

Neural Stem Cells: A Functional Definition Based on Proteomics

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Abstract

Neural stem cells can be isolated from the adult mammalian brain throughout the entire lifetime of an organism, including humans. Unfortunately, no unique molecular marker could be identified expressed by all types of neural stem and progenitor cells in every developmental stage. Genomic and proteomic screening approaches suggest a functional definition of adult stem cells by their molecular inventory. These include (i) the responsiveness to growth factors, (ii) the reuse of developmental signaling cascades in adult progenitors, (iii) the interaction with molecules of the extracellular matrix, (iv) the expression of genes regulating transcription and translation, (v) mechanisms for controlling cell number, and (vi) the protection against cellular stress, such as hypoxia, or DNA damage.

Keywords: Neural stem and progenitor cell, proteomics, genomics, molecular marker, differentiation.

1. Introduction: Stem and progenitor cells in the brain

In the last 10 to 15 years, the view on the regenerative potential of the brain changed dramatically with the characterization of stem and progenitor cells residing in the adult brain throughout the entire lifetime of the mammalian organism, including humans.

Neural stem and progenitor cells have been characterized by their developmental potential which is the ability to produce progeny with is on the one hand more restricted with regard to the multitude of cells they can produce, but on the other hand gains functionality by differentiation [1-3]. In the current concept, brain cells are all derived from several divisions of (i) the totipotent zygote, which has the potential to give rise to all body cells, via (ii) the pluripotent embryonic stem cells, which cannot constitute the whole body anymore, but it can differentiate into all varieties of cells, (iii) the

multipotent stem cell, which persists in the organism and gives rise to the cells of the same germinal layer, (iv) progenitor cells of the respective tissue, which are restricted to specific functional cells of the respective organ, and finally, (v) the differentiated tissue-specific cells, which are terminally differentiated and can exert the specific cellular tasks. With regard to the brain, these are neurons, astrocytes, and oligodendrocytes, which are thought to originate all from a common neural progenitor cell (Figure 1) [4-8].

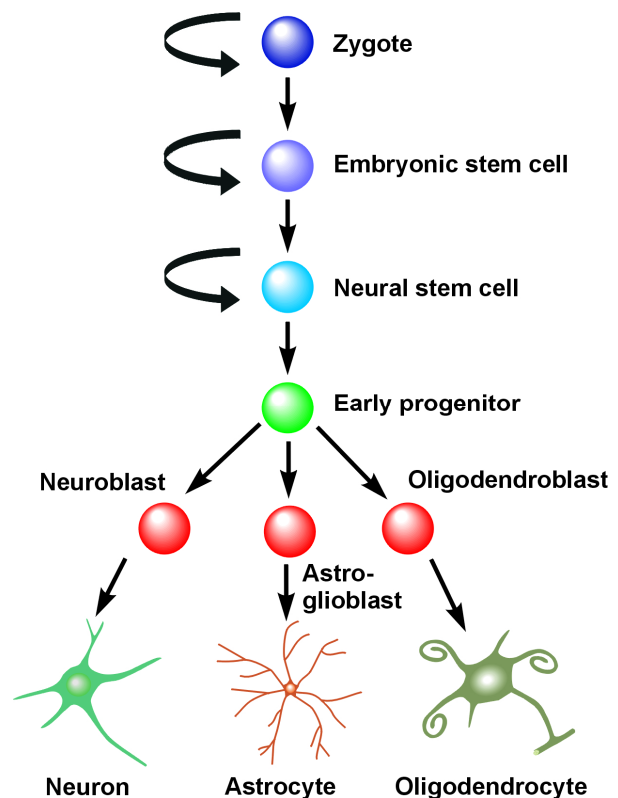


Figure 1. Hierarchical concept of the biological origin of neural stem cells [9]. All brain cells are derived from common precursor cells, which are characterized by their proliferation and differentiation potential (Copyright © Nova Science Publishers, Inc., Hauppauge, NY, USA. Reprinted with permission.)

2. The search for common molecular markers of neural stem cells

Whereas hematopoietic stem cells (HSCs) can be defined by the expression of a specific set of molecular markers, such as the typical combination of CD34⁺, CD59⁺, Thy1/CD90⁺, CD38^{low}, C-kit/CD117^{low}, lin⁻ for human hematopoietic stem cells [10-12]. However, also HSCs may be present in the bone marrow without this typical phenotype.

Unfortunately, no such definition has been found for neural stem cells [6, 13, 14], thus the definition of these cells relies on the functional properties self-renewal and multipotency [5, 15]. Although presumptive markers have been proposed (reviewed in [16]), none of them comprehends all subtypes of neural stem cells at any given point of time or region in development (Figure 2).

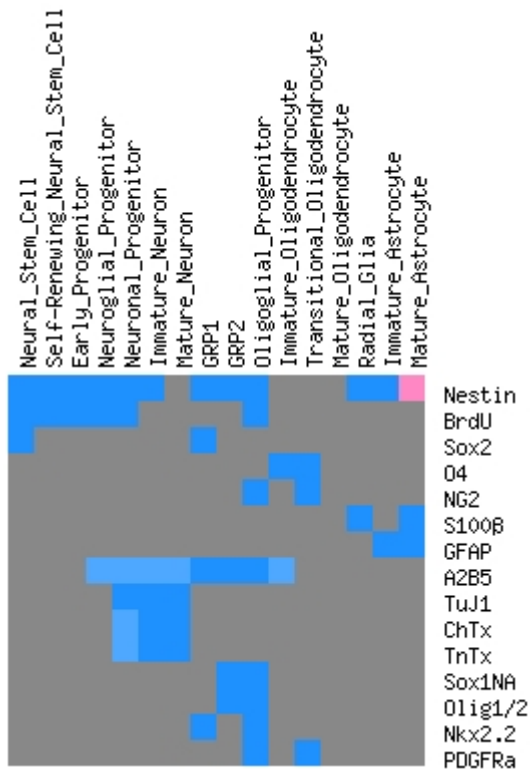


Figure 2. Pseudoclustering of molecular markers expressed in neural stem cells and their progeny. There are no unique proteins expressed, therefore, a definition solely based on these markers is not possible. (blue boxes: expression confirmed, light-blue boxes: expression disputed, red box: expression unclear) (GRP, glial-restricted precursors) (Modified from [9]).

In microarray-based screening approaches, specific gene expression patterns have been proposed for the differentiation stages of neural stem cells [17, 18]. Genomic approaches also defined the overlapping set of expressed genes in several types of stem cells, including neural stem cells [19-22].

3. Definition of adult stem cells by common functional properties

With regard to proteomic analyses, proteomic inventories of neural stem cells have been created using two-dimensional gel electrophoresis [23, 24]. Additionally, the events underlying cellular differentiation *in vitro* have been studied in human fetal cortex [25], rodent subventricular zone [26-28], hippocampus [23, 29], olfactory bulb [30], and cerebellum [31, 32], as well as in neural stem cell-like cell lines [33, 34] and the porcine brain [35].

Comparing the protein expression patterns, no single characteristic molecule could be identified, but the functional definition, as specified for the genomic approaches, is supported (Figure 3) [9, 36]. In the light of these results, adult stem cells have all the same prerequisites, but the exact molecular composition dependent on cell and tissue-specific factors. These common prerequisites involve:

(i) The responsiveness to growth factors such as EGF, FGF-2, EPO, G-CSF, VEGF, LIF, TGF- β , NGF, as well as to neurotransmitters such as glutamate, GABA, or NO. Again, not all neural stem and progenitor cells react to each of these molecules, but, for example, the expression of VEGF receptors in the rodent brain is highest in the zones of spontaneous neurogenesis, i. e. hippocampus, subventricular zone, and olfactory bulb [37]. A prerequisite to growth factor responsiveness is the expression of specific membrane-spanning receptors, which are able to initiate intracellular signaling cascades.

(ii) The reuse of developmental signaling cascades in adult progenitors. Nearly 20 such pathways have been identified in neural stem cells, including Shh, Pax, Hox, Wnt, Notch/Delta, TGF- β , NFkappaB, or JAK/STAT [38]. Interestingly, the activation of these pathways in the adult may have different functions compared to the embryonic developmental pathways.

(iii) The interaction with molecules of the extracellular matrix. During differentiation and maturation, stem cells migrate, extend processes, attach to the extracellular matrix, and adhere to nearby microenvironmental surfaces. Therefore, they require the expression of motor proteins, lytic enzymes, and enzymes for providing metabolic energy. Several extracellular matrix proteins mediating cell-cell interaction have been identified in neural stem cells, such as integrins and cadherins.

(iv) The expression of genes regulating transcription and translation. Although no specific proteins in the categories of transcription factors, DNA or RNA binding proteins, and chromatin remodeling enzymes can be named, stem cells need these molecules to change the cellular phenotype from its undifferentiated form to the new functional requirements of a differentiated stem cell.

(v) Mechanisms for controlling cell number. Mainly three processes regulate stem numbers:

Mitosis (proliferation), differentiation, and apoptosis (programmed cell death). An essential process is also asymmetric cell division, which regulates the stem cell pool. In this context, typical proteins regulating cell number are caspases and cyclins.

(vi) The protection against cellular stress, which involves metabolic deprivation, NO and O₂ toxicity, DNA damage, mechanical distortion, hypo- and hyperthermia, or hypoxia. Proteins in this category comprehend molecular chaperones, NO detoxifying enzymes, and components of the proteasome.

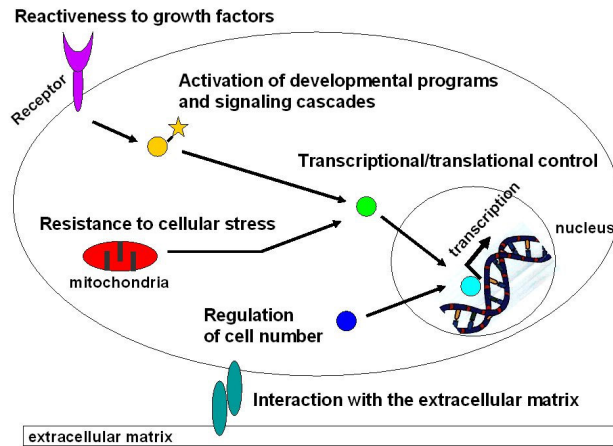


Figure 3. Novel definition of adult neural stem cells by combining molecular marker expression and functional aspects (Modified from [36]).

Of note, although adult neural stem cells express these molecules, different proteins can be found in the cells. This heterogeneity in the protein expression pattern is supported by protein expression studies comparing neural stem and progenitor cells from different brain regions [30, 39, 40].

4. Conclusions

Although it is not possible to identify a single molecular marker expressed by all types of neural stem cells at any given time in development and adulthood, nor a specific set of expressed markers fulfilling these requirements, it is possible to define neural stem and progenitor cells by common functional properties. A major advance in the field was the introduction of functional genomic / proteomic approaches, mainly based on two-dimensional gel electrophoresis for protein separation and mass spectrometry for protein identification.

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