel development, after intravitreal implantation. This observation suggests that stem cells, containing a population of endothelial progenitors, may be useful in the treatment of a broad range of ocular diseases in which blood-vessel proliferation contribute to loss of vision [65]. In this study, the applied HSC fraction could not only inhibit angiogenesis when engineered to express an anti-angiogenic, but can also rescue and stabilize a mature vasculature destined to degenerate. Since the growth of vessels and nerves is mutually dependent, preventing the vascular degeneration is believed to provide a trophic effect that promoted survival of the photoreceptors. Remarkably, the vascular rescue seen in this model seems to depend critically on the age of the animal. The regeneration potential was shown only in young mice (P2 to P15 mice) in which the retinal vasculature was not yet developed. The question therefore arose as to whether or not Lin^- HSCs/gene-modified Lin^- HSCs can provide the vascular rescue in the mature retina of the adult mice. Adenoviral transfected MSCs were observed to infiltrate the adult mouse retina and rescue photoreceptor cells in the dystrophic retina of *rho*$^{-/-}$ mice, after subretinal transplantation. These engrafted cells morphologically integrated into the RPE, as well as retinal neurons and glia [74]. However, this study could not overcome the technical difficulties and the retinal lesion due to the subretinal transplantation.

Recombinant vectors based on lentiviruses (rLVs) are widely accepted as the vectors of choice for stable, efficient transduction of both non-dividing and slowly dividing primary mammalian cells, including bone marrow-derived HSCs and mesenchymal stem cells (MSCs) [reviewed in 81, 82]. They possess several advantages: First, rLVs, which integrate into the host genome, are more resistant to transcriptional silencing,

commonly associated with cells transduced with oncoretroviral vectors [83]. Second, the self-inactivating (SIN) safety modification of lentiviruses-which permanently disables the viral promoter within the viral long-terminal repeat (LTR) after integration-allows the internal promoters to control transgene expression in the targeted cells. Regarding rat MSCs, a comparative study of transduction efficiency of adenovirus (AdV), adeno-associated virus (AAV), lentivirus (LV), and nonviral vectors showed that LV was the most effective with transduction efficiencies of up to 95 %, concurrent with low levels of cell toxicity [84]. High and moderate levels of cell transduction using LV did not affect the differentiation ability of the cells. In the same study, AdV could also effectively transduce those rat MSCs, but a significant increase in cell death was however seen with increasing viral titer. Lipofection of plasmid DNA gave moderate transfection capacities but was also toxic for the MSCs. Electroporative gene transfer was transfection-ineffective, and resulted in high cell death. However, the integration, infiltration and gene/protein expression of these gene-modified MSCs were not yet demonstrated *in vivo.*

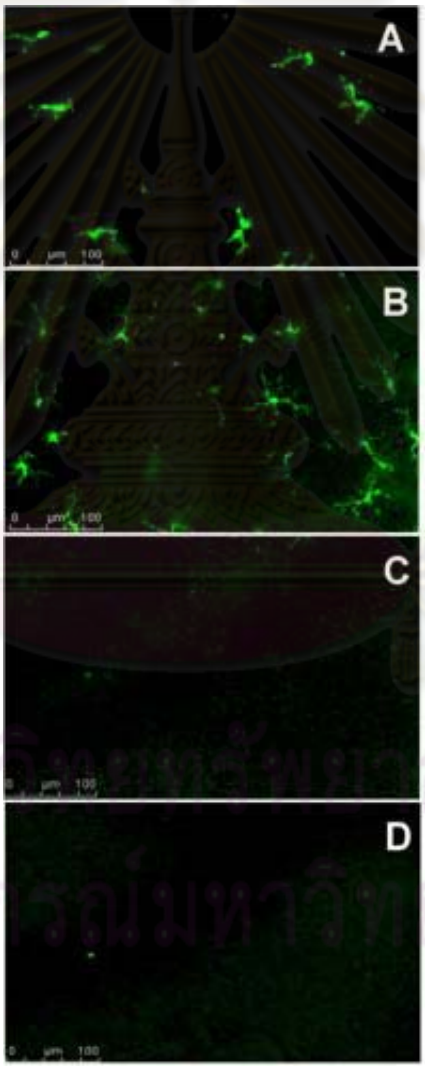

Fig.1 Engraftment of GFP-genetically modified bone marrow derived cells, which were systemically transplanted into lethally irradiated recipient mice, in the retina 2 months after transplantation. All images were obtained from whole-mount preparation of the chimeric mouse retina using a confocal laser scanning microscope (Leica, Germany). (**A**) GFP^+ donor cells (*green*) engrafted in the ganglion cell layer of the retina. They showed an amoeboid shape, implying the morphology of activated microglia. (**B**) In the inner plexiform layer of the retina, only a ramified GFP^+ cells were detected, presenting the so-called resting microglia. In the outer plexiform layer (**C**) and the outer nuclear layer (**D**), no GFP+ cells were observed. Scale bars: 100 m.

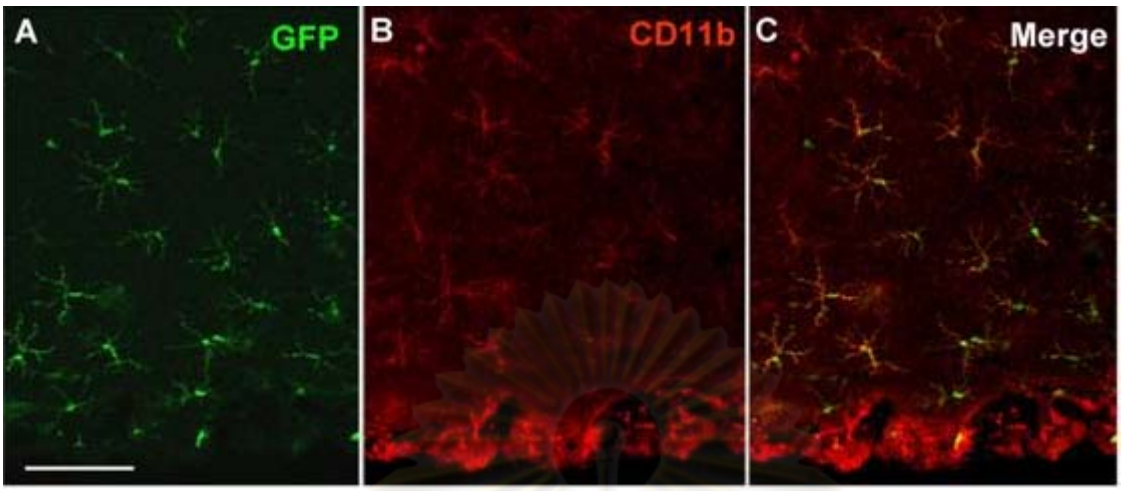

Fig. 2 Engraftment of GFP-genetically modified bone marrow derived cells (BMDCs), which were systemically transplanted into lethally irradiated recipient mice, in the retina 8 months after transplantation. All images were obtained from whole-mount preparation of the chimeric mouse retina using a confocal laser scanning microscope (Leica, Germany). (**A**) Ramified GFP^+ BMDCs (*green*) were found in the peripheral marginal retinal area (the *ora serata*). These cells showed an immunreaction for CD11b (**B**), as demonstrated by the overlay of the confocal images **A** and **B** (shown in **C**). Scale bars: 100 m.

Prospects for BMSC-based gene therapy in inherited retinal diseases

To date no effective therapy is available for treatment of inherited retinal diseases such as RP and RS. Progress over the past decade in basic research in ophthalmology has initiated the development of novel therapeutic strategies for retinal degenerations. One of the most promising therapeutic options is BMSC-based gene therapy. Although gene-modified BMSCs that were intravitreally/subretinally transplanted convincingly promoted the retinal regeneration, these therapeutic strategies remain limited by technical difficulties and the retinal degeneration caused by *in vivo* cell-transplantation. Moreover, the rescue effects seem to depend very much on the age of the retina. Cell-based gene transferring systems that provide high cell-survival and stable gene/protein expression may provide the possibility of overcoming those disadvantages. According to an *in vitro* comparative study, lentiviral vector turned out to be the most effective system to transduce BMSC with low cell toxicity, and without any effect on the differentiation ability of the cells. Several *in vivo* studies have demonstrated that systemically transplanted, GFP-transgenic BMSCs can target the adult mouse retina, engraft in different layers of the retina, and long-term express GFP. They were moreover shown to synthesize various neurotrophic factors which may promote the retinal regeneration or may retard retinal degeneration. Taken together, BMSCs may serve as a transferring system for a therapeutic gene in inherited retinal disease, since they can infiltrate into the different layers (vascular and avascular zone) of the retina, provide a stable, long-term gene/protein expression, and produce some neurotrophic factors which may mediate the retinal regeneration/preservation. However, further studies need to be performed in order to demonstrate proof-of-principle that BMSC-based gene therapy can mediate significant quantifiable improvements in morphology and function of the retina in experimental models. Concerning the clinical trials, we are still far from “bench” to “bedside”.

The author has no conflict of interest to declare.

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