Introduction

The retina suffers from many dystrophies and age related degenerations that end in sever visual function loss.

Recent ophthalmic researches imply that stem cell technology applications hold a promise for treatment of such dystrophic or degenerative conditions.

Stem cells are undifferentiated cells that pose a self renewal capability giving rise to multiple cell types. They exist in embryo as well as throughout the different organs of adults.

Adult stem cells are derived from adult tissues and cultured in a way that favoures their growth and isolation from a large non-stem cell population of the patients own tissues.

Stem cells have been cultured from the limbus, conjunctiva, ciliary epithelium and the retina that have all been proved to accommodate potentially useful stem cells.

In animal models of retinitis pigmentosa transplantation of stem cells has resulted in some alleviation of the pathology. Transplants into the fovea in cases of macular degeneration resulted in clinical improvement.

Using endogenous stem cells derived from ciliary epithelium and glial cell growing in the retina with appropriate provocation could result in a proper therapeutic approach.

This review of literature will include the following points:

- The molecular basis of retinal dystrophies and degeneration.
- Cell biology of retinal dystrophies and degeneration.
- Stem cell biology.
- Stem cell culture.
- Recent technology involved in the field.
- Methods of applications in the treatment.
- Results of this approach.
- Complications.
- The future.

Aim of the Study

The aim of this study was to detect the role of stem cells in degenerative retinal diseases and its methods of application and future directions concerning stem cells.

Chapter (1):
THE MOLECULAR BASIS OF RETINAL DYSTROPHIES AND DEGENERATION

Monogenic diseases of the retina and vitreous affect approximately 1 in 2,000 individuals, or more than 2 million people worldwide. Consequences for affected individuals are variable and can range from legal blindness in the most severe forms of retinal degenerations (Leber congenital amaurosis, LCA) to less severe or rather mild retinal dysfunctions (Night blindness, achromatopsia) ***(Audo et al., 2009).***

For most of them, no treatment can be offered. In the past 20-25 years, the knowledge about the molecular basis of retinal diseases has tremendously progressed and evidence for the contribution of genetic factors but also environmental circumstances is continuously accumulating. After a time period that was mainly characterized by the identification of genes and disease-causing mutations for the monogenic retinal and vitreoretinal traits in families, monogenetic and multifactorial diseases are in the interest of clinical, genetic and basic research. Still, a reliable molecular diagnosis is possible for only half of the affected individuals or families with monogenic forms of retinal diseases ***(Bech-Hansen et al., 1998).***

In addition, the predictive value of a mutation or risk allele for multifactorial disorders is problematic since the phenotypic and/or symptomatic consequences are highly variable. Nevertheless, the knowledge about the molecular mechanisms has also improved diagnostic assessment of patients by genetic testing. It is the ultimate goal to better understand the molecular etiology of these diseases and to develop approaches for therapeutic interventions ***(Dryja et al., 1993)*.**

The diseases discussed in this article can be categorized in four major groups: (i) rod dominated diseases, (ii) cone dominated diseases, (iii) generalized retinal degenerations (affecting both photoreceptor cell types, rods and cones), and finally (iv) exudative as well as erosive vitreoretinopathies ***(Bessant et al., 1999)*.**

Retinal and vitreoretinal diseases classification also considers whether the ocular phenotype is associated with pathologies of other tissues (syndromic forms) or only affects the retina, retinal pigment epithelium and the vireous body (nonsyndromic forms) (fig. 1). In addition, the mode of inheritance was used as one characteristic feature of the different disease phenotypes in order to categorize them. The aim was to provide a comprehensive overview about the molecular basis of retinal and

vitreoretinal diseases and to discuss selected aspects in more detail ***(Fuchs et al., 1995).***

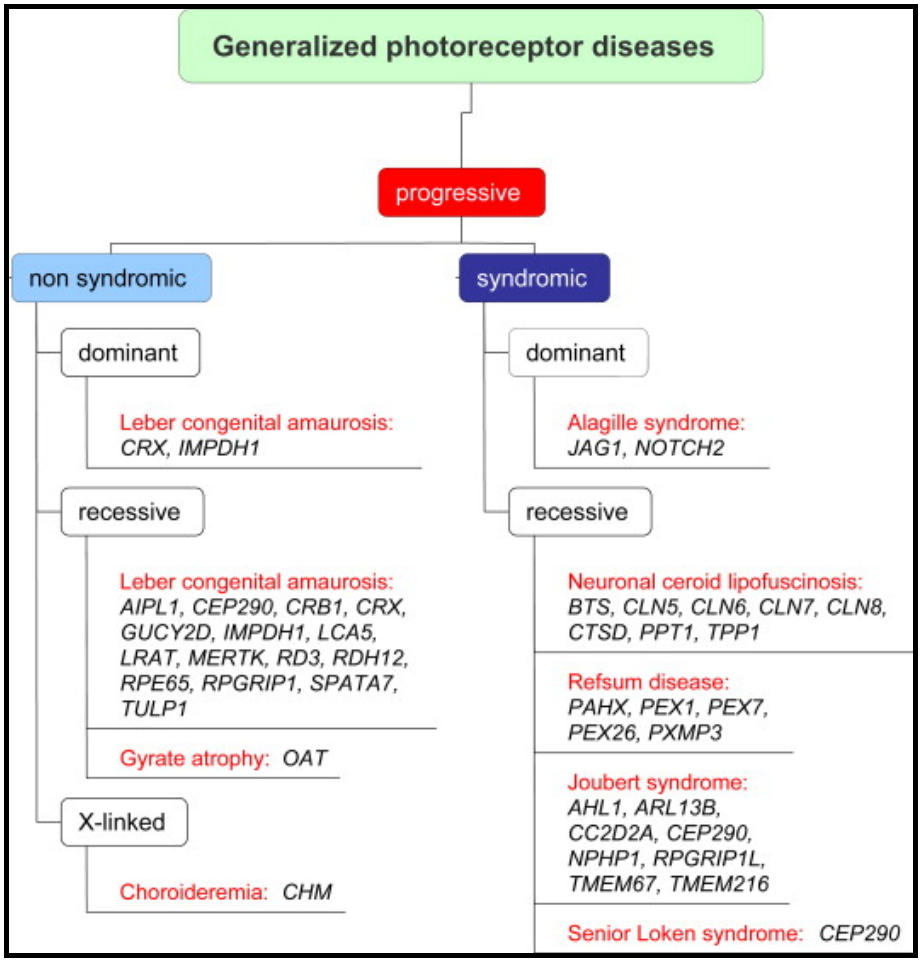

Figure (1): Classification of Retinal and vitreoretinal diseases ***(Fuchs et al., 1995).***

Classification of retinal and vitreoretinal diseases:

I- Non-syndromic retinal and vitreoretinal diseases.

1. Diseases of rod photoreceptor cells (stationary and progressive).

a. Congenital stationary night blindness (stationary rod diseases).

b. Retinitis pigmentosa and progressive rod-cone diseases.

2. Cone and cone rod diseases (stationary and progressive).

a. Stationary cone dysfunctions.

b. Cone and cone rod dystrophies (CODs and CORDs).

c. Macular degenerations (monogenic and age-related).
- Monogenic macular dystrophies.
- Age-related macular degeneration (AMD).
- Vasculogenesis and angiogenesis.

3. Generalized photoreceptor diseases (non-syndromic).

a. Leber congenital amaurosis (LCA).

b. Choroideremia.

c. Gyrate atrophy of the choroid and retina.

4. Vitreoretinopathies.

a. Erosive vitreoretinopathies.

- Enhanced S cone syndrome (ESCS).
  - Snowflake vitreoretinal degeneration.
  - Autosomal dominant vitreoretinochoroidopathy (ADVIRC).
  - Wagner syndrome and erosive vitreoretinopathy.

b. Exudative vitreoretinopathies (EVRs).

II. Syndromic retinal diseases.

1. Usher syndrome.

2. Bardet Biedl syndrome.

3. Senior Løken Syndrome/Nephronophthisis (SLSN).
4. Refsum disease.
5. Joubert syndrome.
6. Alagille syndrome (ALGS.
7. Alstrom syndrome (ALMS).
8. Neuronal ceroid lipofuscinosis (NCLs).
9. Primary ciliary dyskinesia (PCD).
10. Stickler syndrome

(Gal et al., 1994).

In order to diagnose and counsel patients and their families, knowledge about their disease-causing mutation is essential ***(Li et al., 2009).***

Especially for genetic counselling, targeted clinical characterization and gene therapeutic interventions, the precise molecular diagnosis is very important. Unfortunately, the disease-causing mutation is often difficult to identify due to the genetic heterogeneity and clinical variability of retinal diseases ***(Pusch et al., 2000).***

Several strategies do exist and novel technologies are emerging that increase the chance to identify the causative mutation in patients. For this purpose, knowledge of the pedigree is of utmost importance. It often provides significant clues to the selection of genes implicated for molecular genetic testing ***(Sullivan et al., 1999).***

In cases of autosomal dominant retinitis pigmentosa (RP), the likelihood to identify the causative mutation among the five most frequently affected genes is approximately 50%. These five genes are rhodopsin (RHO), peripherin 2 (PRPH2), retinitis pigmentosa 1 (RP1), pre-mRNA processing factor 31 (PRPF31), and inosine monophosphate dehydrogenase 1 (IMPDH1). The prime candidate for autosomal dominant retinitis pigmentosa (adRP) is rhodopsin, which accounts for up to 30% of the cases ***(Kennan et al., 2005)*.**

Whereas the other candidates have frequencies between 5 and 20%. Moreover, these five autosomal dominant retinitis pigmentosa (adRP) genes comprise a total of only 40 coding exons, a number that seems manageable to be analyzed by conventional sequencing or other appropriate methods. Nevertheless, in cases of incomplete penetrance (carriers of the mutation are occasionally not affected), the collection of candidate genes is much smaller ***(Van Genderen et al., 2009)*.**

Only three of the 19 adRP genes have been associated with incomplete penetrance (PRPF31, PRPH2, and RP1). To optimize mutational screening procedures for such cases, a detailed case history including a family pedigree over several generations is needed to recognize incomplete penetrance of the phenotype. In cases where the pedigree suggests X-linked inheritance within the family,

RPGR and RP2 are the only candidates associated with RP so far. In addition, almost all cases can be explained by mutations in either RPGR (up to 80% of the cases) or RP2 (up to 20% of the cases) ***(Neidhardt et al., 2008).***

A screening strategy starting with a mutation hot spot in RPGR followed by sequencing of other exons has been shown to be successful in most cases and to reduce costs and efforts ***(Neidhardt et al., 2008).***

Additional loci on the X chromosome have been mapped, but the corresponding genes have not yet been identified. Interestingly, Retinitis pigmentosa GTPase regulator (RPGR) mutations may also affect female carriers and thus should not be associated with classical X-linked recessive inheritance. The genetic basis for this observation is still unclear ***(Wycisk et al., 2006).***

Since skewed X-inactivation has not been identified in affected female carriers so far, genetic or environmental modifiers may be relevant too. In contrast to X-linked RP, the candidate selection is more difficult in cases with autosomal recessive inheritance ***(Yamamoto et al., 1997).***

Promising candidate genes for autosomal recessive retinitis pigmentosa (arRP) are PDE6A and PDE6B that together explain about 5-10% of the cases ***(Daiger et al., 2007).***

USH2A is a gene frequently found to be mutated in arRP (up to 20% of the cases), but is also associated with Usher Syndrome ***(Hartong et al., 2006).***

Furthermore, ABCA4 and RPE65 often show RP-associated mutations, but classically cause Stargardts disease or LCA. Thus, a thorough clinical characterization of the phenotype including syndromic features will further facilitate the selection of candidate genes for molecular genetic testing in a patient with arRP ***(Zeitz et al., 2006).***

Following these screening strategies, the samples without identified mutations may be included in research programs to identify novel loci and disease-associated genes. Methods that are used to further characterize such samples often include array technologies (single nucleotide polymorphism arrays) with the aim to identify linkage intervals in families with multiple affected members or homozygous regions in the genome of consanguineous families. Furthermore, array technologies may be used to directly screen for mutations ***(Koenekoop et al., 2007).***

In particular, genetic testing for the large gene ABCA4 and for all recessive or dominant RP genes requires efficient diagnostic tests in order to examine the DNA sample of a patient in an affordable amount of time. One such tool for mutation detection represent micro arrays. This technique has been established for several retinal diseases ***(Cremers et al., 2007).***

Also for congenital stationary night blindness (CSNB), the large number of genes and mutations associated with this disease are challenging for confirmation of the clinical diagnosis. Recently, a micro array has been developed which provides a fast and rather inexpensive tool to screen DNA samples from patients for known mutations in one of the above mentioned genes ***(Cremers et al., 2007).***

However, with this diagnostic test only known sequence alterations can be detected as in the case of ABCA4 ***(Klevering et al., 2004)***.

The most sensitive technology currently available for routine diagnostics is direct DNA sequencing in combination with multiple ligation-dependent probe amplification (MLPA), the latter of which can be used to reveal copy number variations, such as deletions or duplications ***(Klevering et al., 2004).***

Another challenge for genetic testing is the interpretation of the results of these tests. Many of the DNA sequence variations lead to amino acid substitutions and it is difficult to predict the pathogenic effect for many of them. Therefore, functional assays are needed to characterize these sequence alterations. For example, ABCA4 possesses an ATPase activity and it is possible to analyze specific missense mutations in cell culture assays

and answer the question of a potential pathogenic effect ***(Sun et al., 2000)*.**

Due to the extensive individuality of pathogenic and non pathogenic sequence alterations, this approach is difficult to realize in a routine diagnostic setting. In addition, deep intronic mutations may be responsible for a significant number of cases and these are extremely difficult to be analyzed in functional assays ***(Jiang et al., 2009).***

Today, the new (next generation) sequencing technologies enable us to collect up to 500 million base pairs per single sequencig run at comparably low costs. This will complement or even replace other methods to identify mutations in genetically heterogeneous diseases, such as retinal degenerations and dysfunctions ***(Voelkerding et al., 2009)*.**

Chapter (2):

CELL BIOLOGY OF RETINAL DYSTROPHIES AND DEGENERATION

Age-related macular degeneration (AMD), the leading cause of worldwide blindness in the elderly, is a bilateral ocular condition that affects the central area of retina known as the macula. The macula lutea, which derives its name from the deposition of yellow xanthophyll pigments, is located temporal to the optic disc and is bounded by the temporal superior and inferior vascular arcades. Although the macula comprises only four percent of retinal area, it is responsible for the majority of useful photopic vision. The fovea lies at the center of the macula and is approximately 2mm in diameter. The fovea is particularly well seen in vertical section view using ocular coherence tomography techniques in living eyes. The fovea contains the highest density of cone photoreceptor cells and is the only region of the retina where 20/20 vision is attainable. The macula accounts for almost 10% of the entire visual field. Thus, lesions developing in this region can have a major impact on visual function ***(Uhlmann et al., 1991).***

The initial clinical diagnosis of early AMD is based on seeing drusen the hallmark indicators of disease and pigmentary changes in the macula. Drusen look like yellow-white spots in the retina. They are extracellular

deposits located between the retinal pigment epithelial (RPE) basal lamina and the inner collagenous layer of the elastin-containing Bruch's membrane (Fig 2) ***(Uhlmann et al., 1991).***

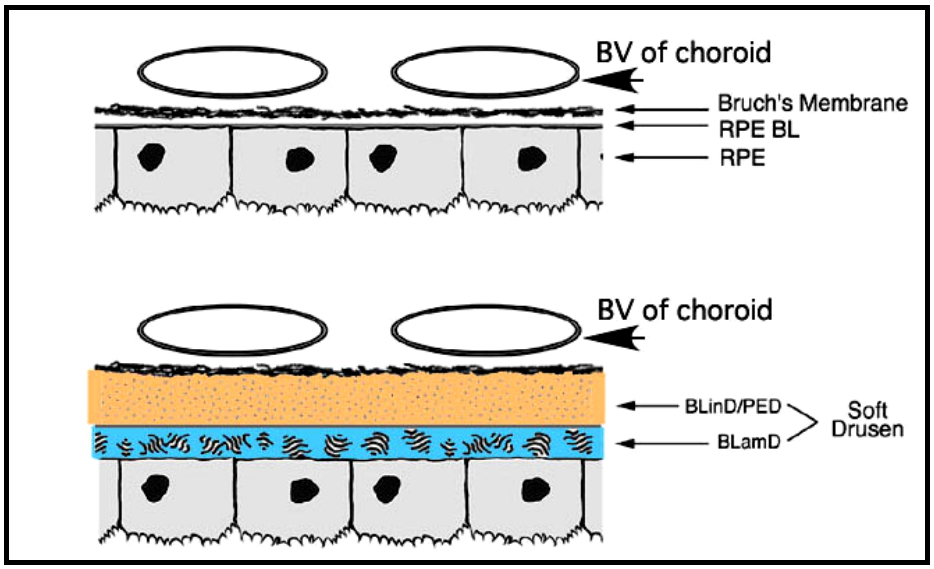

Figure (2): Schematic images depicting the choroid-RPE-retina interface in a normal retina and an AMD interface. The choriocapillaris-RPE interface in unaffected (top) and affected (bottom). The majority of early AMD-associated extracellular lesions – including drusen, basal laminar deposit (BLamD), and basal linear deposits (BLinD) – form along this interface ***(Uhlmann et al., 1991).***

AMD has a tremendous impact on the physical and mental health of the geriatric population and their families. Prior to 1990, AMD of all forms was often referred to as "senile macular degeneration" or SMD, a reflection of the fact that the vision loss associated with AMD manifests late in life when most affected individuals are looking forward to enjoying retirement activities and maintaining independence. Instead, millions with AMD suffer bilateral