Experimental Eye Research 126 (2014) 1–4

Contents lists available at ScienceDirect

Experimental Eye Research

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

Review

Cell culture of retinal pigment epithelium: Special Issue

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ARTICLE INFO

Article history:
Received 3 July 2014
Accepted in revised form 12 July 2014

Keywords:
retinal pigment epithelium
cell culture
translational ophthalmology
in vitro models
ocular cell lines
cell differentiation
signature genes

ABSTRACT

This series of review articles highlights how *in vitro* models of RPE can be effectively used to understand essential functions of the RPE that are not only fundamental to epithelial biology, but also have direct relevance to the visual system. The issue contains reviews from experts in the field covering aspects of basic cell and epithelial biology, namely: the barrier properties of the RPE (Rizzolo, 2014), epithelial polarity (Lehmann et al., 2014), cytoskeleton (Bonilha, 2014), and lysosomes (Guha et al., 2014), as well as properties more unique to the RPE, *e.g.*, vitamin A metabolism (Hu and Bok, 2014), bioenergetics (Adijanto and Philp, 2014), phagocytosis (Mazzoni et al., 2014), ion transport (Reichhart and Strauß, 2014), and melanin/lipofuscin (Boulton, 2014).

1. Introduction to Special Issue on cell culture of RPE

Over the past five decades there has been increased use of cell culture models to elucidate genes and signaling pathways that regulate normal functions of the retinal pigment epithelium (RPE), such as polarity, phagocytosis, transepithelial transport, and vitamin A metabolism. RPE cell cultures also have been used in toxicology and drug screening, and to model the effects of oxidative stress on this cell type (see Table 1). To draw physiologically relevant conclusions from these investigations, the culture conditions employed must adhere to best current standard practices, and the properties of the RPE cells used must emulate the most meaningful criteria for evaluating such cultures as experimental systems. As one step towards this ideal, we the co-editors of this Special Issue of *Experimental Eye Research* have enlisted a group of experts to contribute reviews on specific facets of RPE cell biology, biochemistry, and physiology as studied *in vitro*. With this Special Issue, our main goal is to promote relevant and reproducible studies using RPE cells. The rationale for this project is, in part, based on the following, not mutually exclusive, specific concerns:

1) Recent evaluations of the authenticity of ocular cell lines (Boatright et al., 2013);
2) The reproducibility, in general, of published cell biology research studies (Ioannidis, 2005);
3) The validation of basic investigations to identify and characterize therapeutic targets, and also of preclinical drug testing and toxicological assays, using cultured RPE.

The fact that dissociated cells must proliferate and eventually re-establish properties of an epithelial tissue, such as apical junctional complexes, cell polarity, and the expression of specific signature markers that reflect the overall state of differentiation, applies not only to cultures derived from RPE harvested from fresh eyes, but also to immortalized cell lines, such as ARPE-19 (Fig. 1). It has been documented that both the latter cell line and “normal” RPE cell strains may adopt a variety of morphological and biochemical phenotypes, more or less resembling the desired outcome—the equivalent of freshly harvested, intact, and healthy RPE tissue—depending on culture conditions (Burke et al., 1996; Luo et al., 2006). It has come to the point in the annals of RPE

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[2] The statements herein do not reflect opinions or policies of the Dept. Veterans Affairs or the U.S. government.

http://dx.doi.org/10.1016/j.exer.2014.07.010
0014-4835/

Table 1
Progress in RPE cell culture.

Milestone	References	Comments
(By 1900): RPE described as a distinct structural entity based on: • Pigmentation • Polygonal uniformity of cells Functional attributes recognized: • Regeneration of visual purple • Embryological origin	(Wolfensberger, 1998)	No significant advances in first half of 20th century
Role of RPE in photoreceptor outer segment renewal	Young and Bok, 1969 Feeney, 1973	"Renaissance" of the RPE (Spencer, 1972)
Embryonic chick RPE expanded in culture: • Putative "dedifferentiation" *in vitro*, manifested as loss of melanin, was reversible • Clonal colonies exhibited cobblestone appearance and regained pigmentation with time in culture	Cahn and Cahn, 1966	Investigators exploited availability and ease of isolation of chick RPE, its avid adaptation to culture, and pigmentation as a diagnostic feature; no obvious relation to role of RPE in visual health and disease
Routine propagation of RPE from human donor eyes • Multiple passages • Lifetime of strains extended to months or more	Mannagh et al., 1973	
Cultured human RPE used for experimental studies	Del Monte and Maumenee, 1980 Flood et al., 1980 Edwards, 1982 Boulton et al., 1983	Included donors with retinitis pigmentosa
Cultured fetal human RPE • Pure strains initiated from explanted spheres of RPE-Bruch's membrane-choroid isolated without enzymes • High proliferative rate • Confluent monolayers melanized and formed fluid-filled domes on plastic substrate	Aronson, 1983	
Concurrent progress with RPE cultured from non-human mammalian species:		
• Rat	Edwards, 1977	
• Cat	Stramm et al., 1983	
• Cattle	Basu et al., 1983	
• Pig	Akeo et al., 1988	
Application of more defined growth and maintenance media with lowered serum component	Oka et al., 1984 Pfeffer et al., 1986	
Use of permeable supports	Heth et al., 1987 Chang et al., 1991	
ARPE-19, spontaneously immortal human line	Dunn et al., 1996	Alternative to repeated isolation of primary cultures
Generation of differentiated RPE from embryonic stem cells derived from non-human primate • Expressed subset of RPE-specific signature genes and proteins	Haruta et al., 2004	

culture where the question may be raised of whether, as a community, we should continue to fund and publish work performed on cell lines and strains that have transcriptomes/proteomes considerably divergent from those of native adult or human fetal RPE (hfRPE). Indeed, the introduction to many papers on cultured RPE begins with the obligatory designation of the RPE as constituting the outer blood-retinal barrier and describing how this tissue exhibits the basic phenotype common to all epithelia, *i.e.*, polarized sheets of cells with apical junctions that separate two compartments and regulate the directional secretion and transport of substances into or out of these compartments. However, all too often this "mantra" seems to be subsequently forgotten, because

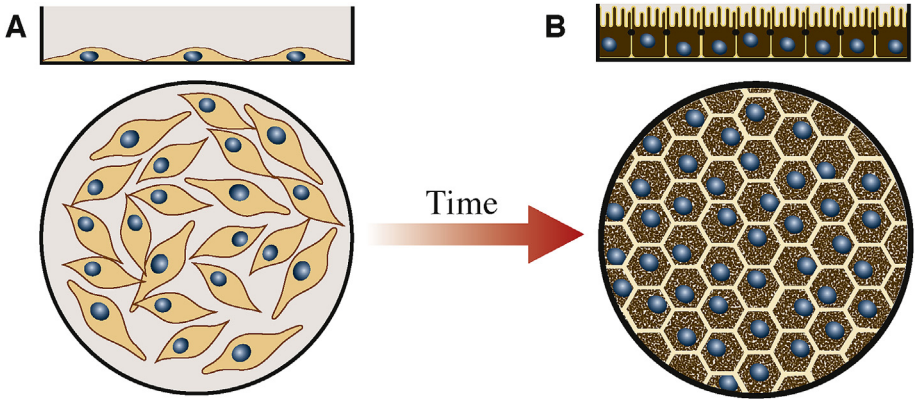

Fig. 1. Subconfluent RPE cells lack structure and function properties of differentiated RPE. (A) Dedifferentiated RPE cells lack basic features of epithelial cells including apical junctional complexes, polarized distribution of membrane and cytoskeletal proteins, and expression of RPE-specific signature genes. (B) With time in culture the RPE monolayer re-establishes a phenotype similar to RPE *in situ*.