Progress in Retinal and Eye Research 42 (2014) 130–144

Contents lists available at ScienceDirect

Progress in Retinal and Eye Research

journal homepage: www.elsevier.com/locate/prer

Stem cells as source for retinal pigment epithelium transplantation

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ARTICLE INFO

Article history:
Available online 13 June 2014

Keywords:
Retinal stem cells
Retinal neurospheres
RPE65
LRAT
Bestrophin 1
Beta5-integrin
OA1
CRALBP
MITF

ABSTRACT

Inherited maculopathies, age related macular degeneration and some forms of retinitis pigmentosa are associated with impaired function or loss of the retinal pigment epithelium (RPE). Among potential treatments, transplantation approaches are particularly promising. The arrangement of RPE cells in a well-defined tissue layer makes the RPE amenable to cell or tissue sheet transplantation. Different cell sources have been suggested for RPE transplantation but the development of a clinical protocol faces several obstacles. The source should provide a sufficient number of cells to at least recover the macula area. Secondly, cells should be plastic enough to be able to integrate in the host tissue. Tissue sheets should be considered as well, but the substrate on which RPE cells are cultured needs to be carefully evaluated. Immunogenicity can also be an obstacle for effective transplantation as well as tumorigenicity of not fully differentiated cells. Finally, ethical concerns may represent drawbacks when embryo-derived cells are proposed for RPE transplantation. Here we discuss different cell sources that became available in recent years and their different properties. We also present data on a new source of human RPE. We provide a protocol for RPE differentiation of retinal stem cells derived from adult ciliary bodies of post-mortem donors. We show molecular characterization of the *in vitro* differentiated RPE tissue and demonstrate its functionality based on a phagocytosis assay. This new source may provide tissue for allogenic transplantation based on best matches through histocompatibility testing.

Contents

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[1] Percentage of work contributed by each author in the production of the manuscript is as follows: Evelina Bertolotti: 35%; Alberto Neri: 10%; Monica Camparini: 5%; Claudio Macaluso: 10%; Valeria Marigo: 40%.

http://dx.doi.org/10.1016/j.preteyeres.2014.06.002
1350-9462/

1. Introduction

The retinal pigment epithelium (RPE) is a highly specialized epithelium with a neuroectodermal embryonic origin like the retina. While the retina was first described by Galen in the second century A.D., discovery of the RPE required the use of the first rudimentary microscopes in the 18th century and was described by Carlo Mondini of Bologna in his "Commentationes Bononienses" (1790) as "a real membrane formed by innumerable globules which makes an excessively delicate network" (Marmor and Wolfensberger, 1998). The histology of the RPE was then elucidated at the end of the 19th century and further characterized in more recent times.

The eyes derive from two evaginations of the forebrain that generate the optic vesicles connected to the brain by the optic stalks. The optic vesicles then invaginate to form the optic cups with the outer layer destined to become the RPE. The outer stratum is a monolayer of cells that differentiate during embryonic/fetal development and is characterized by pigmentation, which appears during the 5th week of human embryogenesis. RPE differentiation is induced by several factors including the signaling molecule Activin, a member of the TGFβ family, which is secreted by adjacent mesenchymal cells. These signals induce expression of transcription factors, such as microphthalmia-associated transcription factor (MITF), orthodenticle homolog 2 (OTX2) and paired box 6 (PAX6), that are essential for RPE specification and to drive expression of proteins necessary for the distinguishing functions of the RPE (Bharti et al., 2012; Fuhrmann et al., 2000; Housset et al., 2013). The fully differentiated RPE consists of a polarized monolayer of pigmented cells with a basal side adherent to the Bruch's membrane, which separates the RPE from the choroid, and an apical membrane facing the photoreceptor cells.

In this paper we summarize molecular and functional characteristics of the RPE tissue since the characterization of these features is required when RPE is generated *in vitro*. We also discuss RPE impairment and diseases that will be amenable to cell replacement strategies. We review several stem cell sources to produce RPE *in vitro*. Finally, we present a new protocol for the differentiation of adult human retinal stem cells into RPE sheets.

2. Molecular and functional characteristics of the RPE and related diseases

2.1. Molecular and structural characteristics of RPE cells

RPE cells are characterized by asymmetrical distribution of molecules at the cell surface and compartmentalization of the organelles in the cytoplasm (Fig. 1). Morphological differences between apical and basal membranes are infoldings at the basal membrane and microvilli at the apical side. The apical projections not only increase the apical cell surface but also envelop photoreceptor cell outer segments (POS) and mediate the turnover of the tip of the photoreceptors cells through phagocytosis. Organelles and cytoskeleton filaments are localized differently along the apicobasal axis. The nucleus and mitochondria are found at the basal side of RPE cells and pigmented melanosomes are transferred to the apical zone where they orient parallel to the incoming light. During differentiation, adherens junctions form among adjacent cells and are mediated by cadherins, namely cadherin 2 (N-cadherin) and cadherin 3 (P-cadherin) (Burke et al., 1999; Lagunowich and Grunwald, 1989; Murphy-Erdosh et al., 1994). Formation of adherens junctions is followed by the formation of circumferential bundles of actin filaments to build zonula adherens junctions (Nabi et al., 1993; Owaribe and Masuda, 1982; Williams and Rizzolo,

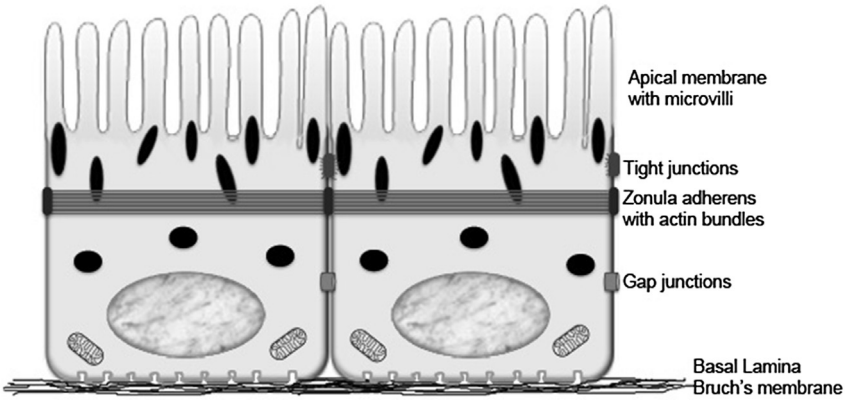

Fig. 1. Schematic representation of RPE cells and their polarized phenotype. RPE cells show asymmetric distribution of proteins in apical and basolateral membrane domains. The apical membrane is characterized by microvilli and is separated from the basolateral membrane by tight junctions. Actin filaments form the circumferential microfilament bundles that attach to the zonula adherens. Gap junctions mediate communication between RPE cells. The basal membrane is characterized by infoldings and attaches to its basal lamina and to the Bruch's membrane. Melanosomes are represented with black round and oval shapes and mitochondria with ellipsoidal shapes below the nuclei.