PERSPECTIVE

Cerebrospinal fluid and neural stem cell niche control

Neurogenesis from inner brain neural stem cells (NSCs) is a process which takes place continuously in mammals throughout their life. However, the main ontogenic difference is the intensity of neurogenesis, which commences as a very intensive and global activity in the early embryonic brain (neural tube), persists in fetal and newborn stages, and declines significantly in adulthood, becoming restricted to specific places with low neurogenic activity such as the subventricular zone (SVZ) and the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus.

According to Lim and Alvarez-Buylla (2014), NSCs have their origin in modified astrocites (activated B cells in **Figure 1**); these generate the transit-amplifying cells (C cells in **Figure 1**), which finally become migrating neuroblasts (A cells in **Figure 1**). NSCs have two basic behavioural characteristics. Firstly, they are able to self-renew so as to maintain a constant cell population or, in some cases, to expand it; this involves a mitotic ability specific for these cells in the central nervous system. The other basic characteristic is the potential to differentiate into glia (gliogenesis) or neurons (neurogenesis) in order to create a nervous system (in the embryo) or to replace neurons in specific places of the adult brain (the olfactory bulb from the SVZ or the hippocampus from the SGZ).

NSCs operate inside a complex structure called the "cellular niche", which evolves from the embryo (neural tube) to the adult brain (SVZ and SGZ of the DG). The main properties of this niche are conditioned not only by the presence of NSCs and other types of nervous tissue cells, including the ependymal cells, which recently have been proposed as key regulatory elements in SVZ neurogenic activity *via* Notch and pigment epithelium-derived factor (PEDF) signalling (Bátiz et al., 2015), but also by the complex surroundings of diffusible signals which influence NSC behaviour (Lim and Alvarez-Buylla, 2014). Another critical component in the NSCs niche structure is the extracellular matrix, which provides the niche not only with mechanical cues but also regulates the extracellular signalling microenvironment; it is able to exert specific regulative influences on NSCs and also control the intercellular signalling traffic and bioavailability by their complex glycoprotein and proteoglycan composition (Faissner and Reinhard, 2015).

A study of the cellular niche in the central nervous system reveals the existence of specific ontogenic differences in location and structural organization. In early brain development, when neurogenesis is a dramatic process, it is a global process affecting the entire brain anlage wall, which is organized as a Neuroepithelial pseudostratified epithelium with peripheral newborn neurons. However, in the adult brain NSCs are focally restricted to specific locations including SVZ or SGZ: a self-renewing mitotic place, a migratory pathway (rostral migratory stream or RMS in SVZ), and a final destination where neurogenesis concludes (the olfactory bulb in SVZ and the granular layer of the DG in the hippocampus).

From several studies published in the last few years (Lehtinen et al., 2011), a common factor can be observed in every NSC niche: a direct contact of NSCs with the contents of the brain ventricular system, namely, cerebro-spinal fluid (CSF). Neuroepithelial cells (which are the primary neural stem cells at the beginning of brain development) are all in direct contact with the embryonic CSF (E-CSF) produced by them (before choroid plexus development), acting as an inner medium for cell communication. During later development, the radial glia (derived from primitive neuroepithelial cells), maintain contact with the ventricular cavity whilst constituting a source and migratory guidance for new neurons. Finally, the adult brain neural stem cells (NSCs originating in the radial glia) located in the SVZ and SGZ of the DG, both close to the lateral ventricle, seem to maintain contact with the ventricular cavity. In fact, in the SVZ niche the existence has been shown of a single cytoplasmic prolongation for each undifferentiated precursor, which comes into contact with the ventricular cavity through the ependymal cell layer and extends single cilia inside the cavity, which is considered an aerial to recover signals from the CSF. The hippocampal niche still seems to be not fully understood, and is described in the adult mammal brain as a niche restricted to the SGZ of the DG, unrelated to the ventricular system. However, there are recent studies which show the involvement in this hippocampal niche of a periventricular zone (termed the subependimal zone) in direct contact with the CSF, where self-renewing NSCs migrate under CA3 (The region of the hippocampal neuronal layer close to the DG) to the DG and become part of the subgranular zone (Clarke and Van Der Kooy, 2011).

Direct contact between NSCs and E-CSF is necessary for normal survival, replication and neural differentiation behaviour of NSCs during development, and is considered a major source of diffusible signals in NSC niche regulation (Gato et al., 2005; Gato and Desmond, 2009). However, these inductive properties change dramatically in the adult, where CSF has been described as NSC migratory guidance, remaining mitogenic but having lost most of its neurogenic inductive properties. This has led some researchers to relate ontogenic changes in E-CSF and CSF composition with a dramatic reduction in the neurogenic potential of the adult brain.

Consequently, there are currently two specific questions which should be addressed: (1) what is E-CSF composition, especially during early development? (2) How is E-CSF able to influence NSC niche behaviour?

The answer to the first question began to appear in the last century, with a description of the involvement of E-CSF in brain growth and morphogenesis at the earliest stages of brain development, by means of hydrostatic pressure generated inside the brain cavity. The morphogenetic mechanisms involved are only partially understood, and they have been associated with the apical secretion of osmotically active molecules by neuroepithelial cells, together with the role of ion pumps and aquaporins (Gato and Desmond, 2009). However, this field requires further research in order to provide us with new perspectives in brain ventricle expansion diseases such as certain types of hydrocephaly or the arnold-chiari malformation.

Furthermore, several studies on the protein composition of E-CSF, and CSF at new-born stages showed a really complex fluid which changes abruptly after appearing coincidentally with the loss of neurogenic properties (Zappaterra and Lehtinen, 2012). Several molecules able to regulate cellular behaviour have been detected in the E-CSF, and these are able to justify, at least in part, their regulatory properties. These include growth factors, apolipoproteins, extracellular matrix molecules, morphogens and transcriptional factors. We can, then, consider E-CSF, especially from the embryo and early fetal stages, as a cocktail of molecules able to transmit inductive signals between different periventricular cell populations, including the microenvironment of brain cellular niches. The increase in proteomic technique sensitivity together with the development of protein detection microarrays will in the near future allow us to make a complete analysis of this fluid, which plays a key role in neurogenesis.

Studies published so far have identified several E-CSF growth factors with mitogenic properties, such as EGF, FGF2 or IGF1 or neuronal inductors like Retinoic Acid or their regulatory proteins as is the retinol binding protein (Martín et al., 2006; Alonso et al., 2011; Zappaterra and Lehtinen, 2012) Recently,

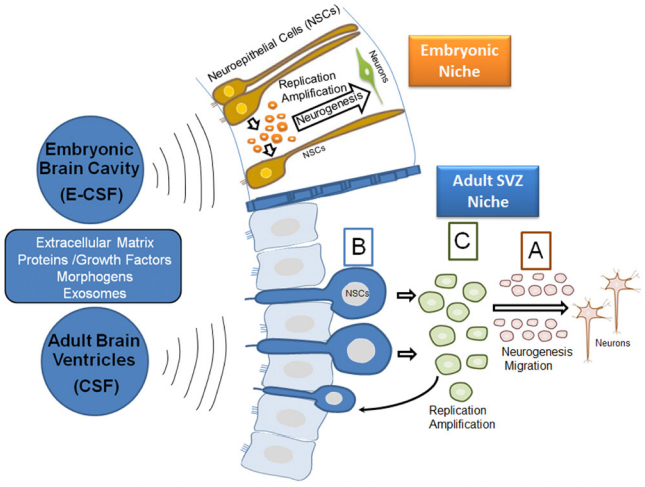

Figure 1 Diagram showing embrionic and adult subventricular zone (SVZ) niche.

several research studies have focused on another kind of CSF component (also present in other brain structures) termed "exosomas". These are a specific type of secreted extracellular vesicles which include in their molecular structure micro RNAs, proteins and signalling peptides, and are considered relevant intercellular messengers traveling through CSF and directly involved in neurogenesis (Bátiz et al., 2015). Given the complexity of the biological role of E-CSF, an in-depth study of its active components and specific functions represents an area of research which will give a valuable insight into brain development and regeneration. Of special interest is the study of specific differences between embryonic and adult CSF, given the ontogenic changes in the inductive properties found there, and which represent the key to activating an intense and complete neurogenic program in adult brain NSCs (Zappaterra and Lehtinen, 2012).

There has recently appeared another interesting hypothesis regarding use of the neurogenic potential of E-CSF in the activation of NSCs in the mammalian adult brain. NSCs emerge from a unique cellular lineage which evolves ontogenically; all originate from the neuroepithelial cells of the neural tube, and in later development neuroepithelial cells remain as radial glia, which finally persist in the adult brain as modified astrocytes known as Neural Precursors. E-CSF is a carrier for generic instructive cues able to increase NSC activity at any time, including adult neural precursors. Some research (Lehtinen et al., 2011; Alonso et al., 2017), has recently shown that there exists in the SVZ a reservoir of neurogenic potential which can be activated by E-CSF, inducing a complete neurogenesis process which concludes with the maturing of the new neurons. This is another interesting research line which must be explored in the future in order to ascertain how E-CSF is able to activate neurogenesis in the hippocampal niche. Similarly, it is necessary to examine whether the neurogenic process induced by E-CSF concludes in live animals with functional changes in their behaviour. These kinds of studies will be the basis for experimental neuroregeneration on animals with a neurodegenerative process or acute injuries in adult mammal brain.

This line of research seeks the developing of neuroregenerative tools to activate endogenous neurogenesis which, however, must be useful in humans. To date there are no studies of E-CSF in humans, given the existence of technical and ethical limitations. Nevertheless, knowledge of E-CSF mechanisms involved in NSC neurogenesis in mammal animal models will prove crucial in order to develop an artificial CSF able to reproduce its neurogenic potential, opening up the possibility of improving human neuroregeneration (Kaneko and Kako, 2011).

Fortunately, in the last decades the number of research teams working on CSF properties has increased, with the corresponding possibility of fresh expectations and research which seeks to explore the precise utility of E-CSF in brain regenerative therapy applied to neurodegenerative and brain vascular disorders (**Figure 1**).

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