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Research Highlight

Hippocampal stem cells: so they are multipotent!

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Although neurogenesis continues throughout life in the mammalian brain, the issue of whether the stem cells that drive the process *in vivo* are self-renewing and multipotent remains unclear. In a recent landmark paper by Bonaguidi et al. (2011) published in *Cell*, the authors provide clonal evidence that neural stem cells in the dentate gyrus of the adult hippocampus are indeed multipotent and undergo symmetric cell divisions.

The concept of adult tissue-specific or somatic stem cells is not a new one and is widely accepted (Morrison and Spradling, 2008). It is also accepted that progenitor cells exist in the adult mammalian brain and these generate neurons, astrocytes, and oligodendrocytes in specific anatomical regions (Gage, 2000; Doetsch, 2003). The perception of a stem cell rather than a restricted progenitor is coupled with self-renewal and often multipotent lineage potential. Analysis of population dynamics and stem cell potential indicated that new neurons and glia are continually generated within the brain throughout life. To date it has not been unequivocally demonstrated that individual neural stem cells generate both neurons and glia while retaining stem cell character in their endogenous *in vivo* setting. This is due to a technical challenge of being unable to trace individual cells and their progeny *in vivo*.

In a recent study published in *Cell*, Bonaguidi et al. (2011) used elegant and very careful analysis of genetically labeled progenitor cells in the dentate gyrus of the adult hippocampus. They provide compelling evidence that some neural stem cells have a long-term ability to generate neurons and glia as well as undergo self-renewing symmetric divisions (Figure 1). Clonal analysis *in vivo* under homeostatic conditions is notoriously difficult and has not been sufficiently demonstrated in the brain to date, hence, the major significance of this work. Various labeling techniques including retroviral infection, *Cre*-mediated lineage tracing, and metabolic labeling have all failed to conclusively show that individual cells rather than populations of cells can be multipotent *in vivo*. To circumvent this problem, Bonaguidi et al. (2011) combined a tried and tested inducible *CreER*^{T2} driven from a *nestin* promoter (*nestin-CreER*^{T2} allele) to recombine the Z/AP *Cre*-reporter allele and indelibly label hippocampal progenitors. They then tinkered with the levels of tamoxifen (TAM) induction to obtain a very sparse genetic labeling of neural stem cells (they claim eight cells in each dentate gyrus of the animals). The extremely low frequency of labeling and computer predictions that each labeling event can be used to clonally trace cells enabled fate potential analysis of individual cells and their progeny.

The difficulties in selective labeling and identification of stem cells in the adult brain have also made it difficult to define the neurogenic lineage in the adult hippocampus, including the cellular hierarchy. Viral labeling and previous genetic lineage tracing imply that quiescent radial and a population of more active horizontal (non-radial) stem cells differentially contribute to adult hippocampal homeostasis (Suh et al., 2007; Lugert et al., 2010). The approach of a single 'suboptimal' TAM-induction of *nestin-CreER*^{T2} Z/AP mice used by Bonaguidi et al. (2011) labeled cells 70% of which had a radial neural stem cell-like morphology. These radial cells were quiescent and did not express MCM2, a mitotic cell marker. Their observation that radial glial-like cells in the adult dentate gyrus are mostly inactive or dormant is consistent with previous studies showing that these cells are difficult to label with retroviruses or thymidine analogs and that they generally do not express mitotic markers (PCNA or Ki67). Furthermore, the labeling approach avoided labeling the more mitotically active horizontal stem and progenitor cell populations, although 30% of the recombined cells did fall outside the radial stem cell category.

Tracing the genetically labeled clones *in vivo* revealed heterogenic potential. Approximately 17% of the labeled clones analyzed after 1 month did not contain cells with a radial morphology and this increased to 33% of the clones by 2 months. It is unclear how many of these non-radial cell-containing clones generated progeny, but some of those did consist of only neurons, some only astrocytes, and some both. It is likely that the ancestral cell either lost or never had radial stem cell character. The majority of the other traced cells formed clones that contained at least one radial progenitor cell. Thirty percent of the progenitors that displayed a radial morphology remained quiescent and did not give rise to progeny after 1 month. This reduced to ~20% by 2 months, implying that some radial cells can remain in an inactive state for many weeks having the ability to generate progeny. The majority of the labeled clones that retained a radial stem cell were multilineage clones. Thus, the founder cell of the clone retained progenitor status and gave rise to neurons, astrocytes, or both. These data provide an

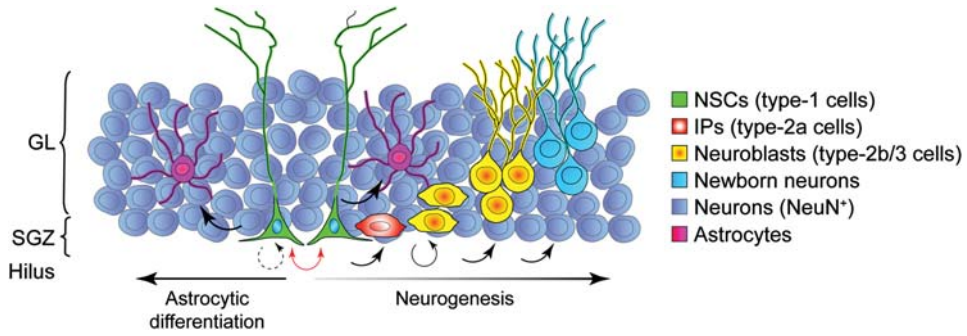

Figure 1 Clonal analysis of quiescent radial neural stem cells (NSCs) in the subgranular zone (SGZ) indicates that individual cells can be self-renewing and undergo symmetric self-replicating divisions (red arrow) but also asymmetric divisions to generate neurons (neurogenesis) or astrocytes. Some radial cells generate multilineage clones containing neurons and polymorphic astrocytes in the granule cell layer (GL). Previous evidence indicates that after stem cell divisions, amplification in the lineage may be limited to one or two cell cycles in the intermediate progenitor (IP) or early neuroblast stages.

unequivocal demonstration of multipotency of single cells. Consistent with previous observations, the predominant differentiated cell type generated was neuron, and neuron-containing clones accounted for <60% of the total; 30%–35% of the stem cells generated neuron-deficient clones; while 13%–19% produced both neurons and astrocytes. Symmetric self-renewing stem cell divisions that did not result in differentiated progeny were rare, accounting for <5% of all clones analyzed. Although low, this number is also highly significant as the finding provides conclusive evidence that neural stem cells in the adult brain can not only generate differentiated progeny through asymmetric cell division but, like their embryonic counterparts, also expand *in vivo*. Importantly, radial neural stem cells never generated oligodendrocytes in the dentate gyrus. Therefore, the neural stem cells traced here include not only cells that remain quiescent for months (up to a year) but also uni- and bipotent stem cells some of which can undergo symmetric as well as asymmetric self-renewing divisions.

So what remains to be shown? The evidence provided by Bonaguidi et al. (2011) that the adult hippocampus contains true stem cells may go some way to satisfy the doubts of skeptics and confirm the beliefs of many in the field, but it does not answer all of the questions. Radial cells rarely enter the cell cycle and even in this study, strange as it seems, using a suboptimal TAM induction regime, mitotic-labeled radial cells were never observed. Interestingly, if the induction protocol was changed to a 4-fold higher dose of TAM, more cells were labeled including MCM2 expressing, mitotic radial cells. As most of the detailed analysis focused on snapshots at 1 and 2 months post-induction, the time course of quiescent cell activation remains to be shown. It is unclear why the stronger induction paradigm labeled more cells but it likely reports cells that expressed the *nestin* transgene at lower levels than the quiescent radial stem cells or perdurance of $CreER^{T2}$ protein in some cells after the transgene was inactivated as cells progressed along the lineage. In this respect, it will be important to assess what proportion of the neural stem cells in the dentate gyrus of adult mice are in a quiescent state at any one time (Lugert et al., 2010). The clonal analysis performed here may skew the view toward a subpopulation of stem cells and, as the authors state, does not exclude that other cells in the subgranule zone may have stem cell properties. In many tissues, active and dormant stem cell populations coexist and work together to maintain homeostasis (Li and Clevers, 2010). In the dentate gyrus, evidence has been put forward for a population of stem cells that are less quiescent than those studied here (Suh et al., 2007; Lugert et al., 2010).

Stem cells are regulated by their niche which controls maintenance, proliferation, and differentiation. The niche signals that control these processes in the dentate gyrus are starting to be elucidated and in an additional twist, Bonaguidi et al. (2011) show that the tumor suppressor PTEN represses the transition of quiescent radial stem cells into proliferation. The signaling pathways that control PTEN activity in this context are not clear but would potentially be an important druggable target for *in vivo* expansion of endogenous progenitor pools. Furthermore, the activity of stem cell populations in the hippocampus is modulated by pathophysiological stimuli *in vivo* (Lugert et al., 2010). Previous findings indicate that chemically induced epileptic seizures and physical activity as well as aging affect activity of radial neural stem cells in the hippocampus. It will be important to unify the findings in the field, to address if the populations studied here and in previous studies respond in a similar fashion to these cues and to elucidate the molecular nature of the signals involved. The analysis by Bonaguidi et al. (2011) provides a strong argument for multipotent neural stem cells in the adult hippocampus but does not address the dynamics within lineage progression. As the radial stem cell enters the cell cycle so infrequently but can generate clones of up to 20 cells, amplification must occur at a different stage. Classically, neural stem cells progress from a quiescent state to a neuron through a series of transient amplifying intermediate cell types (TAP or IP) (Doetsch, 2003). In the hippocampus, this remains controversial and various approaches of viral labeling and thymidine analog have come to different conclusions about the cell and degree of amplification in the lineage (Seri et al., 2001). Based on observations describing active stem cells, mitotic intermediates, and the viral demonstration that expansion is very limited with

immature neuroblast undergoing one or maximally two divisions, there is still a need for clarification (Seri et al., 2001). This is particularly important in light of many experiments showing that different cell types within the hippocampal lineage can be affected by different environmental stimuli (Lugert et al., 2010). In addition, the clonal analysis of quiescent cells did not support the recent data leading to the proposition of a 'disposable stem cell' theory (Encinas et al., 2011). Using similar tools, *nestin-CreER^{T2}* expressing hippocampal stem cells were shown to undergo a limited number of mitotic divisions before differentiating into astrocytes and diminishing the stem cell pool. Clearly, the different protocols may have played a role, but it is strange that a stronger induction paradigm resulted in labeling stem cells that had a more limited potential for maintenance and expansion. One could anticipate that genetic background may have affected the output as neurogenesis is strain-dependent but even here the author claimed to have used the same strains (C57Bl6) (Bonaguidi et al., 2011; Encinas et al., 2011). It remains open whether the mechanisms regulating PTEN or even PTEN expression itself was affected in one of the studies as inactivation of PTEN in quiescent neural stem cells resulted in an increase in terminal astrocytic differentiation (Bonaguidi et al., 2011). PTEN is known to be susceptible to loss of heterozygosity in tumor formation including glioma. Comparatively, it also remains to be shown how aging affects the various hippocampal stem cell populations, particularly the quiescent multipotent cells analyzed by Bonaguidi et al. (2011). The current approach of clonal or sparse mosaic genetic labeling *in vivo* opens up a means to study the intermediate stages of differentiation and it is to be hoped that this approach will be widely accepted and adopted by the community to also address gene function *in vivo*.

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