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Role of epithelial–mesenchymal transition in proliferative vitreoretinopathy

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ABSTRACT

Proliferative vitreoretinopathy (PVR) is a potentially blinding fibrotic complication. It is caused by the formation and contraction of epiretinal membranes (ERMs) that ultimately lead to retinal folds and traction retinal detachments. While multiple cell types have been identified in ERMs, retinal pigment epithelial (RPE) cells have long been implicated as a key player in the pathophysiology of PVR. Clinical and experimental evidence has shown that RPE cells undergo epithelial–mesenchymal transition (EMT) to adopt a fibroblastic phenotype. Cell–cell adhesions maintained by adherens and tight junctions are important for the maintenance of RPE phenotype, and disruption of these junctional complexes results in EMT via activation of signaling pathways such as β-catenin/Wnt and Hippo signaling, as well as transcription factors involving Zeb1, Snail, and ZONAB. Upon EMT, RPE cells can further differentiate into myofibroblasts in the presence of TGF-β with cytoskeletal tension mediated by RhoGTPase. These fibroblasts and myofibroblasts derived from RPE cells can contribute to ERM formation by cell migration, proliferation and matrix modification, and play a key role in ERM contraction. It is not solely the proliferation of these cells that results in PVR but rather the contraction of these cells in the ERM.

1. Introduction

Proliferative vitreoretinopathy (PVR) is an ocular fibrotic complication that occurs following corrective surgery for rhegmatogenous retinal detachment or posterior segment trauma (Pastor et al., 2002). Aberrant wound healing and/or persistent inflammation is believed to contribute to the formation of epiretinal membranes (ERMs). Contraction of ERMs results in loss of visual acuity due to retinal wrinkling, retinal folds and traction retinal detachments (TRD). Analyses of ERMs have identified several cell types, including retinal pigment epithelial (RPE) cells, glial cells, fibroblasts, myofibroblasts and immune cells (Pastor et al., 2002). Fibroblasts and myofibroblasts are considered to be the contractile cellular phenotypes within the ERM, and thus, play a prominent role in the contractile phase of PVR. Co-localization of cytokeratin, used as an RPE marker, with vimentin, a mesenchymal marker, in ERM samples obtained from patients with PVR strongly suggests that RPE cells, which are abundant at the early stages (Morino et al., 1990; Yamashita et al., 1986), are capable of undergoing epithelial–mesenchymal transition (EMT) to become fibroblasts (Fig. 1) (Casaroli-Marano et al., 1999). Furthermore, co-localization of cytokeratin with alpha-smooth muscle actin (α-SMA), a myofibroblast marker protein, suggests that RPE cells that have undergone EMT are capable of turning into myofibroblasts (Fig. 1) (Feist et al., 2014), a process referred to as epithelial–myofibroblast transition (EMyT) (Masszi and Kapus, 2011). This review will summarize the molecular mechanisms involved in EMT of RPE cells and the subsequent differentiation into myofibroblasts, as well as the potential role of RPE cells that have undergone EMT/EMyT in PVR.

2. Epithelial–mesenchymal transition (EMT)

EMT is a process in which epithelial cells adopt a mesenchymal phenotype. In addition to changes in cell morphology, various cellular functions, such as cell motility, proliferation, apoptosis, and protein expression, are altered (Kalluri and Weinberg, 2009). EMT plays key roles during embryonic development, and is also involved in pathophysiological processes such as neoplastic metastasis, as well as increased resistance to apoptosis of cancer cells. While the role of EMT in fibrotic diseases was initially debated, it is now widely accepted as a part of the pathophysiological change

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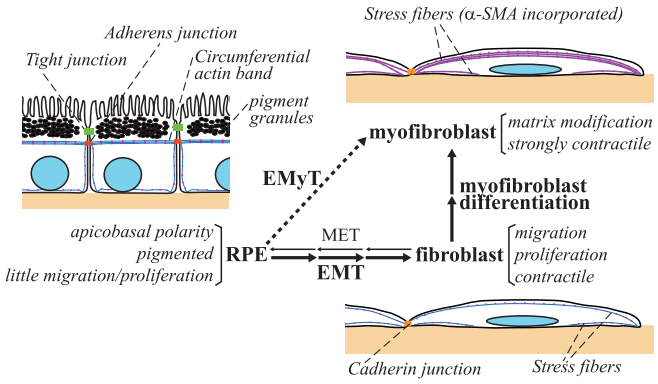

Fig. 1. Transition of RPE cells into fibroblast and myofibroblasts is achieved via epithelial–mesenchymal transition (EMT) and epithelial–myofibroblast transition (EMyT), respectively. RPE cells that undergo EMT lose their epithelial phenotype and function to adopt a fibroblastic phenotype, which can proliferate and migrate. These fibroblasts can further become myofibroblasts, characterized by the expression of alpha-smooth muscle actin (α-SMA)-incorporated stress fibers, under conditions that induce myofibroblast differentiation.

observed in many fibrotic diseases. EMT and the ensuing myofibroblast differentiation can lead to persistent activation of myofibroblasts, a hallmark of fibrotic diseases.

RPE cells under physiological conditions are heavily pigmented with a typical "cobblestone" epithelial appearance and have limited proliferative or migratory capability (Al-Hussaini et al., 2008; Strauss, 2005). However, when cultured in vitro RPE cells undergo EMT characterized by fibroblast-like morphology, mesenchymal protein expression, active proliferation and migration, as well as the loss of epithelial and RPE specific marker proteins and apicobasal polarity (Grisanti and Guidry, 1995; Tamiya et al., 2010). Thus, EMT should be considered an accumulation of partial changes that eventually results in epithelial cells adopting a mesenchymal phenotype, rather than being a one-step phenotypic change from epithelium to mesenchyme (Lamouille et al., 2014). For example, when an RPE sheet is cultured, initially cells at the edge of the sheet become flattened, followed by enlargement of the sheet due to proliferation and migration of these cells, with individual fibroblast-like cells eventually separating from the sheet (Fig. 2A). This is accompanied by a gradual decrease in the expression of RPE specific proteins, such as RPE65, illustrating the gradual nature of the change. During embryonic development mesenchymal epithelial transition (MET), the reverse process of EMT, takes place in multiple locations to establish an epithelial phenotype (Kalluri and Weinberg, 2009). RPE cells cultured in vitro can also be guided to undergo MET to regain the RPE phenotype by culture conditions where cell spreading and proliferation is limited – i.e. reduced serum concentration, low passage number, and high plating density (Maminishkis et al., 2006; Sonoda et al., 2009; Toops et al., 2014). This is possibly due to the cells being maintained in a partial EMT status, which allows MET to readily occur. However, RPE cells that contribute to PVR are not under such restricted conditions, especially for cell spreading, and achieve full EMT overtime. Such cells either maintain a fibroblastic phenotype or differentiate into myofibroblasts instead of reverting back to RPE.

3. Role of cell–cell contact in regulating EMT

Cell–cell adhesion, maintained by junctional complexes such as adherens junctions (AJs) and tight junctions (TJs), plays a critical role in maintenance of epithelial layer integrity, and thus, is required for apicobasal polarity and barrier function (Niessen, 2007). One of the mechanisms by which these complexes maintain an epithelial phenotype is by sequestering EMT signaling effectors at the plasma membrane/cytoplasm to prevent nuclear localization (Fig. 3) (Bernascone and Martin-Belmonte, 2013). Therefore, disruption of cell–cell adhesion leads to EMT. In the RPE sheet culture model, cells at the center of the sheet where cell–cell contact is intact maintain an RPE phenotype both in appearance and function, while cells at the edge of the sheet where cell–cell adhesion is lost undergo EMT (Fig. 2A) (Tamiya et al., 2010). Disruption of cadherins, the key protein of AJs, at the center of the RPE sheet by calcium chelation results in expression of the mesenchymal protein vimentin and initiation of proliferation (Fig. 2B). The role of cadherin in maintaining the RPE phenotype is, at least in part, due to its function to sequester β-catenin to the AJs. β-catenin is known to play dual roles (Fagotto, 2013). The first role is to function as part of the AJ complex in the presence of epithelial

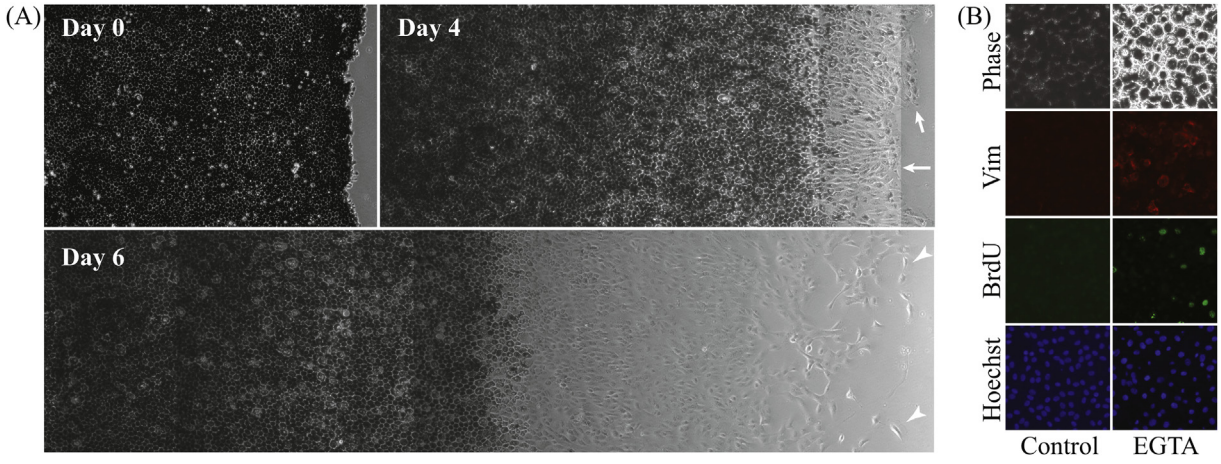

Fig. 2. EMT of RPE cells results from disruption of cell–cell adhesion. (A) The gradual change from RPE phenotype to fibroblast phenotype via EMT. On day 0 (top left), shortly after attachment of the RPE sheet to the matrix, all cells maintain an RPE phenotype with heavy pigmentation and cobblestone appearance. By day 4 (top right), the cells at the edge of the sheet (arrows) become flat and initiate collective migration as a sheet of cells. By day 6 (bottom), the spindle shaped cells at the migratory front separate from the sheet (arrowheads). In contrast, even cultured under the same condition, the cells in the center of the sheet maintain the RPE phenotype with heavy pigmentation and cobblestone appearance. (B) Cells at the center of the sheet can undergo EMT, as evidenced by the expression of the mesenchymal protein vimentin and initiation of proliferation (represented by the BrdU uptake), when cell–cell adhesion is disrupted by chelating calcium with EGTA. Adopted from Tamiya et al. IOVS 51, 2755–2763, 2010.