Stem Cell Biology and Regenerative Medicine

Stephen H. Tsang *Editor*

Stem Cell Biology and Regenerative Medicine in Ophthalmology

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Preface

In the last few decades, stem cell research has developed groundbreaking technologies to both study and treat diseases. This research has proven fruitful for the field of ophthalmology, especially in recent years. With its relative immune privilege, the eye has proven an ideal testing ground for stem cell therapies.

This book describes just a few of these developing treatments. The authors of this book describe a wide range of possible applications, from oculofacial plastic surgery to the restoration of sight lost by degenerative disorders and glaucoma, to cancer research. Indeed, stem cell research seems to have reached a critical mass in ophthalmology. As recently as 2011, the FDA approved trials for stem cell-based treatments for macular degeneration; other clinical trials may follow, as discussed in the last chapter of this book.

These changes have not happened overnight. From a scientific standpoint, several discoveries have made stem cells a viable treatment source for humans. In 1981, when embryonic stem cells were first synthesized in the laboratory, it became possible to imagine generating graft tissues or animal models to test drugs from stem cells. Fifteen years later, the Yamanaka research group discovered that mouse skin samples could be reprogrammed through gene therapy into induced pluripotent cells. Both ES and iPS cells are pluripotent, or reprogrammable. Moreover, iPS cells are autologous, meaning they are derived from the subject's own tissue. By modifying cell culture media or performing gene therapy, researchers have been able to generate many types of tissues using ES cells and iPS cells.

Autologous tissues can also be generated using the progenitor cells which exist naturally inside the body. Unlike pluripotent stem cells, progenitor cells can differentiate into a limited number of tissues. These "local" cells can be adapted to replace and repair diseased tissue. Promising progenitor cells include: adipose tissue stem cells, ciliary stem cells, mesenchymial stem cells, corneal stem cells, and lens stem cells.

A significant area of stem cells research has been the retinal degenerative disorders. These conditions all involve degeneration of the retinal pigment epithelium, a tissue that sustains living photoreceptors. Researchers have hoped to restore this tissue with differentiated stem cells. To date, several studies have found visual

rescue in mice treated with stem cell-derived RPE and photoreceptors. Intricate new surgical techniques have had to be developed to perform these transplant procedures.

Transplant surgeries can be used to replace many kinds of damaged tissue. Recently, adipose tissue-derived stem cells have attracted interest as source of tissue for oculofacial surgeries such as facial reconstruction, wound healing, and skin rejuvenation. The ease of gathering these autologous stem cells makes them particularly advantageous for plastic surgeries.

Stem cell-derived tissues such as lens and corneal tissue may be suitable for transplant. Media outlets have already begun reporting on the potential that severe corneal epithelial diseases may be treatable with corneal stem cells. Successful efforts have also been made to generate lens progenitor cells and lentoid bodies from ES stem cells.

Research on mesenchymial (or, marrow) stem cells may allow treating vascular disorders of the eye. Recent findings suggest that these can be transplanted into the eye to improve angiogenesis. Bone marrow cells may have potential for treating ischemic retinal diseases, and perhaps even some non-ischemic retinal diseases.

The treatment of glaucoma may involve special challenges. It has recently been discovered that transplantation of stem cells into the retina can potentially replace damaged neurons, or provide neurotrophic factors to surviving neurons. These may be useful for treating glaucoma, providing that neurons are able to integrate. A number of signaling and transcription factors are currently being studied to this end.

Gene therapy has continued to evolve alongside stem cell therapy. New gene therapy techniques using gene addition, or enhancing gene replacement, have improved the efficiency of directly treating disease-causing genes. These gene therapy methods minimize the risk of mutagenesis and may be used along with stem cells to replace diseased patient cells with new disease-free cells.

Finally, stem cell research has improved our understanding of the pathogenesis of eye diseases. The mechanisms leading to various types of cancer are still unknown. This book contains a discussion of the evidence that cancer stem cells can lead to uveal melanoma.

Stem cell research never stops changing and growing. There may come a time when the research discoveries of today alter the landscape of ophthalmologic practice. The last chapter of this book describes the types of safety trials that may be used to assess stem cell-based treatments' viability. In the meantime, these cells of great potential continue to offer challenges to researchers and hope to patients with serious eye pathologies.

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Contents

Abbreviations

3D	Three dimensional
AAV	Adeno-associated virus
ACAID	Anterior chamber-associated immune deviation
adRP	Autosomal dominant retinitis pigmentosa
ADSC	Adipose-derived stem cells
AMD	Age-related macular degeneration
AMED	Amniotic membrane matrix-based ES cell differentiation
AO	Adaptive optics
arRP	Autosomal recessive retinitis pigmentosa
ARVO	Association for Research in Vision and Ophthalmology
AT region	Adenine-plus-thymine region
BAC	Bacterial artificial chromosome
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
bHLH	Basic helix-loop-helix
BMP	bone morphogenetic protein
BMSC	Bone marrow stem cells
CAL	Cell-assisted lipotransfer
CAL	Conjunctival allograft
CAU	Conjunctival autograft
c-CLAL	Cadaveric conjunctival limbal allograft
CE	European Conformity
CEC	Corneal endothelial cell
CESC	Corneal endothelial stem cell
CLAU	Conjunctival limbal autograft
CMZ	Ciliary marginal zone
CNTF	Ciliary neurotrophic factor
CNV	Choroidal neovascularization
CoDA	Context-dependent assembly
CRX	Cone–rod homeobox
CTFR	Cystic fibrosis transmembrane conductance regulator

DNA	Deoxyribonucleic acid
EGFP	Enhanced green fluorescent protein
EPC	Endothelial progenitor cell
ERG	Electroretinogram
ES	Embryonic stem
ESC	Embryonic stem cell
FA	Fluorescein angiography
FAF	Fundus autofluorescence
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
fMRI	Functional magnetic resonance imaging
GCL	Ganglion cell layer
GFAP	Glial fibrillary acidic protein
GUCY2D	Retinal guanylate cyclase 2D
HCS	Hematopoietic stem cells
HES	Hairy enhancer of split
hESC	Human embryonic stem cell (see ES)
HIV	Human immunodeficiency virus
ICG	Indocyanine green
ILM	Inner limiting membrane
iPS	Induced pluripotent stem
iPSC	Induced pluripotent stem cell
ITR	Inverted terminal repeats
KLAL	Keratolimbal allograft
LCA	Leber's congenital amaurosis
LESC	Limbal epithelial stem cell
lr-CLAL	Living related conjunctival limbal allograft
LSCD	Limbal stem cell deficiency
LTR	Long terminal repeats
MEN	Multiple endocrine deficiency
mERG	Multifocal electroretinogram
mfERG	Multifocal electroretinogram (interchangeable)
MMP 1	Matrix metalloprotease 1
MS	Melanomasphere
MSC	Mesenchymal stem cell
MSFE	Melanosphere forming efficiency
NICD	Notch intracellular domain
NRL	Neural retina-specific leucine zipper
NSC	Neural stem cell
OCP	Ocular cicatricial pemphigoid
OCT	Optical coherence tomography
OPEN	Oligomerized pool engineering
OSD	Ocular surface disease
PAX-6	Paired boxed protein-6

PCP	Planar cell polarity
PCR	Polymerase chain reaction
PDM	Placental decellular matrix
PET	Positron emission tomography
PRO	Patient reported outcomes
rAAV vector	Recombinant adeno-associated virus vector
RCS	Royal College of Surgeons
REST	RE-1 silencing transcription factor
RGC	Retinal ganglion cell
RHO	Rhodopsin
RNA	Ribonucleic acid
RP	Retinitis pigmentosa
RPC	Retinal progenitor cell
RPE	Retinal pigment epithelium
RPGRL	Retinitis pigmentosa GTPase regulator
RVD	Repeat variable di-residue
SDIA	Stromal cell-derived inducing activity
SFEB/DLFA	Dkk-1, Lefty-A, FCS, and Activin cells
siRNA	Small interfering RNA
SJS	Steven–Johnson Syndrome
ssAAV vector	Single-strand adeno-associated virus vector
SVF	Stromal vascular fraction
TAC	Transient amplifying cell
TAL	Transcription activator-like
TALEN	Transcription activator-like effector nucleases
USH2A	Usher syndrome 2A
UV	Ultraviolet
VEGF	Vascular endothelial growth factor
VEP	Visually evoked potential
xlRP	X-linked retinitis pigmentosa
ZFN	Zinc finger nuclease

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Chapter 1
The Eye as a Target Organ for Stem Cell Therapy

Mark A. Fields, John Hwang, Jie Gong, Hui Cai, and Lucian V. Del Priore

Abstract Retinal degenerations are a heterogeneous group of disorders that are characterized by progressive cellular dysfunction, cellular disarray, and eventually cell death. Early in the course of disease therapeutic intervention consists of pharmaceutical treatment to prevent cell death or gene therapy to correct the underlying mutation. Due to the nature of pathologies involving these disorders, particularly in late stage of disease, cell replacement therapy or electric stimulation of remaining cells by artificial retinal prosthesis is the only viable option. Stem cell therapies for retinal degenerative diseases such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP) are a promising therapeutic option and will require replacement of lost photoreceptor cells and retinal pigment epithelium (RPE). Current clinical trials are underway to evaluate the potential of stem cell therapy in humans. The use of induced pluripotent stem (iPS) cells hold great promise as a potential reservoir of cells for the treatment of retinal disorders as well as a clinical tool to help understand disease pathology. Advances in stem cell technology will translate these therapies into viable clinical options for the treatment of retinal degenerative diseases and other disorders.

Introduction

Retinal degenerations are a heterogeneous group of disorders that are characterized by progressive cellular dysfunction, cellular disarray, and eventually cell death. Numerous classification systems exist for these disorders, but no one classification

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system captures the complexity of the disease processes, the diversity of their pathology, and the common themes in treatment that underlie these diseases. Many current classifications distinguish between macular diseases and peripheral retinal degenerations, but this classification system does not represent the complexity of the disease process in a complete fashion. Prior to the discovery of gene mutations that increase the risk profile for age-related macular degeneration (AMD), retinal degenerations were often classified as either hereditary or nonhereditary diseases, but the simplicity of this classification has been called into question based on the observation that certain alleles increased the risk of AMD [1–4]. Thus, for the purpose of this discussion, retinal degenerations will be classified by whether they are Mendelian disorders (e.g., most if not all forms of retinitis pigmentosa (RP), Leber's congenital amaurosis, and Best's disease) or non-Mendelian retinal disorders, including AMD.

Because of the complexity of the disease processes, it is possible to dedicate an entire chapter of this book to each disease and still not cover all the details of each condition. However, regardless of the cause of the retinal disorder, it is important to recognize that severe vision loss is typically associated with cellular dysfunction or death. Early in the course of many diseases there is cell dysfunction without cell death. In these early stages, gene therapy, pharmacological treatment to manipulate the cell death pathway, and/or treatment with locally administered growth factors, such as ciliary neurotrophic growth factor, may all prove to be useful. However, late stages of retinal disease, which are usually accompanied by severe vision loss, will require a different approach. For example, in advanced stages of many forms of RP, severe vision loss is due to death of photoreceptors, loss of the native retinal pigment epithelium (RPE) monolayer on Bruch's membrane, migration of pigmented cells into the retina, and transsynaptic degeneration leading to inner retinal disturbance. In advanced geographic atrophy in AMD, there is loss of RPE and photoreceptors and secondary atrophy of the choriocapillaris. Reversal of vision loss in these late stages of disease, after cell loss has occurred, will likely require cell therapy with transplantation of photoreceptors, RPE and/or choriocapillaris cells; or direct electrical stimulation of the inner neural retina with multi-electrode arrays.

In this review we will discuss the clinical and pathological features of retinal degenerations that are important to their potential treatment with stem cell therapy; the unique combination of eye anatomy and imaging capabilities that makes it an excellent target organ for early stem cell therapy in humans; and the status of human trials.

Clinical and Pathological Features of Retinal Degenerations

Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a group of Mendelian hereditary disorders characterized clinically by bilateral progressive loss of peripheral vision, a marked ring-like constriction of the visual field, night blindness, and late loss of central

vision. As a group the population prevalence of RP is about 1:4,000, so the estimates are that approximately 100,000 in the USA have this disease. Investigators have identified at least 45 loci for mutations that can cause retinitis pigmentosa, and these genes collectively account for disease in a little over half of all patients [5–7]. Of the cloned genes for retinitis pigmentosa it is estimated that dominant retinitis pigmentosa account for about 50 %, recessive retinitis pigmentosa account for about 40 % and X-linked retinitis pigmentosa account for approximately 80 % of cases, indicating that many genes remain to be identified [6, 8]. Rods are the predominantly affected photoreceptors and dysfunction causes night blindness and peripheral field loss beginning as early as the teenage years [9]. Disease progression leads to central acuity loss and legal blindness in the majority of patients [10]. Classic findings on funduscopic exam include perivascular bony spicule pigmentation, attenuated arterioles, and waxy optic disc pallor, typically associated with vitreous cells and posterior subcapsular cataracts. However, many of these findings may be absent in early stages of disease [11, 12]. Electroretinogram (ERG) testing is important for diagnosis and may provide prognostic information [10]. The genetics of retinitis pigmentosa are extremely complex with diverse modes of inheritance [12]. Potential interventions include vitamin A therapy and carbonic anhydrase inhibitors, but treatment options are extremely limited in the majority of cases with no effective form of therapy. Results evaluating vitamin A efficacy have shown limited benefit but potential risks exist with oral vitamin A supplementation, including the risk of hepatotoxicity [13]. Carbonic anhydrase inhibitors have shown clinical benefit in reducing macular edema and improving visual acuity in some patients with retinitis pigmentosa [14].

Genetics

The genetics of retinitis pigmentosa are extremely complex with diverse modes of inheritance including dominant, recessive, X-linked, mitochondrial, and digenic forms [12]. The disease may manifest solely with visual symptoms or may be accompanied by a constellation of systemic findings in patients with syndromic retinitis pigmentosa. The diversity in genetic transmission and clinical presentation is not entirely surprising given that retinitis pigmentosa constitutes a broad group of diseases that arises from diverse biological pathways.

Retinitis pigmentosa demonstrates multiple modes of segregation [15]. Autosomal dominant transmission occurs most frequently and accounts for 20 % of retinitis pigmentosa cases. Symptoms are generally less severe with adult-onset with variable penetrance of symptoms. Autosomal recessive disease occurs in 13 % of cases and is characterized by earlier onset of symptoms and severe vision loss. X-linked recessive disease accounts for 8 % of cases and has the poorest visual prognosis with early onset and rapid progression of symptoms [12]. Visual deficits typically present within the first decade of life and progress to partial or complete blindness by the third or fourth decade. In approximately 20 % of nonsyndromic cases, the mode of