Experimental Eye Research 126 (2014) 77–84

Contents lists available at ScienceDirect

Experimental Eye Research

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

Review

Cultured primary human fetal retinal pigment epithelium (hfRPE) as a model for evaluating RPE metabolism

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ARTICLE INFO

Article history:
Received 6 November 2013
Accepted in revised form 19 January 2014
Available online 28 January 2014

Keywords:
primary cultured human fetal RPE
metabolism
differentiation

ABSTRACT

Mitochondrial dysfunction has been shown to contribute to age-related and proliferative retinal diseases. Over the past decade, the primary human fetal RPE (hfRPE) culture model has emerged as an effective tool for studying RPE function and mechanisms of retinal diseases. This model system has been rigorously characterized and shown to closely resemble native RPE cells at the genomic and protein level, and that they are capable of accomplishing the characteristic functions of a healthy native RPE (e.g., rod phagocytosis, ion and fluid transport, and retinoid cycle). In this review, we demonstrated that the metabolic activity of the RPE is an indicator of its health and state of differentiation, and present the hfRPE culture model as a valuable *in vitro* system for evaluating RPE metabolism in the context of RPE differentiation and retinal disease.

1. Introduction

Interposed between the neural retina and the choroidal blood supply, the retinal pigment epithelium (RPE) plays a critical role in maintaining metabolic homeostasis in the outer retina which is essential for normal photoreceptor activity and health (reviewed in Adijanto and Philp, 2012). The RPE regulates ion, pH, and fluid homeostasis in the inter-photoreceptor space (Hughes et al., 1998), transports nutrients to and metabolic waste products from the retina (Bergersen et al., 1999), processes retinol into 11-cis-retinal (Lamb and Pugh, 2004), phagocytizes shed photoreceptor outer segments (POS) (Kevany and Palczewski, 2010), secretes neurotrophic factors to maintain photoreceptor integrity (Steele et al., 1993), and forms the outer blood retina barrier (Rizzolo et al., 2011). All of these specialized functions of the RPE depend on the expression and polarized distribution of receptors, transporters, channels and enzymes, many of which are markers of differentiated RPE.

In various systems, cells undergo a metabolic shift from glycolytic to oxidative metabolism as they advance through stages of differentiation (Agathocleous and Harris, 2013; Agathocleous et al., 2012; De Pauw et al., 2009; Porter et al., 2011). A glycolytic phenotype allows the cells to rapidly generate metabolic intermediates and energy needed to actively proliferate and migrate (Lunt and Vander Heiden, 2011). However, as cells mature and differentiate, they rely on oxidative metabolism to sustain specialized cellular functions (Agathocleous and Harris, 2013). Disruption of mitochondrial biogenesis in the RPE of a postnatal mouse resulted in a shift in cell metabolism, from oxidative respiration to aerobic glycolysis, that was accompanied by a loss of RPE specific genes (Zhao et al., 2011). With the association between RPE mitochondrial dysfunction and many genetic and age-related ocular diseases (Feher et al., 2006; Nordgaard et al., 2008; Rath et al., 2008; Tyni et al., 2004; Udar et al., 2009), one may postulate that restoration of normal RPE metabolism could be a useful therapeutic strategy for these blinding diseases.

In this review, we demonstrate how RPE metabolism and differentiation can be evaluated in a primary human fetal RPE culture model. We show how siRNA-mediated gene knockdown (KD) can be used to study key metabolic pathways involved in RPE differentiation and highlight the tools that can be used to evaluate changes in RPE metabolism.

Abbreviations: hfRPE, Human fetal retinal pigment epithelium; MCT, Monocarboxylate transporter; P0, Passage 0; BEST1, Bestrophin1; KD, Knock down; CLDN10, Claudin10; LDH, Lactate dehydrogenase; SDHD, Succinate dehydrogenase isoform D; TCA, Tricarboxylic acid; ETC, Electron transport chain; NMDG, N-Methyl-D-glucamine; ITS, Insulin-transferrin-selenium; THT, Taurine-hydrocortisone-triiodo-thyronin; HI, Heat inactivated; OCR, Oxygen consumption rate; ECAR, Extracellular acidification rate; PPP, Pentose phosphate pathway; AU, Absorbance unit; HK2, Hexokinase 2; G6PD, Glucose-6-phosphate dehydrogenase; NMR, Nuclear magnetic resonance.

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0014-4835/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.exer.2014.01.015

2. Human RPE cell culture as a model for studying RPE differentiation and metabolism

Number of primary cell culture systems (fetal and adult) (Blenkinsop et al., 2013; Gamm et al., 2008; Hu and Bok, 2001; Maminishkis et al., 2006; Sonoda et al., 2009; Valtink and Engelmann, 2009) and cell lines (e.g., RPE-J, D407, and ARPE-19) (Davis et al., 1995; Dunn et al., 1996; Nabi et al., 1993) have been used to evaluate basic RPE function as well as the molecular mechanisms underlying RPE-associated diseases. While the RPE culture system is convenient because it can be easily mass-produced and experimentally manipulated, validation of these culture models for known properties of RPE function and physiology is essential. Among the most extensively characterized RPE cell culture model is the primary human fetal RPE first established in Dr. Dean Bok's laboratory (Hu and Bok, 2001; Pfeffer, 1991) and later modified in Dr. Sheldon Miller's laboratory (Maminishkis et al., 2006; Maminishkis and Miller, 2010). These two model systems differ in the methods used for isolation of fetal RPE cells and in the medium used to culture these cells. Regardless, with both approaches, hfRPE cells re-establish pigmented epithelial monolayers with apical microvilli that express key mRNA, miRNA (Adijanto et al., 2012; Liao et al., 2010; Strunnikova et al., 2010; Wang et al., 2010), and proteins essential for RPE function (Adijanto et al., 2012), exhibit proper protein polarity of ion channels and transporters (Adijanto et al., 2009; Maminishkis et al., 2006), phagocytize rod outer segments (Castorino et al., 2011), metabolize retinol (Flannery et al., 1990; Hu and Bok, 2013), secrete growth and neurotrophic factors in a polarized manner (Castorino et al., 2011), establish tight and adherens junctions (Economopoulou et al., 2009; Peng et al., 2010, 2011), and mediate vectorial transepithelial transport of fluid (Castorino et al., 2011; Li et al., 2009; Maminishkis et al., 2006). Genomic analysis by microarray revealed that the mRNA expression profile of cultured human fetal RPE cells closely resembles native fetal RPE cells and adult native RPE cells (Strunnikova et al., 2010).

In Dr. Miller's hfRPE culture model, native hfRPE cells from donors at 16–22 weeks of gestation were manually dissociated from the eyecup and trypsinized and seeded onto flasks (Passage 0; P0). hfRPE cells were cultured for 4 weeks during which time the cells re-established a highly pigmented monolayer before they were dissociated from the flasks and re-seeded onto 12 mm transwell filters (Passage 1; P1 at 200k cells/well) where RPE cells again proliferate and migrate. Upon contact with adjacent cells, RPE cells initiate a "re-differentiation" program (or "re-morphogenesis"; see (Burke, 2008) for review) to establish tight junctions and begin expressing key RPE genes and proteins (e.g., bestrophin1 (BEST1), MCT3, RPE65) (Adijanto et al., 2012). This process typically takes 3–4 weeks, but can be accelerated or slowed depending on cell seeding density. Passage 2 (P2) primary hfRPE cells seeded at high density (30%; 160k cells/cm^2 or more) can acquire many properties of differentiated RPE in 10–14 days, while cells seeded at lower densities (~15%; 80k cells/cm^2) typically take 3–4 weeks to differentiate. While hfRPE cells from different donors exhibit some variability in the time it takes them to differentiate, it seldom deviates far from this range. It is important to note that primary RPE cells can only undergo a limited number of divisions before they lose their capacity to re-differentiate (Adijanto et al., 2012; Grisanti and Guidry, 1995) and hfRPE cells seeded at less than 5% density will not differentiate into a functional RPE monolayer, regardless of the time spent in culture. As a reference, Fig. 1 presents the morphology of P2 hfRPE cells seeded at a range of densities (from 2% to 60%) cultured for 21 days in a 96-well plate. In this batch of cells, hfRPE cells seeded at 30% and 60% densities achieved proper cobblestone morphology. Cells seeded at 15% density require more time to differentiate, and hfRPE cells seeded at 7.5% and below had completely lost their ability to differentiate and are referred to as dedifferentiated hfRPE cells. Notably, P2 dedifferentiated hfRPE cells do not re-differentiate even when seeded at high densities (30–60%) in passage 3. In the experiments presented in this review, we compared the metabolism of P2 hfRPE seeded at or above 30% density and cultured over 2–3 weeks to achieve RPE differentiation

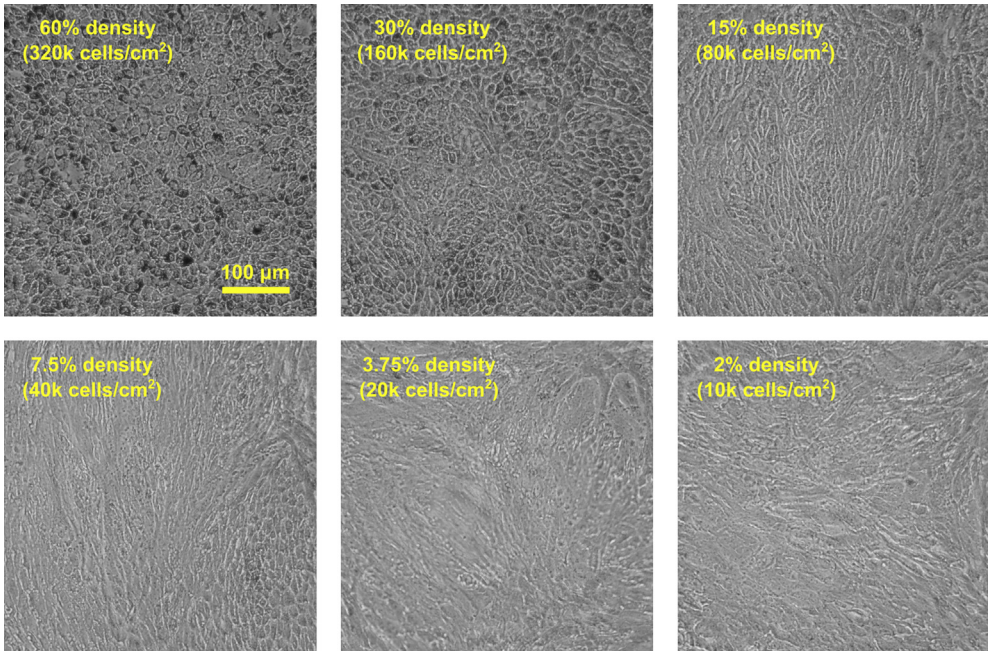

Fig. 1. Morphology of hfRPE cells seeded at various densities. Fully differentiated P1 hfRPE cells on transwells were trypsinized and re-seeded into 96-well plates at 60, 30, 15, 7.5, 3.75, and 2% densities. They were cultured in complete hfRPE culture medium (Advanced MEM (Gibco; cat# 12492) supplemented with Penicillin-Streptomycin, Glutamax®, THT, and 5% HI FBS). Media were replaced three times a week and images were taken at 10× magnification on 21 days post seeding.