Mesenchymal Stem Cell Transplantation for Retinal Degenerations and Dystrophies: Present and Future

Emma Ghazaryan, Shurong Wang, Yan Zhang, Yuxi He and Guanfang Su

Eye Hospital, 2nd Teaching Hospital of Jilin University, Changchun, China

Abstract: Retinal degenerations are the main causes of irreversible blindness in developed countries. Up to date the main pathological mechanisms of these diseases are not fully understood and consequently there is no complete treatment option for those diseases. In this aspect stem cells have drawn attention of many researchers and health care professionals. Considering ethical issues, safety and facile isolation Mesenchymal Stem Cells (MSCs) are more preferable for practical use. They have been used for several preclinical and clinical trials. In general the results were promising, however broader practical use should be preceded by resolving many problems and questions. In this review we will describe mesenchymal stem cells, especially those derived from Bone-Marrow (BMSC), their main features, privilege, mechanisms of action and their potential use for the treatment of retinal degenerations. We will also discuss the results of several pre-clinical and clinical trials.

Keywords: Retinal Degenerations, Stem Cell, Mesenchymal Stem Cells, Transplantation, Preclinical and Clinical Trials

Introduction

Retinal degenerations, which are mainly due to the genetic defects, diabetes, aging and environmental factors, are the main causes of irreversible blindness in developed countries. The final result of all types of retinal degenerations and dystrophies is the loss of photoreceptor cells, which leads to irreversible vision loss. Generally, all types of retinal degenerations progress through similar main mechanisms, with differences at the first step of the pathologic pathway. They all progress to the apoptosis of photoreceptors, total ablation of the Outer Nuclear Layer (ONL) of the retina and consequent neural remodeling of the inner neural retina (Marc et al., 2003). In the meanwhile, some types of retinal cells can escape apoptosis and preserve their main functional ability as latent cells, which made possible restoration of lost vision with electrical implants (Da Cruz et al., 2013). This gives hope that transplantation of new photoreceptors will reanimate retina and restore vision.

Stem Cells

Stem cells are undifferentiated cells with the ability of self-renewal and differentiation into more specialized cells.

The ideas about existence of stem cells came first after bombing in Hiroshima and Nagasaki in 1940 s. Those who died over a prolonged period from lower doses of radiation had compromised hematopoietic systems that could not regenerate either sufficient white blood cells to protect against otherwise nonpathogenic infections or enough platelets to clot their blood. Later, it was demonstrated that mice that were given doses of whole body X-irradiation developed the same radiation syndromes; at the minimal lethal dose, the mice died from hematopoietic failure approximately two weeks after radiation exposure. Soon thereafter, using inbred strains of mice, scientists showed that whole-body-irradiated mice could be rescued from otherwise fatal hematopoietic failure by injection of suspensions of cells from blood-forming organs such as the bone marrow. In 1961 the hematopoietic stem cells were isolated from bone marrow and since then are used in clinical practice for hematologic diseases. Friedenstein et al. (1976) described mesenchimal stem cells and called them fibroblast precursors. Later, Thomson et al. (1998) discovered embryonic stem cell, Yamanaka and Takahashi (2006) showed that they were able to transform typical fibroblast from adult skin into pluripotent stem cell and they named it induced Pluripotent Stem cell (iPS).

Now three main types of stem cells are in use: Embryonic Stem Cells (ESC), Adult stem cells, Induced Pluripotent Stem cells (iPS). IPS and ESC grow faster, being pluripotent and can differentiate...
into any kind of cell upon appropriate stimulation, but they have practical problems, halting their clinical use. They raise ethical issues and have high risks of immune rejection and tumorogenesis. In contrast to ESC and iPS, MSCs are more suitable for stem cell therapy, because of facile isolation and in-vitroculture, prolonged self-renewal ability, autotransplantation, low risk of tumor formation (Caplan, 2009) and a lack of ethical issues. There have been many studies showing that these cells are safe and have good plasticity, without producing tumors (Herzog et al., 2003; Phinney et al., 2006; Zhang and Alexanian, 2014). As such, they are preferred for transplantation therapy.

Mesenchymal stem cells can be isolated from different organs and tissues: Bone-marrow, adipose tissue, teeth pulp and also amniotic fluid, umbilical cord, placenta, etc (Erices et al., 2000; Prusa et al., 2003). The most researched and used are Bone-marrow derived Mesenchymal Stem Cells (BMSC). Generally BMSC are found in bone-marrow niche where they make trophic basis for hematopoietic cells (Bianco et al., 2001). Several researchers suggest, that these BMSC are located around the vessels in the stroma of BM and they play roles as pericytes, that can be mobilized from BM with special stimulus and migrate to the site of injury to perform their cellular functions (Wu et al., 2007; Caplan, 2008). The ratio of BMSC is decreasing with age: For newborns it’s 1:104 of nucleated cells of BM; for teens it goes down to 1:105 and for adults it goes further down to 1:106 or less (Caplan, 2009). This is consistent with that regeneration is faster and more complete in children than in adults and degenerative diseases start to manifest mainly in aged people.

**Bone Marrow-Derived Mesenchymal Stem Cells**

Bone marrow-derived Mesenchymal Stem Cells (BMSCs) are described with several features: They are isolated from bone-marrow, adherent to the bottom of plastic culture dishes, obtain spindle shape like fibroblasts and express a combination of cell surface markers (CD105, CD90, CD29, etc and negative for CD31, CD45, CD11b, etc). Under appropriate conditions, they can give rise to muscles, adipose tissue or bone cells. Although above characteristics are well among scientists, more detailed characterizations show that MSC from different species, organs and tissues, despite having many common features, have differences in the expression of cell surface markers, cytokine production and growth factors production, in addition to changing certain characteristics after ex-vivo culturing (Javazon et al., 2004; Martins et al., 2009; Bayati et al., 2013).

MSC are able to recover injured tissues and organs through following mechanism: Cellular transdifferentiation and cell replacement, paracrine trophic function (Crisostomo et al., 2008). They also can be used as non-viral, safe, prolonged acting vehicles for gene therapy or drug introduction (Arnhold et al., 2006; Park et al., 2012).

The beneficial action of transplanted MSCs is suggested to be due to the production of trophic factors (Uccelli et al., 2011), however the ability of integration and differentiation is still questionable (Harris et al., 2006; Xu and Liu, 2008).

MSC are preferred for autotransplantation. Recently researches showed that BMSC don’t express MHC class II antigens, co-stimulatory molecules CD80, CD 84 or CD40 and only express low quantity of MHC class I antigens, so they can be invisible for recipient immune system. This makes MSCs universal also for allogeneic and even xeno-transplantations (Chiu et al., 2005). It was also shown that MSC itself suppresses immune system by suppressing T-cell, B-cell and NK activity (Tse et al., 2003; Ribeiro et al., 2013). This function was even used for clinical experiments, Graft Versus Host Disease treatment, etc (Ning et al., 2008; Puymirat et al., 2009; Baron et al., 2010). MSC derived from adipose tissue, umbilical cord, amniotic fluid and bone-marrow showed generally similar characteristics, so cells for transplantations can be obtained from medical wastes (liposuction, placenta, Umbilical Cord (UC), amniotic fluid after delivery) and used safely for allotransplantations (Oh et al., 2011; Tejaswi et al., 2013; Ribeiro et al., 2013).

**MSCs in Retinal Degenerations**

Retinitis Pigmentosa (RP) and Age-Related Macular Degeneration (AMD) are the most common cases of retinal degenerations. Many scientific groups focused on these diseases, aiming at revealing pathological mechanisms and final treatment options for them. They also serve as main models for stem cell transplantation for retinal degenerations.

**Retinitis Pigmentosa**

Retinitis Pigmentosa is described by the primary or secondary loss of photoreceptors due to gene abnormalities. Its rate is about 1:3000-1:7000. Usually, RP starts with the loss of rods and followed by the loss of cones. RPE cells detach from Bruch’s membrane, migrate to inner retina and associate with abnormal vessels. Extracellular matrix deposits between RPE cells and endothelial cells of the vessels and closely resembles Bruch’s membrane in situ. Further disease progress to the formation of bone
Diabetic or aged people, with many problems within. This is not similar to human body, especially in animal models with only one disease were used. Quantities in other organs. But in all preclinical trials exactly to the injured tissue, remaining in very small.

Inoue et al., 2003; Shi et al., 2007; Shi et al., 2009; Wang et al., 2009; Jackson et al., 2010; Jackson et al., 2010a; Tsuruma et al., 2008; Hill et al., 2008; Li et al., 2009; Wang et al., 2010a; Tsuruma et al., 2014). In addition Internal Limiting Membrane (ILM) may prevent cells from integration. It will be interesting to see whether intravenous injection can be a successful route of the cell therapy for the retina. Many works showed that after IV injection MSCs migrated and localized in injured liver, heart, brain and retina, but in this case more cells were required for injection (Xu et al., 2007; Jackson et al., 2010; Wang et al., 2010b). In fact BMSC express main chemokine receptors (CC, CXC, C and CX3C) and can be attracted by their ligands, especially SDF-1 and IL-8 (Ringe et al., 2007; Shi et al., 2007). It is known that production of SDF-1 is augmented after injuries, so BMSC migrate exactly to the injured tissue, remaining in very small quantities in other organs. But in all preclinical trials animal models with only one disease were used. This is not similar to human body, especially in diabetic or aged people, with many problems within body. In this case it’s not clear, whether stem cells will be able to migrate to relatively small retina? Although many beneficial effects from BMSC transplantation have been shown, it’s not clear whether BMSC can be used to treat diseases at multiple sites of the body with intravenous injection.

After choosing the best route the second problem is when BMSC should be transplanted? For ESC even final stages of disease are eligible for transplantation with normal transdifferentiation and functional recovery (Singh et al., 2013), unfortunately for BMSC transplantation results are not that promising. The problem is due to remodeled retina, Muller glial seal and not permissive extracellular matrix for transplanted cells survival. This was shown in the work of Johnson and coworkers, who showed that after peeling of ILM with Muller cells neurits better integration and transdifferentiation rates were observed (Johnson et al., 2009). In animal models, highest levels of integration were observed in the models of retina laser injuries, which disrupt ILM (Castanheira et al., 2008; Wang et al., 2010a). Also higher integration and transdifferentiation rates were reported, if treatment was started within 24-48 h after injury.

However, the published articles showed rescue of anatomical structure and function of the retina compared to control untreated groups, but none found the toxic reaction or tumor formation after an observational period of over 230d. This made the recruitment of clinical trials with MSC transplantation possible. Several groups have already reported on the safety and feasibility of an intravitreal injection of stem cell (Jonas et al., 2010; Siqueira et al., 2011; Siqueira et al., 2013).

Several pre-clinical and clinical trials are summarized in Table 1.

But before wide use of stem cell transplantation in clinical practice many questions should be answered, particularly:

- How many cells should be transplanted and from which passage of cells?
- Is it necessary to primarily induce cells in vitro to specified lines or should they be induced with in vivo?
- In which stage of disease it is more effective and safer to transplant?
- Which is the best transplantation route?

**Age-Related Macular Degeneration**

Age-Related Macular Degeneration is a multifactorial disease caused by genetic predisposition and environmental factors, occurring with a rate of 18% at the age of 50-60 and over 30% at the age of 70 (Friedman et al., 2004).
Table 1. Summarized table of pre-clinical and clinical trials

<table>
<thead>
<tr>
<th>Author and the year of publication</th>
<th>Type of study</th>
<th>Disease model</th>
<th>Injection route</th>
<th>Cells type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kicic et al. (2003)</td>
<td>Animal model</td>
<td>Retinal degeneration in RCS rats</td>
<td>Subretinal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Otani et al. (2004)</td>
<td>Animal model</td>
<td>Retinal degenerations in or rd1 and rd10 mice</td>
<td>Intravitreal</td>
<td>Bone marrow-derived (lens negative hematopoietic stem cells</td>
</tr>
<tr>
<td>Arnhold et al. (2006)</td>
<td>Animal model</td>
<td>Retinal degeneration in RCS rats</td>
<td>Subretinal</td>
<td>Adenovirally transduced for PEDF expression BMSCs</td>
</tr>
<tr>
<td>Harris et al. (2006)</td>
<td>Animal model</td>
<td>Physical or chemical damage of retina</td>
<td>-</td>
<td>Endogenous hematopoietic stem and progenitor cells</td>
</tr>
<tr>
<td>Arnhold et al. (2007)</td>
<td>Animal model</td>
<td>Retina of the rhodopsin knockout mouse</td>
<td>Subretinal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Inoue et al. (2007)</td>
<td>Animal model</td>
<td>Retinal degeneration in RCS rats</td>
<td>Subretinal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Castanheira et al. (2008)</td>
<td>Animal model</td>
<td>Laser-injured retina</td>
<td>Intravitreal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Sasahara et al. (2008)</td>
<td>Animal model</td>
<td>Animal model of retinitis pigmentosa</td>
<td>-</td>
<td>Endogenous BM-derived microglia</td>
</tr>
<tr>
<td>Gong et al. (2008)</td>
<td>Animal model</td>
<td>Sodium-iodate induced retinal degeneration</td>
<td>Subretinal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Li et al. (2009)</td>
<td>Animal model</td>
<td>Retina injured by ischemia-reperfusion Nd: YAG laser (intravitreal)</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
<td></td>
</tr>
<tr>
<td>Hill et al. (2009)</td>
<td>Animal model</td>
<td>Degenerating neonatal rat retina following intracranial optic tract lesion</td>
<td>Intravitreal</td>
<td>Umbilical cord blood-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Wang et al. (2010b)</td>
<td>Animal model</td>
<td>Laser-injured retina</td>
<td>Intravitreal</td>
<td>Quantum dot-labelled bone marrow-derived stem cells</td>
</tr>
<tr>
<td>Liu et al. (2010)</td>
<td>Animal model</td>
<td>Light-damaged retina</td>
<td>Subretinal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Wang et al. (2010a)</td>
<td>Animal model</td>
<td>Retinal degeneration in RCS rats</td>
<td>Intravitreal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Chung et al. (2011)</td>
<td>Animal model</td>
<td>Retinotomies with developing mouse eye Nd: YAG laser</td>
<td>Intraocular</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Lee et al. (2011)</td>
<td>Animal model</td>
<td>Developing mouse eye</td>
<td>-</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Park et al. (2012)</td>
<td>Animal model</td>
<td>Axotomized retina</td>
<td>Subretinal</td>
<td>Transduced BMSCs for BDNF expression</td>
</tr>
<tr>
<td>Huang et al. (2013)</td>
<td>Animal model</td>
<td>Light-injured retina</td>
<td>Subretinal</td>
<td>Normal MSC and CX3CL1-expressing MSC</td>
</tr>
<tr>
<td>Guan et al. (2013)</td>
<td>Animal model</td>
<td>Sodium-iodate-induced retinal degeneration</td>
<td>Subretinal</td>
<td>Normal MSC or erythropoietin gene-modified MSC</td>
</tr>
<tr>
<td>Tsuruma et al. (2014)</td>
<td>Animal model</td>
<td>Light-injured retina</td>
<td>Intravitreal</td>
<td>Adipose-derived stem cells</td>
</tr>
<tr>
<td>Tsameret et al. (2014)</td>
<td>Animal model</td>
<td>Retinal degeneration in RCS rats</td>
<td>Subretinal</td>
<td>Human bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Siqueira (2012)</td>
<td>Clinical trial</td>
<td>Retinitis Pigmentosa</td>
<td>Intravitreal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Janssen Research and Development, LLC (2010)</td>
<td>Clinical trial Phase 1</td>
<td>Age-related Macular Degeneration</td>
<td>Subretinal</td>
<td>CNTO 2476 (Human umbilical tissue-derived cells)</td>
</tr>
<tr>
<td>Siqueira et al. (2011; Siqueira, 2012)</td>
<td>Clinical trial Phase 1</td>
<td>Advanced Age-Related Macular Degeneration</td>
<td>Intravitreal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Rubens Camargo Siqueira (2012)</td>
<td>Clinical trial Phase 2</td>
<td>Retinitis Pigmentosa</td>
<td>Intravitreal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>Atchaneeyasakul et al. (2012; Atchaneeyasakul, 2012)</td>
<td>Clinical trial Phase 1</td>
<td>Retinitis Pigmentosa</td>
<td>Intravitreal</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>UC (2012)</td>
<td>Clinical trial Phase 1</td>
<td>Dry Age-related Macular Degeneration Retina vein occlusion</td>
<td>Intravitreal</td>
<td>CD34+ bone marrow stem cells</td>
</tr>
</tbody>
</table>

244
It’s the leading cause of irreversible blindness in the western countries and the rate is continuously going up because of the rapid increasing of averaged age (Kolar, 2010). Generally, AMD is classified as dry or non-exudative AMD and wet or neovascular AMD, according to the presence or the absence of Neovascularization (NV). Two late forms of the disease (GA, CNV) are so different in their manifestation and in treatment options, that it is appropriate to consider them as two different diseases and it’s also not clear, what is the switch point between GA and CNV, or in another word, what determines the disease to progress into GA rather than CNV or vice versa? There are several treatment strategies for AMD (AREDS formulations, laser photocoagulation, photodynamic therapy, anti-VEGF drugs, etc.), but none of them is able to stop the progression, not to mention to cure the disease. So for those patients stem cell therapy is believed to be the main choice, however, it is quite different for two forms of disease: In GA use of stem cells is quite similar to that of RP, but in neovascularizations (wet AMD, DR) mechanisms are more complicated and controversial. The main character of wet AMD is neovascularization. Neovascularization is connected with impaired proportion of pro- and anti-angiogenic factors, with overexpression of the pro-ones, especially VEGF. MSCs produce VEGF in big quantities, which contribute to angiogenesis, so in eyes, stem cells may lead to neovascularization and have pathological effects. Supportively, many published works show that endogenous stem cells (especially Lin-HSC, EPC) participate in new vessel formation. Most studies used mice models with bone-marrow reconstituted by bone-marrow-derived stem cells from the Green Fluorescent Protein (GFP) transgenic mice and CNV was induced by laser spots (Espinosa-Heidmann et al., 2003; Sengupta et al., 2003; Takahashi et al., 2004; Tomita et al., 2004; Espinosa-Heidmann et al., 2005; Sengupta et al., 2005; Lecomte et al., 2011). Some studies showed that nearly 50% of cells in the new vessels were GFP positive, indicating they arise from bone-marrow. Hou et al. (2010) used external BMSC and showed their incorporation in new vessels without significant increase in CNV size. What is more important is that they used induced cells to produce Pigmented Epithelium-Derived Factor (PEDF), a strong anti-angiogenic factor. Then they observed a decrease in CNV formation and vessels surrounded by RPE cells, which restricts neovascularization. They proposed to use stem cells only as a safe, prolonged source of anti-angiogenic factors (Hou et al., 2010). Later, blood examination from patients with active CNV showed higher level of BM derived stem cells in peripheral blood, but with low functional ability. There is no significant difference in the CFU-EC between patients with CNV and the control group; in case of bilateral CNV the levels of CFU-EC and SDF-1 were significantly decreased (Yodoi et al., 2007; Machalinska et al., 2011).

It seems that BM derived stem cells don’t contributeto new vessel formation, rather, they contribute to vessel maturation and regulate growth. Endogenous quantity of BMSCs is not enough and they are not functionally active, so they are not able to maturatenewvessels. Consistently, research of Chung et al. (2011) shows that after laser injury in non irradiated mice, injection of extra bone marrow stemcells contribute to resolution of retinal detachment without proliferative component (Chung et al., 2011). In the regards, further experiments are needed to understand the roles of transplanted MSCs in CNV formation.

Conclusion

Being the leading causes of blindness in the western countries, retinal degenerations don’t have a definitive treatment option, which is a significant problem for both the patient and the clinicians. In the last 30 years research was focused on stem cells and their possible application in the treatment of retinal degenerative disease.

Despite the extensive research done in the past three decades and many pre-clinical and several clinical trials conducted, many questions and problems are still not solved, preventing wide application of stem cells in clinical practice. But significant progress has been made toward both prevention and treatment of retinal degenerations and we envision the clinical application of stem cells will open a new era of treating those eye diseases.
Conflict of Interest

Authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Acknowledgement

Researchers want to thank Anushavan Karapetyan and Liu Xin for their comments and assistance during paper writing.

This study was supported by the NSFC Fund, Jilin Province Science and Technology Agency fund. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author’s Contribution

Emma Ghazaryan and Shurong Wang: Conception and design, data collection and manuscript writing.

Yan Zhang: Conception and data collection.

Yuxi He: Data collection

Guanfang Su: Conception, revision and final approval of the version.

Ethics

All authors read and approved the final version and are responsible for any ethical issue that may arise after the publication of this manuscript.

References


Ning, H., F. Yang, M. Jiang, L. Hu and H. Chen et al., 2008. The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: Outcome of a pilot clinical study. Leukemia, 22: 593-599. DOI: 10.1038/sj.leu.2405090


Phinney, D.G., K. Hill, C. Michelson, M. DuTreil and E. Bayly et al., 2006. Biological activities encoded by the murine mesenchymal stem cell transcriptome provide a basis for their developmental potential and broad therapeutic efficacy. Stem Cells, 24: 186-198. DOI: 10.1634/stemcells.2004-0236


Ringe, J., S. Strassburg, K. Neumann, M. Endres and M. Sittinger et al., 2007. Towards in situ tissue repair: Human mesenchymal stem cells express chemokine receptors CXCR1, CXCR2 and CCR2 and migrate upon stimulation with CXCL8 but not CCL2. J. Cell Biochem., 101: 135-146. DOI: 10.1002/jcb.21172


Shi, M., J. Li, L. Liao, B. Chen and R.C. Zhao et al., 2007. Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: Role in homing efficiency in NOD/SCID mice. Haematologica, 92: 897-904. DOI: 10.3324/haematol.10669


