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Intercellular Adhesion-Dependent Cell Survival and ROCK-Regulated Actomyosin-Driven Forces Mediate Self-Formation of a Retinal Organoid

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SUMMARY

In this study we dissected retinal organoid morphogenesis in human embryonic stem cell (hESC)-derived cultures and established a convenient method for isolating large quantities of retinal organoids for modeling human retinal development and disease. Epithelialized cysts were generated via floating culture of clumps of Matrigel/hESCs. Upon spontaneous attachment and spreading of the cysts, patterned retinal monolayers with tight junctions formed. Dispase-mediated detachment of the monolayers and subsequent floating culture led to self-formation of retinal organoids comprising patterned neuroretina, ciliary margin, and retinal pigment epithelium. Intercellular adhesion-dependent cell survival and ROCK-regulated actomyosin-driven forces are required for the self-organization. Our data supports a hypothesis that newly specified neuroretina progenitors form characteristic structures in equilibrium through minimization of cell surface tension. In long-term culture, the retinal organoids autonomously generated stratified retinal tissues, including photoreceptors with ultrastructure of outer segments. Our system requires minimal manual manipulation, has been validated in two lines of human pluripotent stem cells, and provides insight into optic cup invagination in vivo.

INTRODUCTION

Stem cell-derived retinal organoid culture is a promising tool for studying human retinal development and disease (Lancaster and Knoblich, 2014). The growth of retinal organoids from human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) mimics the process of retinal development in vivo. Retinal organoids derived from patient-specific hiPSCs are used in disease modeling and drug testing.

Retinal development in vivo is a multi-step process of cell-fate specification that is regulated by transcription factors, signal transduction molecules, and cell surface molecules. Extracellular matrix (ECM)-mediated epithelialization of the epiblast and the eye field are critical events prior to retinal specification (Bedzhov and Zernicka-Goetz, 2014; Coucouvanis and Martin, 1995; Ivanovitch et al., 2013). Early retinal progenitor cells (RPCs) in the eye field express key transcription factors, including SIX3, RAX, PAX6, and OTX2 (Liu et al., 2010). The eye field evaginates to form the optic vesicle, which later invaginates to form the optic cup with RPCs for neuroretina (NR; $VSX2^{+}$ $PAX6^{moderate}$) in the inner layer and progenitors for retinal pigment epithelium (RPE; $MITF^{+}$ $PAX6^{high}$) in the outer layer (Liu et al., 2010). Ciliary margin (CM) is then specified at NR-RPE boundaries.

Tissue formation relies on cell-cell and cell-ECM adhesions via transmembrane proteins to transduce signals between the intra- and extracellular domains that are linked to actomyosin networks and cell-cell/ECM adhesions, respectively (Jockusch et al., 1995). Disruption of cell-ECM adhesions by cell detachment leads to anoikis (a specific type of apoptosis) (Frisch and Francis, 1994). Similarly, disruption of cell-cell adhesions by inactivation of tight junction (TJ) protein TJP1 or adherens junction (AJ) protein CDH2 causes apoptosis (Katsuno et al., 2008; Oliver et al., 2013; Wheelock et al., 2008). Related spatially and functionally, TJs and AJs are highly dynamic in morphogenesis and are involved in the regulation of apicobasal polarity (Rothen-Rutishauser et al., 2002). The cadherin ring of the AJs couples with a nearby bundle of F-actin to support morphogenesis of epithelial cells.

Numerous morphogenetic events are explained by the cell surface tension model. In equilibrium, surface tension is minimized via coordinated forces generated by cell surface tension and actomyosin-mediated cortical tension, resulting in cell sorting and self-organization (Beysens et al., 2000; Heisenberg and Bellaiche, 2013; Lecuit and Lenne, 2007; Steinberg, 1963). Rho kinase (ROCK) regulates actomyosin networks and cell polarity (Amano et al., 2010). Myosin activation and cell contraction depend on the phosphorylation of myosin regulatory light chains (pMYL) by apical ROCK.

Significant progress has been made in generating retinal structures from hESC or hiPSC cultures in which a regime of extrinsic factors with or without Matrigel are used (Boucherie et al., 2013; Lamba et al., 2009; Meyer et al., 2011; Nakano et al., 2012; Reichman et al., 2014; Zhong et al., 2014; Zhu et al., 2013). However, mechanistic understanding of the in vitro process is only beginning. Deeper

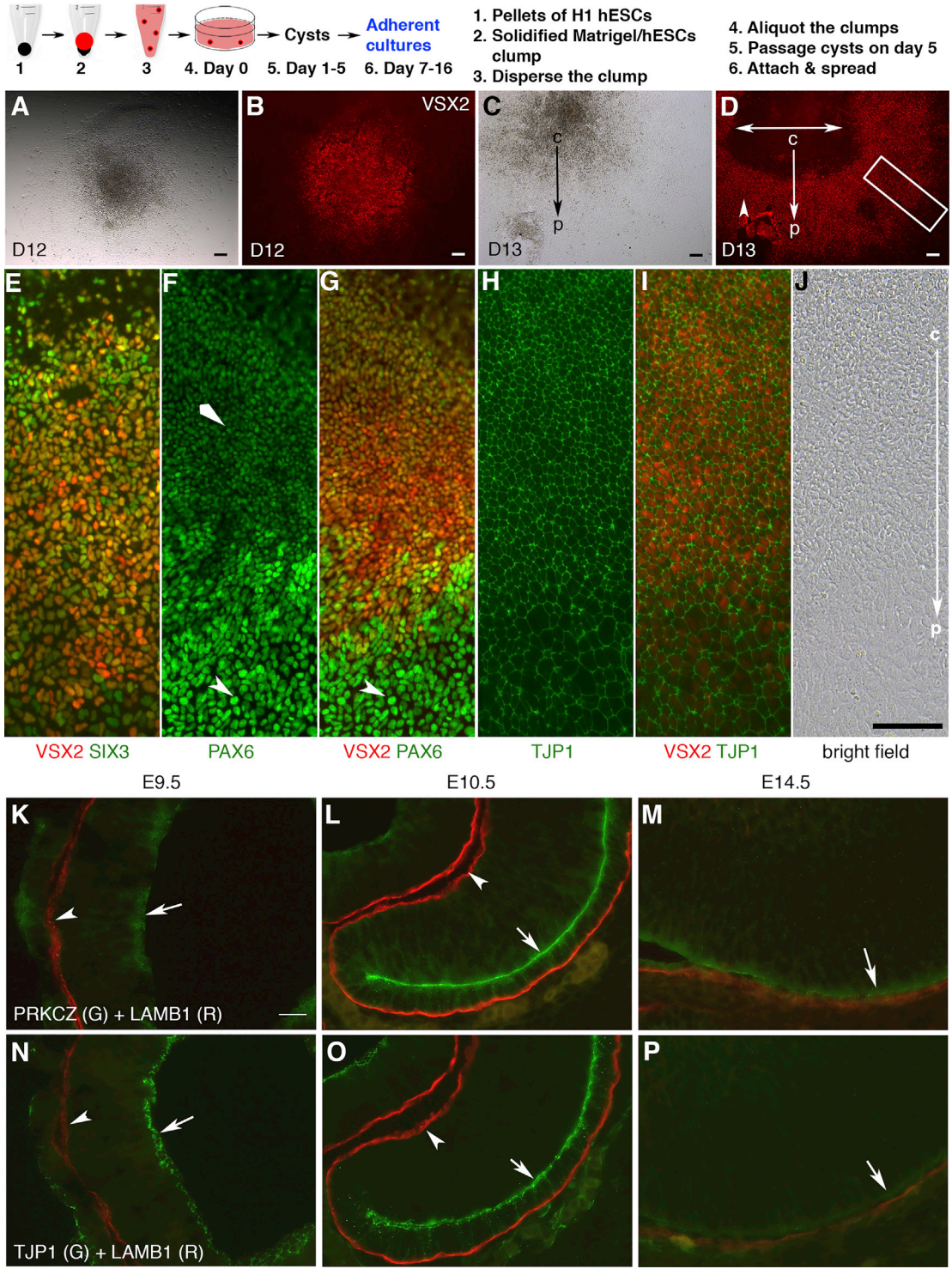

Figure 1. VSX2^{+} RPCs and PAX6^{high} RPE Progenitors Form Patterned Epithelial Monolayer Sheets upon Spontaneous Attachment and Spreading of the Cysts

(A–D) In a procedure shown in the scheme, the cysts spontaneously attached to a culture surface and spread, forming monolayer colonies. Adherent cultures on days (D) 12–16 were used for immunocytochemistry. In a well of 24-well plates, 4–7 patches of VSX2^{+} RPCs were found (n = 5/5, independent wells). VSX2^{+} RPCs assembled as a disk on D12 (B, bright field in A) and as a ring on D13 (arrowhead in D, bright field in C). Double-headed arrow (D) indicates the spreading of VSX2^{+} RPCs.

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