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Engineering human cells and tissues through pluripotent stem cells

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The utility of human pluripotent stem cells (hPSCs) depends on their ability to produce functional cells and tissues of the body. Two strategies have been developed: directed differentiation of enriched populations of cells that match a regional and functional profile and spontaneous generation of three-dimensional organoids that resemble tissues in the body. Genomic editing of hPSCs and their differentiated cells broadens the use of the hPSC paradigm in studying human cellular function and disease as well as developing therapeutics.

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Introduction

When the laboratory of James Thomson, taking cues from developmental biology, first successfully isolated and propagated human embryonic stem cells (hESCs) in 1998 [1], it heralded the beginning of a new field of science in understanding human development, cellular diversity, and disease. In the years following this discovery, efforts to identify the pluripotent nature (capable of forming all developmental germ layers) of ESCs have yielded a sufficient understanding of the pluripotency network to allow the generation of induced pluripotent stem cells (iPSCs) [2,3]. The use of so-called Yamanaka factors to reprogram somatic cells to iPSCs has allowed the development of tools to study a wide variety of diseases alongside genetically normal samples.

The utility of human pluripotent stem cells (hPSCs, encompassing both hESCs and iPSCs) depends on our ability to guide hPSCs to functional cells and tissues of the human body. Two parallel methods have been developed: directed differentiation to enriched populations of cells that match a regional and functional identity of those normally residing in the human body and spontaneous generation of three-dimensional tissues, called organoids that resemble embryonic tissues. Application of genomic editing in hPSCs and their differentiated cells further enables the use of hPSCs in studying early human development, modeling diseases, and developing therapeutics.

Specification of embryonic germ layers from hPSCs

The pluripotent nature of hPSCs allows for generation of diverse cell types, comprising the whole of the human body. It also presents challenges to directing hPSCs to a particular cellular subtype through temporal and spatial coordination of a wide variety of external stimuli. Elegant manipulation of these cascades has allowed formation of all three germ layers [4–6,7••], regionally patterned cells [8–10] and subtype specific cells [10–12,13•].

Early hPSC differentiation relied on a combination of intrinsic programs and modification of the environment to guide differentiation. After the removal of a feeder layer and aggregation of PSCs to embryoid bodies [4], a default pathway allows for neurectoderm formation and subsequent generation of neuron-producing spheres (neurospheres) [4] under the culture condition that favors the growth of neural cells. Analysis of these 3D-neurosphere structures generated during the neurectoderm phase of differentiation revealed architecture that remarkably resembles a developing neural tube [4]. It has subsequently been established that neurectoderm differentiation from hPSC bears striking similarities to *in vivo* human development not only in terms of morphological structures, but also regarding gene expression and the normal developmental timeline [14].

The default neurulation hypothesis arises from early studies in *Xenopus laevis* by Spemann & Mangold, where it was found that signals coming from an organizing center of mesoderm were sufficient to induce neurulation. Through the work of several labs, it was demonstrated that these signals were bone morphogenetic protein (BMP) antagonists [15–17]. Building upon these studies and combining the evidence with more recent work on hPSCs [18–20], Chambers *et al.* developed a strategy to use inhibitors of several anaplastic lymphoma kinases (ALKs) and BMP receptors to drive hPSCs into the neural

lineage [21]. Activated SMAD was reduced in treated nuclei by 24 hours and subsequent activation of neural genes was observed. The colonies displayed rosette-like morphology and a large proportion of the cells (~80%) expressed PAX6 after one week. The authors demonstrated that these progenitors generate neurons, thus advancing the directed differentiation of hPSCs to the neural lineage by refining the protocol into an increasingly chemically defined and controlled paradigm. The so-called dual SMAD inhibition protocol, or simply 2i, marks a shift from following intrinsic developmental programs to a more controllable chemically defined environment.

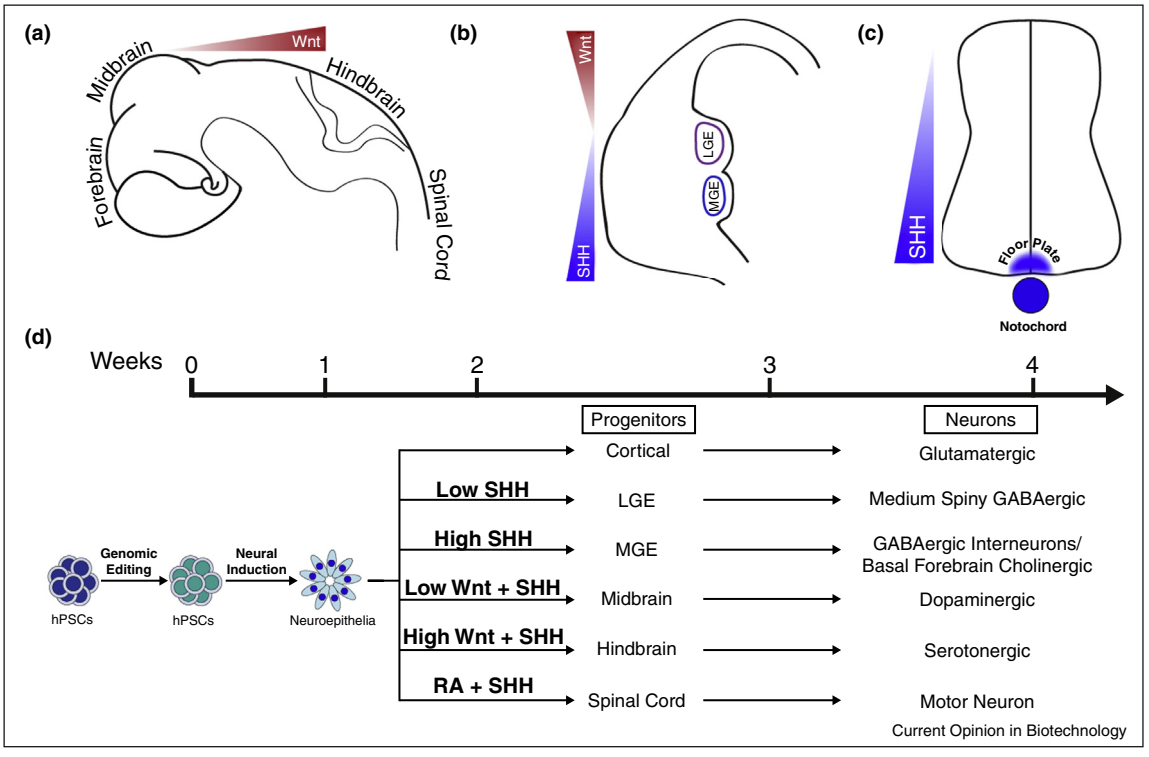
Directed differentiation of functionally specialized cells from hPSCs

During embryonic development, coordination of intrinsic program and environmental cues guides the generation of regionally and functionally specialized cell and tissue types, a process called regional patterning. This principle has been instrumental for directed differentiation to specialized cell types. The neuroectoderm or neuroepithelial cells differentiated from hPSCs carry a dorsal forebrain identity [10,18], consistent with early human brain development. However, the initial neuroepithelial cells are specified to a neural identity but not yet committed to a specialized neuronal fate. They are hence called primitive or naïve neuroepithelia or neural stem cells (NSCs) [10]. These primitive NSCs, in response to temporarily and spatially available patterning molecules, differentiate to cells of the ventral forebrain, mid/hindbrain, and the spinal cord (Figure 1).

Decades of work across numerous deuterostomes and planarians have established the critical role of Wnt signaling in patterning the anterior–posterior axis of bilaterally symmetrical organisms [22]. Similarly, Sonic Hedge-Hog (SHH), secreted from the notochord [23] (Figure 1), determines the dorsal–ventral axis [23,24]. Cellular patterning requires precise feedback and happens in a graded, temporally and spatially dependent manner. Similarly, differentiation of regionally and functionally specialized neuronal types requires application of specific patterning molecules in a specific concentration at a particular time.

Recapitulating neural patterning, Li *et al.* [10] treated the hPSC-derived (differentiated for 2 weeks) SOX1-expressing neurectoderm with a combination of SHH and retinoic acid (RA) in an attempt to guide stem cells to

Figure 1



Summary of regional patterning and neuronal subtype generation. **(a)** Sagittal view of developing mammalian nervous system illustrating morphogen gradient along the rostral–caudal axis. **(b)** Cross section view of developing brain showing the lateral and medial ganglionic eminence (LGE & MGE), illustrating morphogen gradient along the dorsal–ventral axis. **(c)** Cross section of the spinal cord showing notochord; the source of SHH in the developing nervous system. **(d)** Summary of *in vitro* directed differentiation paradigm for subtypes of progenitors and neurons in response to morphogens.