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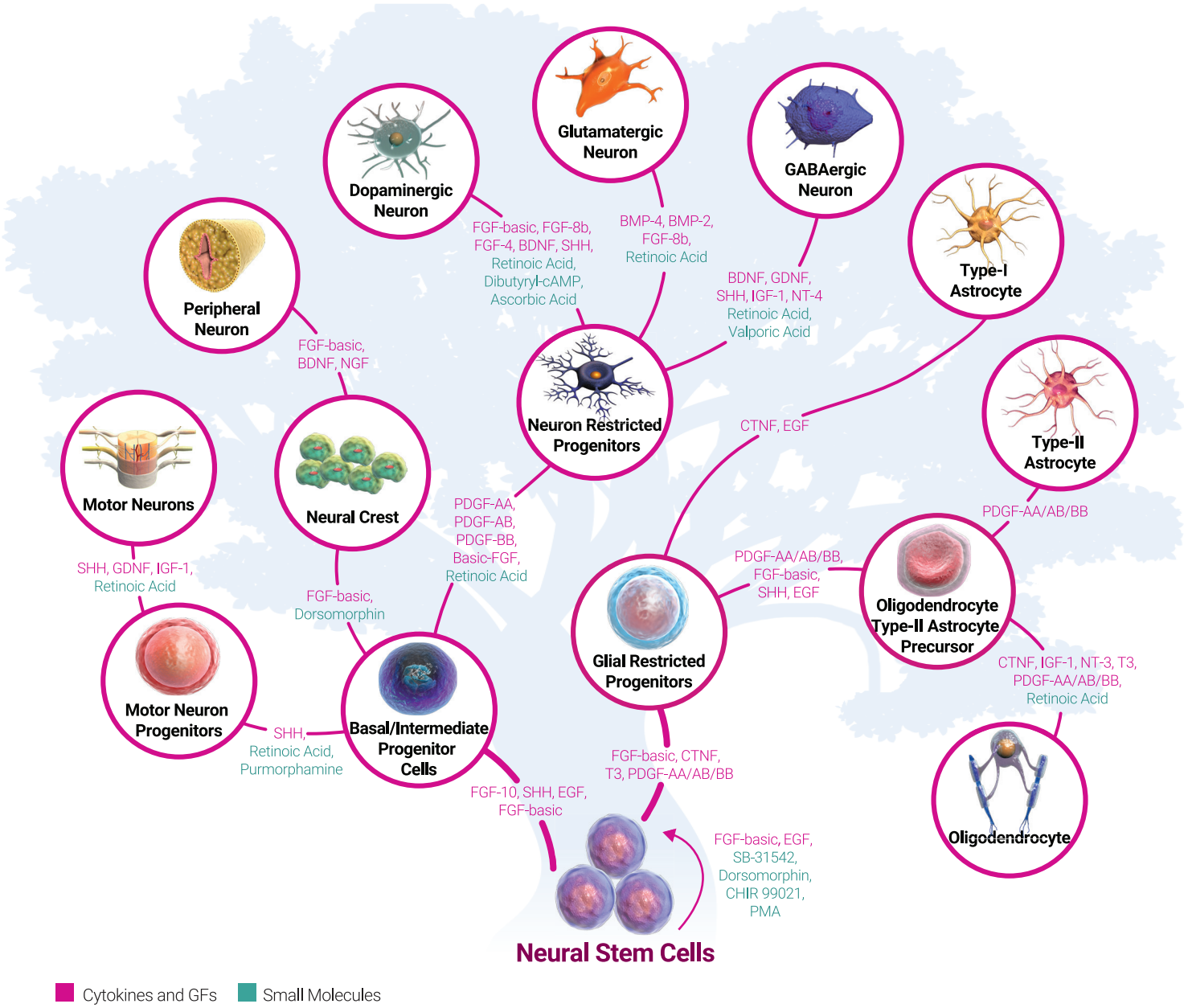
Neural Stem Cells in Developmental Research, Disease Modeling and Regenerative Medicine

Neurogenesis, or the process by which neural stem cells (NSCs) give rise to the neurons of the central nervous system (CNS), initially occurs during embryonic development, termed development or prenatal neurogenesis, and continues throughout adulthood, termed postnatal and adult neurogenesis, with marked differences. During developmental neurogenesis, ectoderm-derived neuroepithelial cells of the ventricular zone (VZ) elongate and generate the primary NSCs of the mammalian CNS, known as radial glial cells (RGCs). In addition to amplifying through self-renewal, RGCs directly and indirectly generate neurons through asymmetrical division resulting in one self-renewing daughter cell alongside either a post-mitotic neuron or an intermediate progenitor cell (IPC), respectively.



While IPCs retain the capacity to self-amplify through symmetrical division, allowing for expansion of cortical size, they primarily differentiate symmetrically into neurons, oligodendrocyte progenitors or astrocyte progenitors. New neurons move to their final destinations through a process known as radial migration, whereupon they mature fully and develop the defining, information-transmitting axons and dendrites. Unlike developmental neurogenesis, adult neurogenesis is restricted to the subgranular zone (SGZ) of the hippocampus and the subventricular zone (SVZ) of the striatum, where new neurons are integrated into existing cortical framework.

The ability of NSCs to generate the neurons and glia that constitute the CNS places them at the forefront of neuro-developmental research, neuro-disease modeling and regenerative medicine; underscoring the need for developing efficient methods for obtaining NSCs.

Currently, three major sources for NSCs have been identified:

1. **Isolation from primary neural tissues** followed by exposure to FGF-basic and EGF to induce proliferation, self-renewal, and expansion.
2. **Differentiation from pluripotent stem cells**, either by embryoid body formation or monolayer culture.
3. **Direct transdifferentiation from somatic cells through induction by:**
   a. Expression of specific transcription factors, usually in combination with small molecules.
   b. Chemical transdifferentiation using a cocktail of only small molecules.
   c. Growth factors in combination with a three-dimensional culture system.

Neuronal stem cells are heavily influenced by their extracellular microenvironment, also known as the stem cell niche. Proximity to more mature neural cells and biochemical signals mediated by cytokines and growth factors, as well as biophysical and mechanical cues, closely regulate NSCs and direct their behavior. These regulatory signals and the small molecules that can be exploited to manipulate the related signaling pathways can be employed *in vitro* to direct the fate of neural cell cultures.

Research in neural development and disease modeling employs a wide variety of neural cell cultures with varying degrees of homogeneity and complexity. A 2-dimensional monolayer of unpolarized NSCs is the simplest culture and is most suited for high throughput screening applications due to relative homogeny and limited differentiating potential. Neural rosettes, which are also considered 2-dimensional, demonstrate more complexity in comparison with the ability to polarize and self-organize. Spheroids and organoids are even more complex, 3-dimensional culture types that better imitate cell-to-cell and cell-to-extracellular matrix (EMC) interactions *in vivo*. The generation of certain neuronal cell types, such as microglia, in organoid culture has, however, eluded researchers thus far. Although 3-dimensional tissue models offer an excellent opportunity to study human brain development and disease, they also carry their own set of challenges. Most notably, the lack of efficient vascularization contributes to restrictions on tissue size due to cell necrosis, while variable morphology both between cultures and within, in specific tissue areas, results in reproducibility issues.

The introduction of engineering-based models, including scaffolding and microfluidic platforms that aim to mimic living tissues and engineered synthetic micro- environments, have further advanced NSC research, allowing the study of neuronal network formation and the modeling of neurological diseases. Biophysical aspects, such as stiffness and mechanical stretch of the EMC, affect NSC development. Whereas stiffer modules lead to differentiation into glial cells, softer and more porous gels lead to enhanced migration and neural cell differentiation. The combination of cell biology-based models and engineering-based models allows for improved tissue architecture and reproducibility.

The most recent development in neural research is bioprinting, an offshoot of 3D printing that allows for the automatic and precise arrangement of cells, ECM, and signaling factors to form living tissues with complex architecture. In addition to overcoming the obstacle of reproducibility, bioprinting of NSCs with the proper differentiation signals, might enable the formation of artificial neural tissues with complex cellular arrangement and a better resemblance to native neural tissues.

The ability of NSCs to secrete soluble neurotrophic factors and to differentiate into various neuronal cell types makes them a promising tool in neural regeneration and cell therapy for diseases associated with the CNS. NSC transplantation has been shown to be effective in a variety of animal models of neurodegenerative diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease and Parkinson's disease (PD), as well as in models of spinal cord injury, stroke, traumatic brain injury, epilepsy and cerebral palsy.

Significant challenges are associated with transplanting NSCs and neurons for therapeutic applications, including lack of cell homogeneity, low survival rate of transplanted cells, and lack of proper differentiation and neurite outgrowth. Engineered biomaterial scaffolds that combine mechanical and biochemical parameters in a defined spatial arrangement are emerging as a promising approach to aid in the therapeutic transplant of NSCs and neurons.

An important tool in NSC and neural developmental research is the ability to identify and characterize the various types of neural cells. This is done by utilizing neuronal lineage markers expressed by the different cells during neurogenesis, including DNA, RNA or protein tags. A list of neuronal lineage markers can be found in the table below.

NEURONAL LINEAGE MARKERS

Cell type	Markers
Neural Stem Cells	SOX1, SOX2, nestin, CD133, PAX6
Neuroepithelial cells	Nestin, SOX2, notch1, HES1, HES3, E-cadherin, occludin
Radial glia	Vimentin, nestin, PAX6, HES1, HES5, GFAP, GLAST, BLBP, TN-C, N-cadherin, SOX2
Microglia	CD11b, CD45, Iba1, F4/80, CD68, CD40
Schwann cells	MPZ, NCAM, GAP43, S100
Oligodendroycte Progenitors and Mature oligodendrocytes cells	GalC, A2B5, PDGFRA, NG2, Olig 1, olig 2, olig 3, MBP, OSP, MOG, SOX10
Astrocytes Progenitors and Mature Astrocytes	CD44, GFAP, EAAT1/GLAST, EAAT2/GLT-1, glutamine synthetase, S100-beta, ALDH1L1
Neuronal Progenitors and Mature Neurons	NeuN, MAP2,NCAM, 160 kDa neurofilament M, 200kDa neurofilament H, synaptophysin, PSD95, βIII tubulin, DCX
Motor Neurons	Isl1, HB9
Glutamatergic neurons	vGluT1, vGluT2, NMDAR1, NMDAR2B, glutaminase, glutamine synthetase
GABAergic neurons	GABA transporter 1, GABAB receptor 1, GABAB receptor 2, GAD65, GAD67
Dopaminergic neurons	Tyrosine hydroxylase, dopamine transporter, FOXA2, GIRK2, Nurr1, LMX1, OTX2
Serotonergic neurons	Tryptophan hydroxylase, serotonin transporter, Pet1
Cholinergic neurons	Choline acetyltransferase, vesicular acetylcholine transporter, acetylcholinesterase

Additional reading:

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