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Developmental insights from early mammalian embryos and core signaling pathways that influence human pluripotent cell growth and differentiation

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Abstract Human pluripotent stem cells (hPSCs) have two potentially attractive applications: cell replacement-based therapies and drug discovery. Both require the efficient generation of large quantities of clinical-grade stem cells that are free from harmful genomic alterations. The currently employed colony-type culture methods often result in low cell yields, unavoidably heterogeneous cell populations, and substantial chromosomal abnormalities. Here, we shed light on the structural relationship between hPSC colonies/embryoid bodies and early-stage embryos in order to optimize current culture methods based on the insights from developmental biology. We further highlight core signaling pathways that underlie multiple epithelial-to-mesenchymal transitions (EMTs), cellular heterogeneity, and chromosomal instability in hPSCs. We also analyze emerging methods such as non-colony type monolayer (NCM) and suspension culture, which provide alternative growth models for hPSC expansion and differentiation. Furthermore, based on the influence of cell–cell interactions and signaling pathways, we propose concepts, strategies, and solutions for production of clinical-grade hPSCs, stem cell precursors, and miniorganoids, which are pivotal steps needed for future clinical applications.

Abbreviations: FGF, fibroblast growth factor; hPSCs, human pluripotent stem cells; hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; NCM, non-colony type monolayer; ROCK, Rho-associated kinase; TGF-β, transforming growth factor β

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Introduction

Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), are potentially important resources for regenerative medicine-based cell replacement. However, this approach has not been efficiently implemented, due in part to currently used inefficient cell culture and differentiation strategies, which are heavily based on cell culture as colonies and aggregated embryoid bodies (EBs) (Chen et al., 2014; Mallon et al., 2006; Thomson et al., 1998; Xu et al., 2001). These methods have led to time-consuming production of relatively low numbers of cells (Hartung et al., 2010; Kehoe et al., 2010; Serra et al., 2012), heterogeneous cellular and molecular states (Bendall et al., 2007; K.G. Chen et al., 2012; Moogk et al., 2010; Stewart et al., 2006), and frequent reports of chromosomal abnormalities (reviewed in Baker et al., 2007; Lee et al., 2013). Certain chromosomal alterations such as trisomies 12, 17, and 20 have the capacity to confer growth advantages as indicated by comparative genetic analyses (Amps et al., 2011; Catalina et al., 2008; Draper et al., 2004; Lefort et al., 2008).

The relationship between heterogeneous cellular states and the incidence of chromosomal abnormalities remains unclear. Thus far, there is no direct proof that the colony-type culture method is responsible for the reported chromosomal abnormalities. However, it is conceivable that the heterogeneity of hPSC colonies (induced by cellular stress) might result in the selection of cells with growth advantages, which are usually bestowed by mutations or chromosomal alterations (Baker et al., 2007; Lee et al., 2013). This cellular stress may be derived from unwanted growth factors or conditions, which could be categorized as abnormal metabolic pathways, deficiency in nutrient or growth factors, and excessive apoptotic or differentiation signals. These suboptimal conditions may directly or indirectly contribute to insufficient cell production, cellular heterogeneity, and chromosomal instability of hPSCs.

It is imperative that we develop efficient culture methods that can overcome the obstacles found in the current culture systems. Moreover, new culture methods should be optimized for production of clinically relevant cell numbers, tissue morphogenesis, high-throughput drug discovery, and efficient assays of cellular stress. A comprehensive analysis of different cell culture protocols for hPSC is available in a recent review (Chen et al., 2014). Here, we will focus on the fundamental principles of developmental biology and core signaling pathways that underlie different growth methods. We will further discuss the relationship between the development of early stage embryos and the mechanisms underlying hESC heterogeneity and chromosomal instability. Finally, we will provide new insights into the development of optimal growth models for hPSCs.

Human pluripotent stem cell colonies and aggregates recapitulate some properties of early-stage embryo development

Unlike mouse embryonic stem cells (mESCs), which possess high single-cell plating efficiency under dissociated conditions, both hESCs and iPSCs cannot grow as single cells under commonly used culture conditions. Human pluripotent stem cells are conventionally cultured as condensed colonies and must be passaged as cell clumps to avoid cumulative apoptotic and differentiation stress. These cells are routinely differentiated toward derivatives of the three germ layers through three-dimensional (3D) EBs. In general, colony-type and aggregated cultures often produce heterogeneous colonies and EBs. Hence, we frequently observe more apoptotic activity in the center of a colony (with more spontaneously differentiated cells at the periphery) (Figs. 1 and 2) and complicated cystic structures after EB agglomeration (Fig. 3). The unique structures of hESC colonies and EBs are reminiscent of the inner cell mass (ICM) and post-implantation blastocysts observed during early embryo development. In the following sections, we will discuss the links between the structures of hPSC colonies and EBs and their developmental counterparts.

Human ES colony structure inherits epithelial features of the ICM

The ICM is comprised of a group of epithelial-like cells, the epithelial features of which are reflected at the intercellular junctions and in their basement membranes (Fig. 1A). Typical epithelial cells are tightly polarized monolayers with a distinct apical surface, basal membrane, and lateral intercellular conjunctions (Figs. 2A, B). At day 4 of compacting human embryos, E-cadherin (E-cad), an important component of epithelial cells, is located on the membranes where cell–cell contacts occur. However, in the ICM, E-cad is diffusely expressed in the cytoplasm, but not on the plasma membrane (Alikani, 2005). Thus, the distribution of E-cad in human preimplantation embryos appears to be an embryonic stage-dependent process. Electron microscopic study has also revealed similar epithelial features in hESCs (Ullmann et al., 2007). Intercellular interactions in both trophectoderm and ICM cells are implemented through gap junctions, tight junctions, and desmosome-like structures (Dale et al., 1991; Gualtieri et al., 1992).

Laminin is another epithelial component of the basement membrane that is expressed in the different stages of early embryos. Matrigel, a basement membrane preparation extracted from a murine Englebreth–Holm–Swarm sarcoma (Kleinman et al., 1982), containing laminins, as well as collagen IV, fibronectin, and vitronectin, was found to support robust hESC growth in defined medium (Ludwig et al., 2006) and is used routinely for the feeder-free culture of hPSCs. Laminin-111, the most abundant form in the Reichert's membrane, was found under the mouse trophoblasts, which was proposed to interact with ICM cells and induce differentiation (Domogatskaya et al., 2012; Miner et al., 2004). At least two laminin isoforms (i.e., laminin-511 and -521) have been detected in the basement membrane of the ICM of mammalian pre-implantation embryos (Domogatskaya et al., 2012; Miner et al., 2004). It has been shown that laminin-511, but not -111, supports mESC self-renewal in vitro without leukemia inhibitory factor (LIF) or mouse embryonic fibroblasts (MEFs) (Domogatskaya et al., 2008). Recently, the use of human recombinant laminin-511 has enabled long-term self-renewal of hPSCs (Rodin et al., 2010). Thus, both E-cad