Human Neural Stem Cell Systems

Subjects: Cell & Developmental Biology

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Definition

Building and functioning of the human brain requires the precise orchestration and execution of myriad molecular and cellular processes, across a multitude of cell types and over an extended period of time. Neural Stem Cells (NSCs) represent the heart of these processes, since they increase the pool of neural progenitors and are the founders of all the neural progeny which will constitute the adult human brain.

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Human Neural Stem Cells: When and Where?

The immense complexity of the human brain is reflected in its cellular organization and in the cognitive and behavioral repertoire that defines us as human. The human brain is the product of an evolutionary and developmental history that resulted in its progressive enlargement and specialization. Our central nervous system (CNS) develops through a dynamic and prolonged process in which myriad cell types are generated by neural stem cells (NSCs) and assembled into an intricate synaptic circuitry. Deviations from the normal course of development can lead to a variety of pathologies, including neurological and psychiatric disorders that affect some of the most distinctly human aspects of cognition and behavior [1][2][3][4].
During early CNS development, the neural tube is comprised of a pseudostratified layer of neuroepithelial cells lining the central cavity [3][5][6]. These cells constitute the ventricular zone (VZ) of the neural tube and are the founders from which all neurons and glial cells of the adult CNS will be generated (Figure 1).

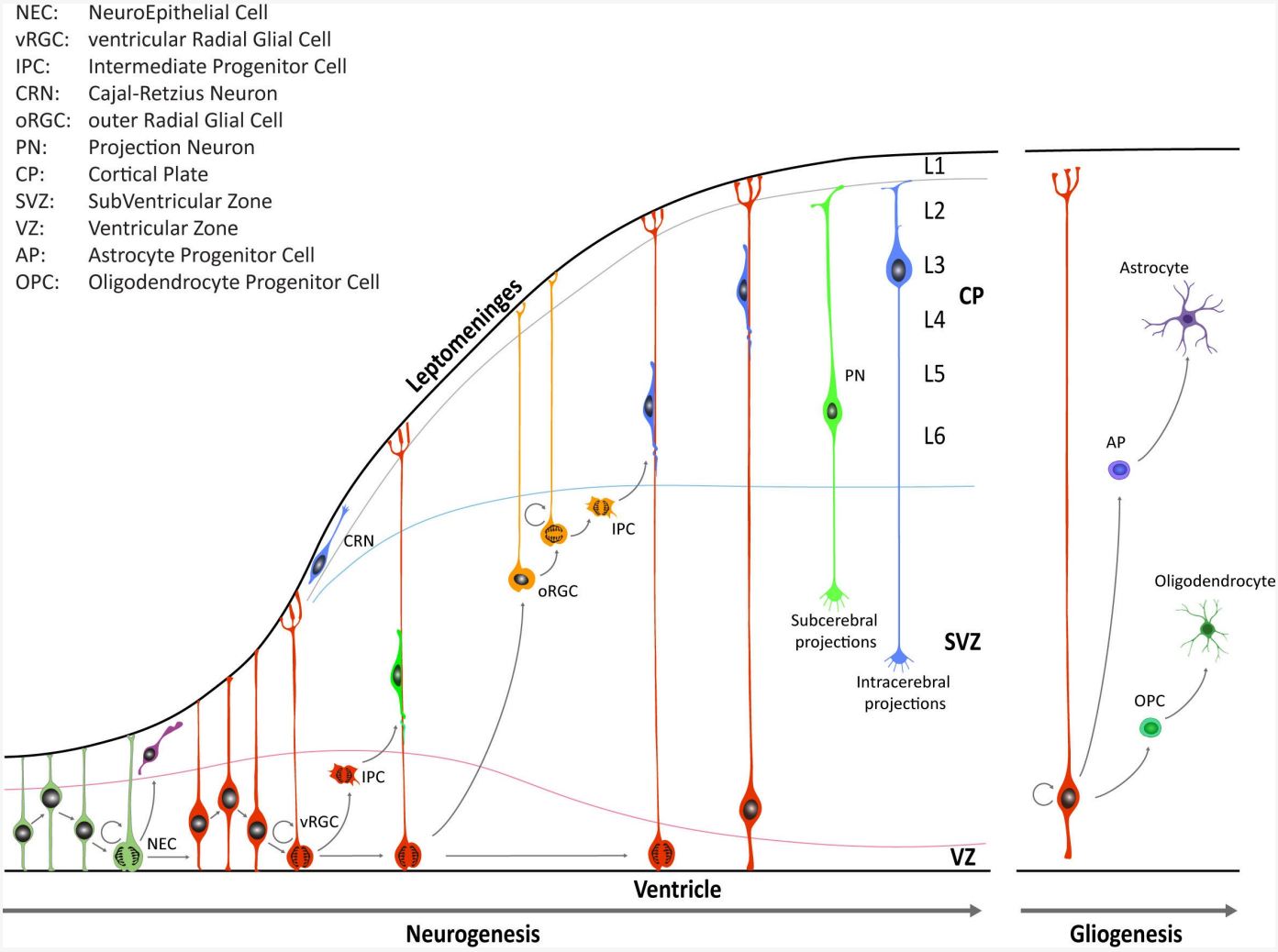

Figure 1. Schematic illustration of neocortical development. Neuroepithelial cells (NECs) undergo symmetric cell division to expand the initial pool and later transition into ventricular radial glia cells (vRGCs). vRGCs begin asymmetric cell division to generate another vRGC and a nascent projection neuron. Neurons then migrates radially from the ventricular zone (VZ) along the RGC basal processes into the cortical plate (CP). Early-born projection neurons (PNs) settle in the deep layers (Layers 5 and 6), and later-born neurons in upper layers. Additionally, some populations of RGC daughter cells convert themselves into intermediate progenitor cells (IPCs) or outer radial glial cells (oRGCs) in the subventricular zone (SVZ). After the neurogenic stages, gliogenesis occurs, generating astrocytes and oligodendrocytes.

Until the seventh post-conceptional week (pcw), depending on the region of the CNS, neuroepithelial cells undergo primarily symmetric divisions in order to expand the stem cell pool [3]. Dividing neuroepithelial cells are characterized by a radial movement of cell nuclei from the apical luminal side (apical surface) to the basal side of the neural tube (basal lamina at the pial surface), in concert with the progression of the cell cycle. Mitosis typically occurs when cell nuclei are close to the apical surface of the neuroepithelium, and this continuous relocation is commonly defined as interkinetic nuclear migration (IKNM). Later on, neuroepithelial cells transition into a distinct class of cells known as radial glial cells (RGCs) which reside in the VZ and in the inner and outer subventricular zone (iSVZ and oSVZ, respectively). RGCs contact, at least initially, both the ventricular and pial surface through their apical and basal process, respectively. These cell populations serve as progenitor cells to generate neurons and macroglia (i.e., astrocytes and oligodendrocytes) and to provide a scaffold for migrating nascent neurons [3][5][6]. RGCs divide, but unlike neuroepithelial cells, the divisions of RGCs are mostly asymmetric, giving rise to either a daughter RGC, an intermediate progenitor cell (IPC, also known as a transit amplifying cell), or a nascent neuron that subsequently migrates out of the VZ or the SVZ to its final location near the pial surface. In the human neocortex, neuroepithelial cells give rise first to ventricular RGCs (vRGCs), which later transit into outer RGCs (oRGCs) [7][8][9]. oRGCs have morphologically distinct features, retain the basal process, but lose apical contact, and their cell bodies translocate into the oSVZ. The oRGCs can be characterized by a distinct transcriptional signature compared with vRGCs and divide in a unique manner, called mitotic somal translocation (MST), a process where the cell soma moves rapidly up the basal fiber before cytokinesis [8]. A third population of RGCs, called truncated radial glia (tRGCs), develops later in neurogenesis [10]. Cell bodies of tRGCs reside near the apical surface and possess basal processes that do not reach the pial surface and appear truncated.
During early neurogenesis, at the beginning of the second trimester, the vRGCs and IPCs give rise to neurons present in the deep layers. On the other hand, oRGCs give rise to later born IPCs, and differentiate predominantly into upper layer neurons (Figure 1). The six cortical laminae are comprised of multiple molecularly defined excitatory neuron subtypes. These cells connect intra-cortically to regulate synaptic activity inside the cortex, as well as subcortically to provide executive regulation of sensory and motor activity [11].

Neural Stem Cells at a glance:

Neuroepithelial Cells: During mammalian embryogenesis, CNS development begins with the induction of the neuroectoderm, which forms the neural plate and then folds to give rise to the neural tube. Within these neural structures there exists a complex and heterogeneous population of neuroepithelial cells, the earliest neural progenitor type to arise. As CNS development proceeds, neuroepithelial cells give rise to temporally and spatially distinct neural stem/progenitor cell populations.
Radial Glia Cells (RGCs): Multipotent neural progenitors with glial-like properties. At the onset of neurogenesis, neuroepithelial cells in the ventricular zone transition into RGCs, bipolar cells with long radial processes extending from the apical surface of ventricular zone to the pial surface. RGCs also act as scaffolds along which newborn neurons can travel from their site of origin to their final destination in the adult CNS.
Intermediate Progenitor Cells (IPCs or Basal Progenitors) : Neural progenitors generated from neuroepithelial cells and RGCs at the apical surface of the ventricular zone. IPCs migrate to the basal side of the ventricular zone forming the subventricular zone. Each IPC divides symmetrically to generate two or four neurons.
Adult Neural Stem Cells: Populations of multipotent neural stem cells mainly present in two specialized niches of the adult mammalian brain, the subventricular or subependymal zone of the lateral ventricle wall and the subgranular zone of the dentate gyrus. They maintain neurogenesis and gliogenesis throughout adult life in rodents and other mammals, but their presence and activity in humans is still debated. They derive from RGCs that in the postnatal brain convert into astrocytic-like NSCs.

In vitro systems of Neural Stem Cells

Our knowledge of NSCs has been revolutionized as optimized culture protocols have been put in place [12]. Since Reynolds and Weiss (1992) [13] made the landmark discovery that NSCs could be maintained in culture via propagation in free-floating neurospheres, several works have progressively i mproved systems for NSC long-term and homogeneous culture. In 2005, it has been shown that NSCs can be expanded as monolayer cultures, named NS cells, with full preservation of their neurogenic potential [14]. NS cells were derived from fetal and adult mouse CNS, but also from neural-committed mouse embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) [14][15][16][17][18][19]. Additionally, NS cells were also derived from *post-mortem* human fetal tissue [20][21]. Although NS cells showed features of neurogenic RGCs, they emerged to be restricted to the generation of GABAergic neurons, independently of the different sources they were derived from [19][20][21].
On the other hand, embryonic neuroepithelial cells possess a great self-renewing potential and wide multilineage differentiation.

Thanks to these unique properties, neuroepithelial cells represent an ideal candidate for *in vitro* studies related to NSC biology, neuronal and glial differentiation, and various neurodevelopmental diseases. Notably, Austin Smith's group has described a population of NSCs derived from 5–7 pcw human hindbrain [22]. These cells, named hindbrain (hb) neuroepithelial stem (NES) cells, are neurogenic and preserve their original regional identity, exhibiting for the first time a stable wide degree of plasticity. More recently, we described the derivation and characterization of neocortical (NCX) NES cells [23]. NCX-NES cell lines were derived from primary neuroepithelium of human *post-mortem* specimens ranging from 5 to 8 pcw. After derivation, NCX-NES cells form neural rosettes, reminiscent of the radial arrangement in the native neural tube. NES cells exhibit stem/progenitor cell characteristics as they express the neuroepithelial marker SOX1 and the pan-neural stem cell markers Nestin, SOX2, and Vimentin. NES cells retain regional identity after long-term expansion, as demonstrated by the expression of FOXG1 and OTX2, key transcription factors demarcating proliferative zones of the early human forebrain. NES cells show great neurogenic potentials, giving rise to mature neurons with extended complex neurites. Furthermore, they generate GFAP-positive astroglial cells, thus demonstrating their multipotential stem cell capacity. We also reported that NCX-NES cells could be expanded for more than 1 year and 38 passages with no evidence of chromosomal instability [23]. Single-cell RNA-sequencing (RNA-seq) on expanded NCX-NES cells and cells from donor-matched brains demonstrated that the majority of cells from the donor-matched brain tissue samples express canonical marker genes of neuroepithelial cells and RGCs of the dorsal forebrain [23]. Remarkably, NCX-NES cells exhibit a close transcriptional signature of early NSCs as their donor-matched genetically identical NCX cells. Together, these data establish NES cell lines as a consistent model of early human brain development. A similar NES population derived from developing human spinal cord has been described, able to maintain regional identity and neuronal commitment of the caudal CNS [24]. Of note, spinal-cord NES cells were successfully tested in cell grafting approaches after spinal cord injury in mice [24].

Human pluripotent stem cells (hPSCs), which include both ESCs and iPSCs, represent an extraordinary *ex vivo* source of neural progenitors. The development of neural induction protocols provides the possibility to generate *in vitro*-derived expandable NSC systems as a platform for studying basic human neurodevelopment, disease mechanisms, and potential therapeutics [12]. Seminal studies have identified rosette-type NSCs that resemble neural tube-stage progenitors, capable to respond to patterning instructions, but not long-term expandable [25]. On the other hand, a long-term population of NES cells (named lt-hESNSCs or lt-NES) was described by Koch et al. (2009) [26]. The study used the embryoid body (EB)-based differentiation protocol and required manual isolation of the rosettes from plated EBs. However, *in vitro* culture conditions bias lt-NES regional identity from rostral (first five passages) to more caudal midbrain-hindbrain identity (later passages) [26]. In this direction, Li et al. (2011) [27] described a small molecule-based neural induction method to derive primitive neural progenitors from hESCs. However, also in these conditions, the NSC population is biased towards a midbrain/hindbrain neural fate [27]. A more recent elegant work improved the isolation and propagation paradigm of neuroepithelial and RG-like cells [28], but the true identity and physiological relevance of hPSC-derived NSCs are open to interrogations because cells could acquire transcriptional and epigenetic programs that diverge from the cell state *in vivo*.

Acknowledgments

We apologize for not citing many original papers due to the space limit. The research in the authors' laboratories was supported by 2017 NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation (#26565) and "PRA—Progetti di Ricerca di Ateneo" from University of Pisa (Institutional Research Grants, Project no. PRA_2018_68) to M.O.

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Keywords

Human neural stem cells;Neurodevelopment;Corticogenesis;In vitro models