Chapter 2

Neural Stem Cell Heterogeneity

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1. Introduction

The concept of neurogenic neural stem cells in the brains of adult mammals including humans is now widely accepted. In rodents these cells have been studied extensively both in vitro and in vivo. Of the two primary neurogenic regions in the rodent brain, the subventricular zone of the lateral ventricle wall generates the most neurons of multiple phenotypes. The newly generated neurons in the subventricular zone migrate to the olfactory bulb replenishing neurons and reconstituting the local circuitry responsible for olfaction. The dentate gyrus of the hippocampus generates a single neuron type, glutamatergic granule cells. These newborn granule cells contribute to specific forms of memory by integrating into existent circuits (Shors et al., 2001; Clelland et al., 2009; Garthe et al., 2009). Over the last few years, what was once considered to be a homogeneous population of astrocytic stem cells in both neurogenic brain regions is now turning out to be a more complex mixture of cells. Heterogeneous populations of cells with stem cell properties are being discovered in both the subventricular zone and dentate gyrus. This heterogeneity combined with potential diversity in signals forming the local niches could provide a situation where these multiple neural stem cell subpopulations contribute of tissue homeostasis and regeneration.

2. Neurogenesis in the subventricular zone

The lateral walls of the forebrain ventricles contain stem cells that generate neuronal subpopulations of the olfactory bulb throughout life (Reynolds and Weiss, 1992; Morshead et al., 1994; Doetsch et al., 1999b; Gage, 2000; Mirzadeh et al., 2008). Although much remains to be learnt about the neurogenic process and the fate determinants controlling maintenance, proliferation and differentiation of stem and progenitors cells in the subventricular zone, morphological, immunological and lineage tracing has recently uncovered a striking hetero-

geneity in the putative stem cell pool. In the first sections of this chapter I will look at some of the key findings and experiments identifying the stem cells and following their fate. I will also ask the question of whether single neural stem cells are multipotent in vivo and look at some for the experimental data addressing this and also cover emerging experimental data showing heterogeneity within the stem cell pool.

3. The subventricular zone and its progenitors

Continued neurogenesis from cells within the subependymal layer of the lateral ventricle wall implies stem cells as a driving force and a regulatory niche. Ultrastructural electronmicroscopic analysis has been instrumental in defining the morphological differences among cells within the subependymal layer of the ventricle wall (Doetsch et al., 1997; Doetsch et al., 1999a; Mirzadeh et al., 2008). Combining electromicroscopy with functional regeneration of the neurogenic niche, astrocytes have been shown to be primary progenitors of the subventricular zone (Doetsch et al., 1999b; Doetsch et al., 1999a; Doetsch et al., 2002). The subventricular zone astrocytes are defined as B-cells. B-cells have a polarized morphology extending an apical process and sensory cilium that projects between the ependymal call (E-cells) lining the lateral ventricle. These B-cell projections organize the E-cells into characteristic pinwheel structure (Mirzadeh et al., 2008). This is likely to be an important structural and signaling center in the stem cell niche. Based on their ultrastructural characteristics and location the B-cell population can be divided into two. B1-cells have their cell body between the chains of neuroblasts (A-cells) and the ependymal lining. B1-cells are quiescent and, based on thymidine incorporation assays and electronmicroscopic analysis, they rarely divide. B2-cells are more displaced towards the parenchyma of the underlying striatum and unsheathe the migrating chains of neuroblasts on route to the olfactory bulb (Doetsch et al., 1997). Unlike the structurally related B1-cells, B2-cells divide more prevalently. C-cells are the committed progeny of the B-cells, likely generated by asymmetric cell division, and they are mitotically highly active but undergo a limited number of divisions before differentiating. The progeny of the transient amplifying C-cells, the A-cells, migrate in chains through tubes formed by B-cells to the olfactory bulb. In adulthood, interneurons of the granule cell layer are the major newborn neuron type in the olfactory bulb, and together with periglomerular neurons, reform local circuits. In addition to neurons of the olfactory bulb, oligodendrocytes are also continuously generated in the subventricular zone and migrate to the corpus callosum. These oligodendrocytes are the product of Olig2-positive transient amplifying cells (a second type of C-cell). The relationship between the neurogenic C-cells and those that generate oligodendrocytes is hotly debated, as is whether they are the products of the same multipotent neural stem cells in the subventricular zone.

4. Heterogeneity within the subventricular zone neural stem cell pool

The mechanisms controlling the fate of progenitors in the subventricular zone remain unclear. The niche and its local interactions, morphogens and growth factors are one potential mode by

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which the differentiation potential of the neural stem cells is controlled (Basak and Taylor, 2009). Assuming that all stem cells with the subventricular zone have the same potential, local differences within the niche or signals interpreted by committed progenitors *en route* to their final destination would be responsible for determining the multiple neuronal fates. The ectopic grafting of stem cells into the subventricular zone indicates some degree of plasticity within the neural stem cell population and suggest niche specific signals as fate determinants (Suhonen et al., 1996). However, even with the same niche, some neural stem cells seem to have autonomous fates and be heterogeneous in their potential (Kohwi et al., 2007; Merkle et al., 2007). By using homochronic/heterochronic transplantation experiments it has been shown that progenitor cells at different ontogenetic stages are intrinsically directed toward specific lineages (De Marchis et al., 2007). In addition, neuroblasts in the rostral migratory stream are also heterogeneous and may be committed to specific neuronal fates even before reaching the olfactory bulb (Hack et al., 2005; Kohwi et al., 2005). Thus, rather than being universally plastic, the neural stem cell pool may be made up of many stem cells with restricted potentials. This is also supported by region specific, viral-mediated genetic labeling of the subventricular neural stem cells in juvenile mice which show diversity in neuronal progeny generated rather than generating all neuron types (Merkle et al., 2007). Granule cells, the major neuron subtypes to be generated during adulthood, are produced from all anteroposterior and dorsoventral locations in the subventricular zone. However, most granule cells are generated from the dorsal and ventral most aspects of the subventricular zone (Merkle et al., 2007). Within this regionalization, the granule neurons generated from the dorsal subventricular zone migrate to a more superficial location in the granule cell layer of the olfactory bulb while those generated ventrally settle deeper in the granule cell layer (Merkle et al., 2007). This regional specification can also be mapped to the location of the stem cells during early postnatal development indicating not only a regional but also a developmentally-regulated fate specification (Merkle et al., 2007). Similarly, periglomerular neurons that migrate to the outer layer of the olfactory bulb also show a region-specific origin. Dorsal regions of the subventricular zone generate the majority of the thymidine hydroxylase-positive neurons whereas Calbindin-positive periglomerular neurons are generated preferentially from the ventral subventricular zone (Merkle et al., 2007). Calretinin-positive periglomerular and granule cells are generated from the medial wall of the lateral ventricle. As this region produces proportionally fewer granule cells in total this suggests that the niche of the medial wall directs the fate of neural stem cells towards Calretinin neuron generation. Although these findings do not rule out niche specific programming of multipotent cell fate, heterotopic transplantation strongly suggests that stem cells retain their differential potential when grafted into a different axial location (Merkle et al., 2007).

5. Mitotically active or quiescent neural stem cells

For many years mitotic inactivity or quiescence has been viewed as a primary stem cell trait. However, recent data in many systems including the intestine and blood suggest that stem cell may not need to be quiescent and some can divide frequently to drive the generation of new cells (Wilson et al., 2008; Essers et al., 2009; Fuchs, 2009; Li and Clevers, 2010). These active

stem cells are the force behind tissue homeostasis and may reside side-by-side with quiescent stem cells that rarely if ever divide but that could be responsible for tissue regeneration. Ultrastructural cellular analysis of the subventricular zone implied that even within the B-cell compartment, B1 cells rarely if ever divide whereas B2 cells are detected in cell cycle (Doetsch et al., 1997). This raised the possibility that in the adult brain stem cells may also either be able to adopt different fates or, different neural stem cells exist which show strikingly different mitotic potential. More recently, mitotically active cells in the subventricular zone were show to be in close proximity to blood vessels suggesting a mitotic influence of the endothelium or blood-born factors (Shen et al., 2008; Tavazoie et al., 2008). This is particularly intriguing as endothelial cells express the Notch ligand Jagged1 and can active neural stem cells regulating maintenance and proliferation both in vitro and in vivo thus implying that activated neural stem cells my have a vascular contribution to their niche (Shen et al., 2004; Nyfeler et al., 2005).

In summary of current and past data, the heterogeneous mitotic activity among neural stem cells suggests at least two potential scenarios. Either individual cells are able to transit between a quiescent and an activated state, or, that there are different stem cells, some which are quiescent and rarely divide, and others that are more mitotically active, dividing frequently and driving the production of new neurons destined for the olfactory bulb. A similar situation of active and dormant stem cells is present in the crypts of the large intestine where previously identified slow or rarely dividing stem cells in the +4 position seem to be the cells responsible for regenerating the epithelial lining of the gut. Conversely, mitotically active cells that are interdigitated with paneth cells at the base of the crypt replenish the epithelial cells lining the villi (Li and Clevers, 2010).

6. Active and quiescent stem cells show differences in Notch signaling

Notch signaling regulates cell fate in many cell systems and across species (Artavanis-Tsakonas et al., 1999; Louvi and Artavanis-Tsakonas, 2006). Lateral signaling between neighboring cells presenting Notch ligands and expressing receptors classically results in binary fate decisions, often in cells undergoing cell division. Notch signaling is active in the subventricular zone and multiple ligands are present on B, C and E cells providing the potential for lateral signaling (Stump et al., 2002; Nyfeler et al., 2005; Imayoshi et al., 2010). Genetic ablation of Notch signaling in stem cells of the subventricular zone results in precocious differentiation and neurogenesis (Imayoshi et al., 2010; Basak et al., 2012). This in turn results in a loss of neural stem cells and a subsequent long-term suppression of neurogenesis. This is a "classical" role for Notch in the regulation of cell fate, whereby loss of Notch signaling during what should be an asymmetric neural stem cell division results in both daughter cells adopting a differentiated cell fate and a concomitant loss of stem cell self-renewal. However, the ablation of Notch from B-cells also results in quiescent B1-cells entering the cell cycle and the active neurogenic pool. This activation of cells that are normally in a mitotically inactive state contributes to a pulse of increased neuroblast production before extinction of the stem cells pool following inactivation of canonical Notch signaling (Imayoshi et al., 2010; Basak et al., 2012). Hence, Notch signaling through its canonical pathway not only regulates stem cell

maintenance in the subventricular zone by repressing neuronal commitment of the stem cell but also suppresses mitotic activity of B1 cells. In addition, canonical Notch signaling is implicated in repressing the mitotic activity in ependymal cells lining the lateral ventricle during ischemic lesions (Carlen et al., 2009). Although the role of ependymal cells as stem cells is highly controversial, it remains possible that, under some degenerative/regenerative conditions, even these differentiated cells may be able to dedifferentiate or transdifferentiate to generate neuroblasts. How this regulation of proliferation function is controlled by Notch is unclear. However, analysis of Notch1 function in the subventricular zone suggests differential receptor usage by neural stem cell in different mitotic states. The Notch gene family contains four genes encoding highly related receptors. These receptors are able to bind all five canonical ligands. At least three Notchs, Notch1, Notch2 and Notch3 are expressed in the subventriuclar zone (Stump et al., 2002; Basak et al., 2012). Notch1, Notch2 and Notch3 are expressed by B-cells whereas Notch1 is also expressed by C-cells, A-cells and E-cells (Nyfeler et al., 2005; Carlen et al., 2009; Imayoshi et al., 2010; Basak et al., 2012). Genetic conditional inactivation of Notch1 from B-cells induces a loss of self-renewal during homeostatic neurogenesis. Notch1-deficient active stem cells fail to self-renew and spontaneously differentiate – similar to ablation of the canonical DNA-binding component of the pathway RBP-J in these cells. However, unlike when RBP-J is deleted, Notch1-deficiency in B1-cells does not result in spontaneous mitotic activity (Basak et al., 2012). The regulation of cell proliferation by Notch signaling has also been implicated in vitro where cultured neural stem cells lacking Notch1 fail to self-renew and differentiate and in the adult zebrafish quiescent progenitors proliferate when treated with the gamma-secretase inhibitor DAPT, which blocks Notch (Nyfeler et al., 2005; Chapouton et al., 2010). Conversely, B1-cells, although they express Notch1, do not seem to depend upon it for a quiescence signal. Thus, it is likely that molecular compensation or signal diversity between the Notch receptors is responsible for the quiescence of B1-cells. This remains to be examined in detail.

It has been difficult to identify and study active neural stem cell in the adult mouse subventricular zone due to an absence of selective markers. Most transgenes used to label neurogenic stem cells utilize the *Nestin*, *GLAST* or *Hes5* promoters (Mori et al., 2006; Balordi and Fishell, 2007; Lagace et al., 2007; Giachino and Taylor, 2009; Imayoshi et al., 2010; Bonaguidi et al., 2011; Basak et al., 2012). These promoters are all expressed by both quiescent and mitotic stem cells. However, combinations of transgenic reporter and surface expression of the Prominin1-associated glycoepitope CD133 and binding of the mitogen epidermal growth factor is able to select active from quiescent stem cells, C-cells and neuroblasts (Pastrana et al., 2009). Conversely, Inhibitor of DNA binding protein 1 (Id1) a target of transforming growth factor-β signaling, is expressed predominantly by quiescent B1-cells. Transgenic mice expressing green fluorescent protein under the control of the Id1 promoter label quiescent B1-cells in the subventricular zone (Nam and Benezra, 2009). These Id1-positive GFAP-positive B1 cells are relatively rare and divide infrequently to generate neuroblasts likely by asymmetric cell division. Interestingly, mitotic activity of subventricular zone neural stem cells requires Id proteins with loss of function resulting in a loss of self-renewal and neurogenesis (Nam and Benezra, 2009). It remains to be shown whether and how the quiescent and active stem cells in the subventricular zone are related to each other or whether they fulfill distinct functions

for example homeostatic neurogenesis and regeneration. It is likely that the elucidation of the diverse neural stem cells in the subventricular zone is going to require the combination of different markers and genetic tools (Beckervordersandforth et al., 2010).

7. The hippocampus continually generates neurons which participate in memory formation

In contrast to the subventricular zone where proliferation and neurogenesis are eradicated soon after birth in humans, the dentate gyrus of the human hippocampus, like in rodents, continues to generate neurons from mitotically active progenitors cells all the way into adulthood. The cellular composition of the neurogenic niche in the dentate gyrus has been studied extensively (Seri et al., 2001; Kempermann et al., 2004; Steiner et al., 2006; Steiner et al., 2008). However, the identity and regulation of neural stem cells in the dentate gyrus remains unclear.

8. The progenitor pool in the dentate gyrus is morphologically and functionally heterogeneous

Self-renewing neural stem cells in the subgranular zone of the adult hippocampal dentate gyrus (also referred to as Type-1 cells) produce intermediate progenitor cells (IPs, Type-2a cells), NeuroD1 and Doublecortin-positive neuroblasts (Type-2b) and subsequently granule neurons (Seri et al., 2001; Kempermann et al., 2004; Steiner et al., 2006). Type-1 neural stem cells have their cell bodies in the subgranular zone and extend a long process through the granule cell layer to the overlaying molecular layer. Type-2 cells are transient intermediate progenitors. They also have their cell body in the subgranular zone but lack a long radial process and have a more rounded morphology with short stubby processes (Seri et al., 2001; Steiner et al., 2006). Neuroblasts by contrast extend a leading process and migrate into the granule cell layer. Whereas radial Type-1 cells are quiescent, Type-2 cells divide readily expanding the progenitor pool. Previous Bromodeoxyuridine labeling experiments suggested that Type-2a cells, which express proneural transcription factors, are the major proliferative progenitor in the adult dentate gyrus (Steiner et al., 2006). In addition, retroviral-labeling experiments showed that neuroblasts that have extended a radial process, exit cell cycle, and only go through one or two cell divisions (Seri et al., 2001). However, recent genetic labeling and lineage tracing of stem cells in the dentate gyrus revealed that Ascl1-positive Type-2a cells do not undergo symmetric cell divisions but generate an addition intermediate cell type, Tbr2-positive Type-2 cells (recently referred to as Type-2ab cells) (Bonaguidi et al., 2012; Lugert et al., 2012). The Tbr2-positive cells divide frequently to amplify the progenitor pool and increase the number of neurons generated from each stem cell division (Lugert et al., 2012).

9. Multiple stem cell populations in the dentate gyrus

The classical view of stem cells in the adult dentate gyrus implicates the quiescent radial glial like Type-1 cells as the primary progenitor. However, retroviral labeling is commonly used to examine neurogenesis in the dentate and to label cells that continue to generate multiple neurons over time (Seri et al., 2001; Suh et al., 2007). As retroviral integration and thus viral gene expression are dependent upon cells passing through the cell cycle, some long-term neurogenic stem cells in the dentate must be mitotically active. Radial Type-1 cells are rarely labeled in these retroviral experiments suggesting that other cells that lack a radial process must also display self-renewing and long-term neurogenic stem cell potential (Suh et al., 2007). This is also supported by lentiviral labeling experiments driving reporter expression from the *Sox2* promoter (Suh et al., 2007). Expression of the transcription factor Sox2 is associated with progenitor cells of the brain and required for their maintenance by regulating Notch, Sonic Hedgehog expression and Wnt activity (Steiner et al., 2008; Favaro et al., 2009; Kuwabara et al., 2009). A population of none radial stem cells with horizontally orientated processes has been identified by Cre-recombinase mediated lineage tracing (Suh et al., 2007). These horizontal cells display stem cell characteristics but are clearly distinct from the previously described Type-1 and Type-2 cells. Horizontal Type-1 neural stem cells like radial Type-1 stem cells in the dentate gyrus have active Notch signaling and are labeled with a Notch signal reporter allele *Hes5::GFP* (Ables et al., 2010; Ehm et al., 2010; Lugert et al., 2010). However, although they express Nestin they do not express the astrocytic protein GFAP. Hence, there remains some debate and despite their similarity in morphology to Type-2 cells, horizontal Type-1 stem cells do not express classic Type-2 cell markers including the proneural transcription factor Ascl1 – a Notch repressed target gene – Tbr2 or Doublecortin (Steiner et al., 2006; Lugert et al., 2010). In addition, horizontal Type-1 cells are more mitotically active than their radial counterparts (Lugert et al., 2010). Therefore, the horizontal *Hes5*-positive cells likely represent the Sox2-positive population of stem cells and those stem cells commonly traced and analyzed by retroviral labeling. Although the relationship between the radial and horizontal stem cells is not clear, horizontal cells rarely generate radial Type-1 cells in viral lineage tracing experiments. Interestingly, activated neurogenic stem cells in the dentate gyrus express Sox1, which, like Sox2 and Sox3, is a member of the SoxB1 family. *Sox1*, like *Hes5*, is expressed by radial and horizontal Type-1 cells (Venere et al., 2012). Lineage tracing shows that Sox1-positive Type-1 cells include the active neural stem cells and support that neurogenic stem cells in the dentate gyrus may switch between active and inactive states (Lugert et al., 2010; Venere et al., 2012).

10. Radial and horizontal hippocampal stem cells respond selectively to external cues

The classical view is that radial Type-1 stem cells divide infrequently to generate transient amplifying progenitors through asymmetric cell divisions. However, as described above there

are additional progenitors in the hippocampal dentate gyrus that can function as stem cells. Hence, the question arises what are the functions of these multiple putative neural stem cells? Do they both contribute to neurogenesis in the adult hippocampus and are they in a lineage relationship with each other? Genetic labeling experiments suggest that both radial and horizontal stem cells may be functionally distinct or at least they respond differently to different pathophysiological cues (Lugert et al., 2010).

Analysis of hippocampal neurogenesis has shown it to be a dynamic process that diminishes with age but can be stimulated and modulated by physiology and pathology (Kuhn et al., 1996; Kempermann et al., 1998; Ben Abdallah et al., 2008; Fabel and Kempermann, 2008; Parent and Murphy, 2008; Steiner et al., 2008; Zhao et al., 2008). Voluntary physical exercise induces increased proliferation and generation of immature neurons. These neurons do not readily integrate into the dentate gyrus but the increased proliferation of the stem cells is significant (Fabel and Kempermann, 2008). Notch signaling also controls neural stem cell maintenance and differentiation within the dentate gyrus (Breunig et al., 2007; Ables et al., 2010; Ehm et al., 2010; Lugert et al., 2010; Lugert et al., 2012). Loss of Notch activity results in the loss of neural stem cells and their precocious differentiation culminating in a loss of neuron production (Ables et al., 2010; Ehm et al., 2010; Lugert et al., 2010). Genetic labeling of neural stem cells in the dentate that display Notch signaling has uncovered diversity in stem cell responses to pathophysiology (Lugert et al., 2010). Physical exercise stimulates proliferation of the radial type1 cells but not the horizontal stem cells (Lugert et al., 2010). Running induces the radial cells to enter the active stem cell pool without expanding the total stem cell population. This suggests that radial cells in physically active animals undergo asymmetric cell divisions to generate committed progenitors that increase the number of newborn neurons whilst maintaining the Type-1 stem cell pool through self-renewal. This also implies that radial stem cells respond to stimuli generated by increased physical activity that are not seen or are not interpreted in the same way by the horizontal stem cells. These findings seem, at first glance, to contradict previous experiments where Nestin expressing progenitors were labeled and suggested that radial Type-1 cells do not proliferate significantly in running mice (Steiner et al., 2008). It is likely that the differences in result reflect the different experimental paradigms used to identify the stem cells of the dentate gyrus. Where as *Hes5* expression identifies a smaller population of cells more restricted to the stem cell pools in the subgranular cell layer, the *Nestin* promoter is expressed by stem cells and more committed progenitors (Bonaguidi et al., 2012). Hence, it remains possible that the different labeling techniques and the extent of cell labeling could effect the quantification and interpretation.

11. Selective loss of active stem cells in the hippocampus of aged mice

Neurogenesis in the mammalian brain diminishes dramatically after birth, even in the dentate gyrus where neurons are continuously generated throughout life. This reduced neurogenesis is associated with a loss of mitotic cells (Kuhn et al., 1996; Kempermann et al., 1998; Ben Abdallah et al., 2008; Steiner et al., 2008). Whereas some reports have suggested an irreversible loss of neural stem cells in the dentate gyrus due to exit from

the stem cell pool and differentiation into astrocytes (Encinas et al., 2011; Encinas and Sierra, 2012), others suggest that the stem cells are not lost but become dormant with age (Lugert et al., 2010; Bonaguidi et al., 2011; Venere et al., 2012). Hence, the reason for the substantial reduction in neuron production remains unclear but may be caused by a culmination of physiological changes.

Genetic lineage tracing of *Nestin* expressing cells revealed that, parallel to the reduced number of neurons generated from the labeled stem cells, radial Type-1 cells in the aged mouse brain enter cell cycle and, following a few cell divisions, differentiate into polymorphic astrocytes that lose radial morphology and presumably stem cell potential (Encinas et al., 2011). This "deforestation" or expenditure of the stem cells likely contributes to the reduction in mitotic progenitors and neurons (Encinas and Sierra, 2012). Surprisingly, in a parallel study using the same genetic tools, clonal analysis indicated that *Nestin* expressing stem cells within the subgranular layer can undergo prolonged neurogenesis. In addition, these clonal experiments revealed an additional degree of heterogeneity within the stem cell population of the dentate gyrus. Some labeled Type-1 cells remained quiescent over many months and failed to generate any viable offspring. Other Type-1 cells divided and generated clones of cells that included progenitors, neurons and astrocytes indicating multipotency (Bonaguidi et al., 2011). Partially supporting the proposal that some *Nestin*-expressing Type-1 cells may exit the stem cell pool, clones were found that contained only differentiated cells. Taken together these data indicate heterogeneity within the stem cells pools and it seems that a combination of entry of stem cells into a dormant state coupled with a partial loss of some progenitors may contribute to the age related decline in neurogenesis (Bonaguidi et al., 2011; Encinas et al., 2011).

In contrast, neural stem cells in the dentate gyrus labeled by Notch activity and Sox2 expression remain in the aged dentate gyrus (Lugert et al., 2010; Bonaguidi et al., 2011; Lugert et al., 2012). Interestingly however, the proportion of the cells that are mitotically active, which is predominantly the horizontal population, are lost. Hence, even in aged mice the number of stem cells remains relatively constant but their mitotic activity reduces and actively proliferating cells are lost, become quiescent, or dormant (Lugert et al., 2010; Bonaguidi et al., 2011; Lugert et al., 2012). This is similar to findings that *Sox1*–positive stem cells remain long-term neurogenic and can enter and exit the active stem cells pools (Venere et al., 2012).

A loss of stem cells in the dentate gyrus would suggest that the neurogenic process cannot be rescued or reversed in aged animals. However, physical exercise and pathological stimulation both stimulate proliferation, neural stem cell activation and under some conditions increased numbers of newly generated neurons (Rao et al., 2005; van Praag et al., 2005; Hattiangady et al., 2008; Jessberger and Gage, 2008; Rao et al., 2008; Zhao et al., 2008). Hence, although loss of stem cells could contribute to the age-related decline in neuron production, some cells with stem cell potential remain even in the dentate gyrus of old mice and these can be activated to proliferate and generate new cells (Lugert et al., 2010; Venere et al., 2012). It still remains unclear whether radial Type-1 cells in old mice enter the cell cycle during physical exercise or whether the few remaining horizontal cells could reactivate in the aged brain or whether a

distinct cell population, previously not studied or labeled with the tools and techniques current available, replenishes the neural stem cell pools.

12. Seizures induce neural stem cell proliferation in the hippocampus

Chronic temporal lobe epilepsy is associated with an increase production of neurons in the dentate gyrus (Parent, 2007; Scharfman and Gray, 2007). Conversely, acute seizures dramatically induce abnormal production of neurons in the dentate gyrus, which may contribute to chronic epilepsy. Whether generation of new neurons in the hippocampus of patients with epilepsy is a result of the disease or contributes to the cause is not clear. In mice, experimentally induced seizures effect neuron production at multiple levels and not least by disproportionately increasing the number of neuroblasts (Type-3 cells) (Jessberger et al., 2005). Both the radial Type-1 and the horizontal stem cells are activated in response to experimentally induced seizures (Huttmann et al., 2003; Lugert et al., 2012; Venere et al., 2012). However, the proportion of radial cells that enter cell cycle is rather modest and the population is not expanded suggesting that their divisions generate more committed progenitors. The horizontal stem cells respond more homogeneously to seizures. The majority of them enter the cell cycle and the total number increases significantly (Lugert et al., 2010). The increase in horizontal cells could be the result of symmetric cell division but also generation of horizontal cells from the radial stem cell pool. Although current tools and techniques have not been able to address the mechanism, the increase in mitotically active stem cells following chronic seize and the differential response of the different stem cell pools has important implications for the cause and progression of temporal lobe epilepsy in humans.

13. Future perspectives

In the future it will be a major challenge to elucidate the heterogeneity within the stem cells pools and to address there cellular function. This will include understanding how these different populations and cell states are regulated and whether their functions are controlled by distinct niche signals or genetic and epigenetic mechanisms. Only the detailed analysis of neural stem cells in the adult brain could uncover their functions in homeostasis, aging and disease. This would raise the exciting possibility that specific neural stem cell subtypes could be directly targeted for therapy.

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