Review

Stem/progenitor cells in the cerebral cortex of the human preterm: a resource for an endogenous regenerative neuronal medicine?

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Stem cells: present and future

Guest Editors: Gavino Faa, Vassilios Fanos, Antonio Giordano

Abstract

The development of the central nervous system represents a very delicate period of embryogenesis. Premature interruption of neurogenesis in human preterm newborns can lead to motor deficits, including cerebral palsy, and significant cognitive, behavioral or sensory deficits in childhood. Preterm infants also have a higher risk of developing neurodegenerative diseases later in life. In the last decade, great importance has been given to stem/progenitor cells and their possible role in the development and treatment of several neurological disorders. Several studies, mainly carried out on experimental models, evidenced that immunohistochemistry may allow the identification of different neural and glial precursors inside the developing cerebral cortex. However, only a few studies have been performed on markers of human stem cells in the embryonic period.

This review aims at illustrating the importance of stem/progenitor cells in cerebral cortex during pre- and post-natal life. Defining the immunohistochemical markers of stem/progenitor cells in the human cerebral cortex during development may be important to develop an "endogenous" target therapy in the perinatal period.

Keywords

Cerebral cortex, human preterm, stem cells, neurogenesis, regenerative medicine, immuno-histochemical markers.

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Introduction

The complex organization of the human cerebral cortex distinguishes humans from other species. The cerebral cortex plays a key role in cognitive functions, intelligence language and consciousness, motor abilities, memory and sensory perceptions. It is subdivided into four regions or lobes, which cover both hemispheres: the *frontal lobe*, containing dopamine-sensitive neurons associated with reward, attention, short-term memory tasks, planning, and motivation; the *parietal lobe*, involved in integrating sensory information from the various senses; the *temporal lobe*, involved in visual memories and language comprehension; and the *occipital lobe*, involved with the sense of sight.

Discovery of adult neural stem cells and neurogenesis

Neurogenesis occurs in two main neurogenic areas of the adult human brain: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus [1].

The first reports of cell division and differentiation in the adult brain emerged from studies on brain development in experimental animals by Leblond and colleagues in the early 1960s. Through incorporation of tritiated thymidine into DNA by dividing cells, they observed that glial cells were dividing throughout the parenchyma [2]. They found dividing cells in the SVZ but in the absence of neurogenesis. In the mid-1960s, Altman et al. [3] first showed the rostral migratory stream (located between the SVZ and olfactory bulbs) of cells that were born postnatally in the SVZ and matured into neurons in the olfactory bulb [4]. They also observed dividing cells in the SGZ and established the evidence for neurogenesis in the DG of rat adult brain [5].

Following studies, based on the application of transmission electron microscopy (TEM) combined with tritiated thymidine staining, confirmed the existence of adult neural stem/progenitor cells in the DG and the olfactory bulb [6].

The application of stereological techniques for labeling dividing cells with bromodeoxyuridine (BrdU) and neurons (NeuN), combined with the application of confocal microscopy, eventually demonstrated that the dividing cells in the DG indeed became neurons [7, 8]. In the late 1990s, Erickson studied human brain tissue postmortem and demonstrated that the human hippocampus retains its ability to generate neurons throughout life [1]. Using immunofluorescent labeling for BrdU and for neuronal markers including NeuN, calbindin and neuron specific enolase (NSE), he demonstrated that new neurons are generated from dividing progenitor cells in the DG of human adult.

Neurogenesis has been demonstrated through carbon-14 dating techniques in the human hippocampus of adult subject. Neurons are generated throughout adulthood and the rates are comparable in middle-aged humans and mice, suggesting that adult hippocampal neurogenesis may contribute to human brain function. [9].

The functional significance of these new neurons is uncertain. Several studies showed that hippocampal adult neurogenesis is important for some forms of learning and memory, and for related mechanisms of neural plasticity such as long-term potentiation (LTP) [10]. In recent years, adult neurogenesis has been suggested to play a critical role in brain homeostasis and disease. Deficient neurogenesis has been implicated in

the pathogenesis of multiple neurological and psychiatric disease [11], including depression, epilepsy [12] and Alzheimer's disease [13]. Epigenetic factors acting during gestation have been proposed, in recent years, as a possible cause of neurogenesis disarrangement and of susceptibility to develop neurodegenerative disorders later in life, including Alzheimer's disease and Parkinson's disease [14].

Stem/progenitor cells and neurogenesis in the cerebral cortex during pre- and post-natal life

According to the definition given by the National Institute of Health (NIH, 2001), stem cells have three main characteristics that distinguish them from other cells types:

1. they are capable of self-maintenance for long periods through the process of cell division;
2. they are not specialized cells;
3. physiological or experimental conditions can be induced to differentiate these cells toward specific lineages with special functions.

The mature human central nervous system (CNS) is composed of two major differentiated cell types: neurons and glial cells. Neurons transmit information through action potentials and neurotransmitters to other neurons, muscle cells or gland cells. Glial cells play important roles of their own in the CNS in addition to providing a critical support role for optimal neuronal functioning and survival.

The symmetric division of neural stem cells (NSCs) underlies their ability to self-renew and serves to expand the NSC pools. In the later stage of neural development, NSCs switch to asymmetric division that produces one NSC and one neural progenitor cell (NPC): apical/basal intermediate progenitor cell or apical/basal radial glial cell, daughter cells with a limited capacity of self-renewal (**Fig. 1**). Radial glial cells give rise to intermediate progenitor, a basal progenitor cell or a newborn neuron. The apical radial glial cells maintain contact with the ventricular and the pial surface. Apical intermediate progenitors maintain contact only with the ventricular surface while both types of basal progenitor cells are not in contact with the ventricular surface. Radial glial cells and the intermediate progenitor cells represent the primary source of cortical neurons
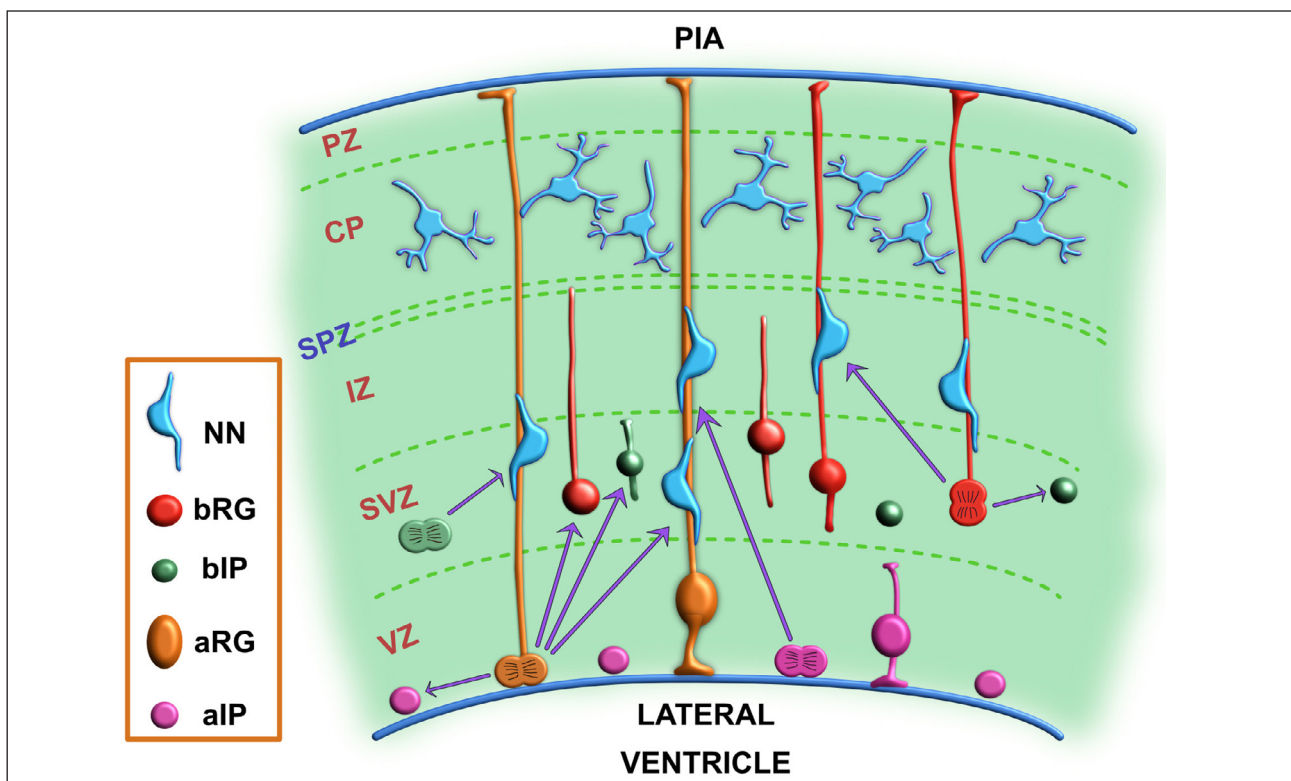

Figure 1. Schematic representation of stem/progenitor cells in human cerebral cortex niche in the early stage of gestation: ventricular zone (VZ), subventricular zone (SVZ), intermediate zone (IZ), subplate zone (SPZ), cortical plate (CP), pial zone (PZ); apical radial glia (aRG), apical intermediate progenitor (aIP), basal radial glia (bRG), basal intermediate progenitor (bIP), newborn neuron (NN).

(**Fig. 1**). The differentiation of NSCs is regulated by internal signals that are controlled by genes that carry information for all the cell structures. Moreover, NSC differentiation is under control of external epigenetic signals, including hormones and molecules secreted by other cells. Extrinsic factors believed to be essential for NSCs pool maintenance and proliferation include Fibroblast Growth Factor (FGF) [15], Epidermal Growth Factor (EGF) [16], Sonic Hedgehog (SH) [17], and Wnt family [18].

Microglia are considered macrophages of the brain and spinal cord and represent the first and principal active immune defense in the CNS. Microglial cells derive from the mesoderm as opposed to the neuroectoderm from which other CNS cells are derived [19]. During development, microglial cells become distributed throughout the gray and white matter [20]. Microglial cells derive from two main sources: early during development from the yolk sac and later on from myeloid precursors that subsequently take up residence in the CNS, forming a stable self-renewing population that persists through adulthood [21, 22].

The developing cerebral cortex may be subdivided into different regions: the ventricular neuroepithelium, the SVZ, the intermediate zone, the subplate zone, the cortical plate, the pial zone (**Fig. 2A**). The ventricular neuroepithelium and the SVZ form an active proliferate zone, the site of origin of cortical neurons [23]. Therefore they are considered the stem/progenitor cell niche in the developing cerebral cortex (**Fig. 2B**).

Neurogenesis and gliogenesis are sequential processes that involve cell proliferation, migration and differentiation, all events that occur simultaneously, originating from proliferating neuroepithelial cells. Neurogenesis is more prominent in early gestation and gliogenesis in late gestation [24].

At gestational week 6, the humans cerebral cortex is formed by two layers: a thick proliferative ventricular zone and a narrow, cell-sparse marginal zone. By gestational week 7, an additional proliferative zone, the SVZ, begins to form between these two layers. Cortical neurons proliferate in the ventricular zone and the SVZ with a rapid phase from gestational week 10 and with a slower phase from gestational
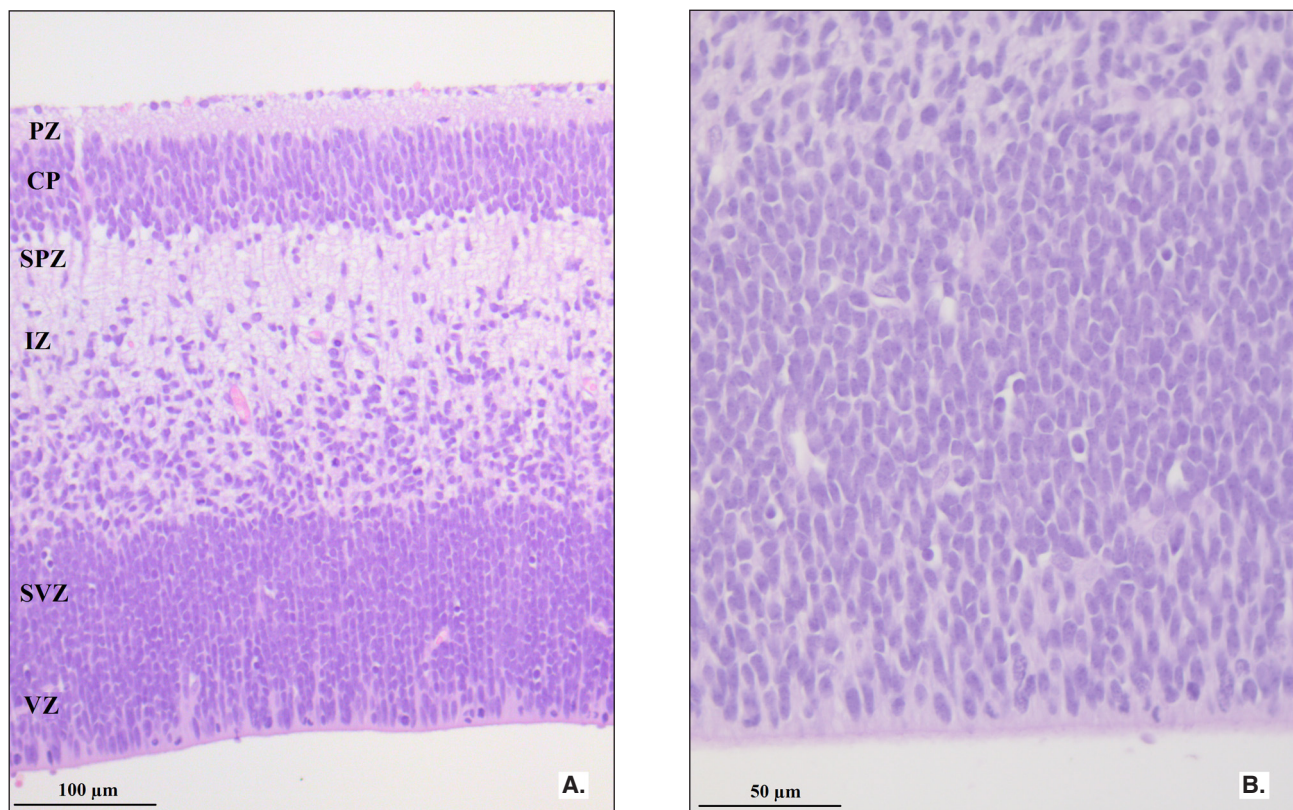

Figure 2. A. Different regions of human cerebral cortex at gestational week 12: the ventricular zone (VZ), the subventricular zone (SVZ), the intermediate zone (IZ), the subplate zone (SPZ), the cortical plate (CP) and the pial zone (PZ). **B.** Higher magnification of stem/progenitor cells niche of VZ and SVZ.

week 22. Three cell types can be identified: 1) round "globular" cells with end-feet attached to the ventricular lumen (NSCs and NPCs); 2) oval "fibrous" cells with end-feet at the ventricular surface and a thin, radial fiber reaching the pial surface (radial glia); and 3) "detached" cells with their processes reaching the pial surface (NPCs) [25].

Radial glia are bipolar-shaped cells that play a key role during CNS development, being capable of generating neuronal and glial cells. Moreover, radial glial cells guide the radial migration of neurons from the ventricular zone toward the pial zone [26, 27].

During the second half of gestation and early postnatal life, the SVZ is the proliferative zone mainly producing glial cells [24].

In the postnatal life, the pial zone becomes sparsely cellular and the cortical layers are well formed.

The subplate zone disappears by the first postnatal month whereas subplate neurons persist in adult brain white matter as interstitial neurons. Germinal matrices with proliferating neuroglial precursors (glioepithelia) may persist in the SVZ around the lateral ventricles even throughout adult life [28].

Immunohistochemical markers of stem/progenitor cells during human cerebral cortex development

Several studies, mainly carried out on experimental models, evidenced that immunohistochemistry may allow the identification of different neural and glial precursors inside the developing cerebral cortex.

Nestin. One of the earliest markers expressed in the primitive neuroephitelium is nestin. Nestin is an intermediate filament protein present in developing astrocytes and developing neurons of mouse cerebral cortex [29] and radial glial cells in animal models [30]. Nestin is also expressed in radial glial fibers during early stages of human cerebral cortex development [31]. In later stages of development, nestin is replaced by cell type-specific intermediate filaments such as neurofilaments (NF) in neurons and glial fibrillary acidic protein (GFAP) in glial cells [32].

Sox2 (Sry-related high mobility group box2) is a member of the extended Sox family [33, 34]. It is one of the earliest transcription factors expressed in the developing CNS [35]. In the developing mouse neocortex, Sox2 expression is restricted to the proliferating cell populations including neural stem and progenitor cells, glial precursors and proliferating astrocytes [34]. Sox2 expression was also detected in precursor cells of the ventricular and subventricular neuroepithelium of the developing human cortex and the migrating neurons [31] (**Fig. 3**).

Vimentin is another protein expressed in neural stem/progenitor cells. It is a type III intermediate filament protein and acts as a crucial cytoskeletal component of mesenchymal cells. In the developing cerebral cortex, vimentin identifies multiple cell types, including primitive neuroepithelial cells such as radial glial cells [36, 31] and astrocytes.

Neuron-specific enolase (NSE) is a glycolytic isoenzyme which is located in central and peripheral neurons and neuroendocrine cells [37] and in developing human cerebral cortex [31].

S100B calcium binding protein (S100B) is a protein of the S100 protein family. It is glial-specific and it is expressed primarily by astrocytes. In mouse developing cerebral cortex S100B protein has been shown to be also expressed in radial glial cells [38].

Wilm's tumor 1 (WT1). Recently a study of our group has evidenced another important marker of radial glial cells: WT1 [39] (**Fig. 4**). WT1 is a transcription factor highly expressed in several human organs during embryogenesis. Previuos studies showed a potential role of WT1 protein in the development of the mouse nervous tissues, including retina, retinal ganglia and the olfactory system [40, 41]. WT1 immunostaining was also detected in the sympathetic system and in the gastroenteric nervous system of human fetuses [42].

Pax6. The transcription factor Pax6 is specifically localized in radial glial cells of the cortex [43]. Recent studies showed that β-catenin/Pax6 signaling plays critical roles in self-renewal and neurogenesis by radial glia during mouse neocortical development [44].

Our studies show the ability of the different immunohistochemical markers to evidence
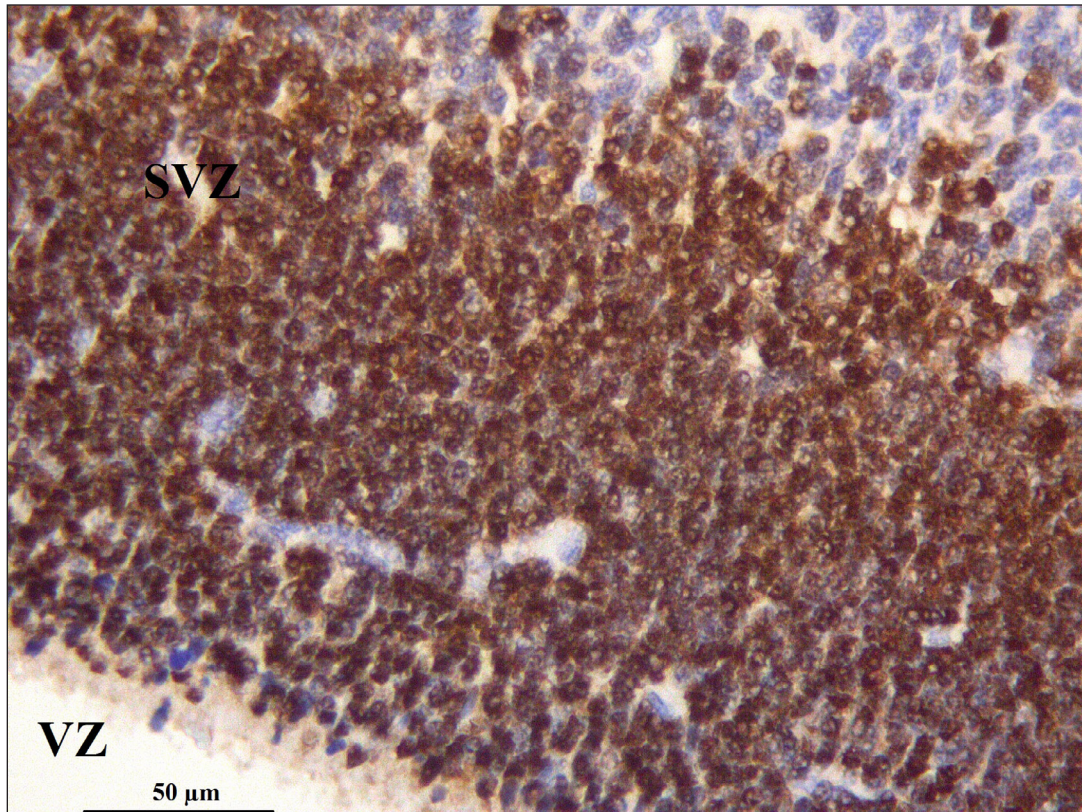
Figure 3. Human cerebral cortex at 11 week of gestation. Sox2 nuclear reactivity of migrating precursor cells localized in the ventricular zone (VZ) and in the subventricular zone (SVZ).
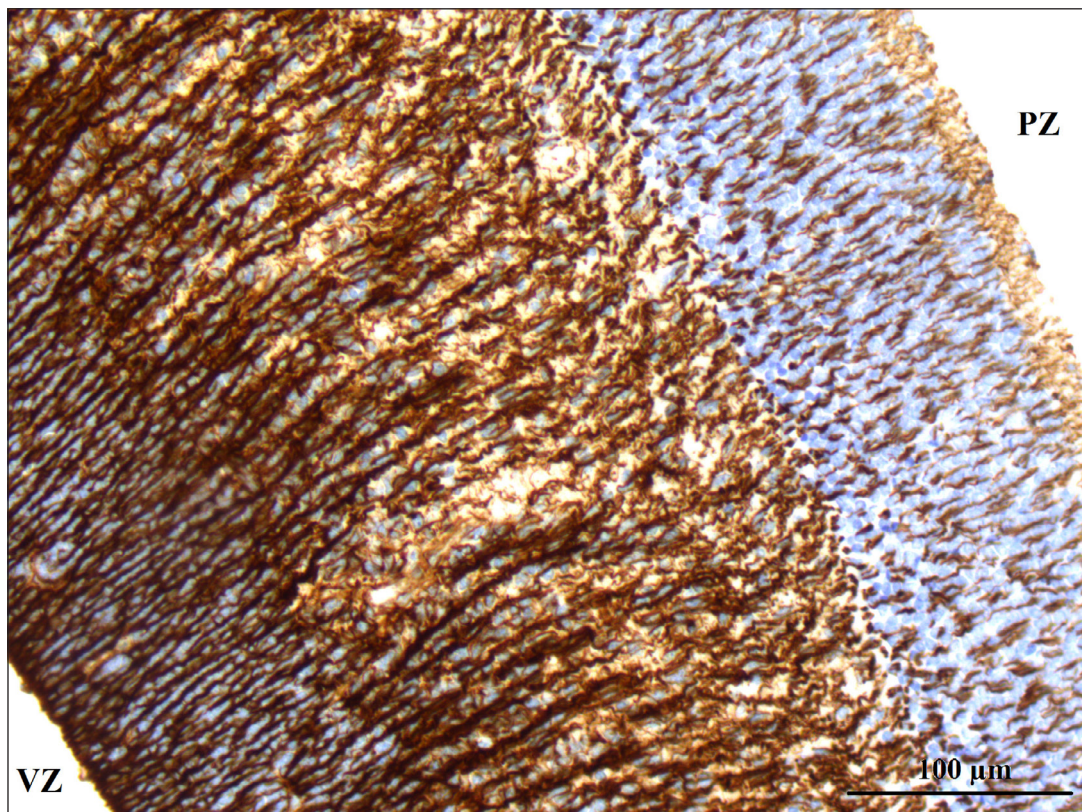

Figure 4. Human cerebral cortex at 11 week of gestation. Immunoreactivity for WT1 in radial glial cells fibers extending from the ventricular zone (VZ) toward the pial zone (PZ).

different zone of the developing human cerebral cortex, allowing the identification of the multiple stages of differentiation of neuronal and glial cells.

The role of perinatal regenerative medicine in prevention of neurodegenerative diseases

Over the past 15 years, the study of stem cells and neurogenesis has been the focus of many research groups, due to the possible role in the development and treatment of several neurological disorders, including epilepsy, Parkinson's disease, and Alzheimer's disease [14, 45, 46].

As previously stated, the development of the CNS represents a very delicate moment of embryogenesis. In the cerebral cortex, neuro-genesis continues until the end of pregnancy. Premature interruption of neurogenesis can lead to motor deficits, including cerebral palsy (CP), and significant cognitive, behavioral or sensory deficits in childhood.

Premature birth is one of the most important public health issues internationally. In fact, every year about 13 million infants are born preterm worldwide. Premature infants frequently develop complications, including intraventricular hemorrhage, hypoxia, ischemia, and sepsis, which reduce cortical growth and development [47, 48]. Children who have had hypoxic-ischemic brain injuries may present alterations of brain development such as mental retardation, learning disabilities, and hearing and visual impairments. [49].

Neurogenesis in the ventricular zone and SVZ of the cerebral cortex would continue in the third trimester of pregnancy and in the first postnatal period recently. It has been hypothesized that preterm birth might suppress neurogenesis. [50]. According with this hypothesis, preterm infants should be considered at higher risk for developing neurodegenerative diseases later in life [14]. Multiple neonatal disorders may impact neurogenesis, causing an imbalance between excitatory glutamatergic and inhibitory GABAergic neurons. The imbalance of excitation and inhibition within neural microcircuitry is associated with epilepsy, autism, neurodevelopmental disorders, and psychiatric illnesses in childhood: all these disorders have been reported to be more common in preterm than in term infants [51, 52].

All these data taken together, we may speculate that prolonging the neurogenesis after birth would allow the development of new neurons transforming a subject susceptible to develop neurological disorders later in life into a resistant child. In fact, whereas *in uterus* neurogenesis occurs in a protected environment, in children and in adults new developing neurons may be vulnerable being exposed to a high number of risk factors, such as toxins and infectious agents [53]. Stem cells secrete factors that stimulate and maintain neurogenesis, thus increasing cell proliferation, neuronal differentiation, and functional integration. As a consequence, stem cell therapy is a promising therapy and it has proven effective in promoting functional recovery in animal models of neonatal hypoxia-ischemia [54]. Understanding the molecular and cellular mechanisms underlying neurogenesis after an insult is crucial for developing tools to enhance the neurogenic capacity of the brain [55].

The classical approach of regenerative medicine is stem cell transplantation. We propose a new approach: "physiological" [56] and "endogenous" [57] regenerative medicine based on stem cells physiologically present at birth in premature infants.

We think that identifying the immunohisto-chemical markers expressed by NSCs and NPCs that play a key role in the development of the cerebral cortex may be important to develop a target therapy in the perinatal period. This type of approach would stimulate endogenous stem cells and prolong the neurogenesis after birth. Thus, individuals susceptible to various neurodegenerative diseases in childhood and in adulthood might become resistant to neuro-degeneration, thanks to their higher burden of glial and neuronal cells.

Declaration of interest

The Authors declare that no conflict of interest exist.

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