Editorial

Stem Cell INVESTIGATION

Redox homeostasis: unlocking the bottleneck in glia-to-neuron conversion

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Recent discoveries in the field of regeneration research inspire great optimism for future therapies aimed at repairing the injured or diseased human brain. Of particular clinical significance is the approach to promote neurogenesis from endogenous parenchymal cells independent of the use of embryonic or induced pluripotent stem cells. Perhaps the most attractive strategy appears to be direct *in vivo* cell reprogramming to induce a neuronal fate. However, the initial enthusiasm in the field has been tempered, as quantity and quality of replenished neuronal populations have remained low. An exciting new study by Magdalena Goetz and co-workers (1) published in *Cell Stem Cell* sheds new light on the problem of cell death at the time of fate conversion and emphasizes the importance of coordinated metabolic reprogramming to increase neurogenesis successfully. In this editorial we summarize these critical new findings and consider them in the context of the adult brain's potential for plasticity and self-regeneration.

Creating an ectopic neurogenic niche to drive neurogenesis *de novo* endogenously

The discovery that adult neurogenesis persists throughout life has stirred great optimism as a potential therapeutic mechanism that can be applied to the entire brain. External stimuli (e.g., physical exercise, exposure to an enriched environment) (2,3); and intrinsic signaling pathways [e.g., neurotransmitters such as serotonin, growth and neurotrophic factors such as the brain-derived neurotrophic factor (BDNF) reviewed in (4)]; modulate neurogenesis in concert with cell-autonomous survival and differentiation factors (5). Although frequently expressed simultaneously within the same cell, transcription factors differ widely in their effects, i.e., on cell proliferation, differentiation, cell death and cell survival, with most of them showing a transient expression pattern [reviewed in (6)]. An intensely studied pro-neurogenic stimulus is physical exercise which has been shown to robustly increase the number of proliferating progenitor cells in the dentate gyrus of the hippocampus (7,8), thus increasing the reservoir of cells that can be quickly recruited into a neuronal fate (9). Interestingly, voluntary running also increases the number of proliferating Neuron-glia2 (NG2) glia precursor cells in the amygdala (10). Enlarging the glia pool may be especially important in demyelinating diseases, while therapeutically enforced neurogenesis is needed in neurodegenerative disorders and in stroke. Cell engineering to drive neurogenesis *de novo* from stem and non-stem cells of the (diseased) brain is desirable, as neurons may be lost in neurological and neurosurgical conditions such as traumatic brain injury, stroke as well as the classical neurodegenerative diseases such as Alzheimer's or Parkinson's disease.

The capacity of the adult brain to self-regenerate upon injury is limited. Replacement of neurons only takes place in a few spatially restricted neurogenic regions, where neural stem cells retain fate plasticity [the subventricular zone and the subgranular zone of the dentate gyrus in rodents, the adult human hippocampus and striatum; reviewed in (11)]. Outside these neurogenic regions, proliferating NG2 glia precursors are the endogenous source of cells with multipotent potential (12,13), but they remain glial-restricted *in situ*. Hence, most of the brain consists of non-neurogenic regions apparently incapable of repair. The knowledge gained from neurogenic niches can be used to drive cell fate of (non-neurogenic) resident progenitor cells. Previous *in vivo* work in rodents has provided insights into initiation of adult neurogenesis in models of targeted apoptotic cell death (14,15), as well as after acute brain injury (stab lesion) (16,17). Although the goal of generating new neurons from endogenous precursor cells was achieved, these approaches required specific forms of tissue damage. However, non-selective injury, as in the case of traumatic brain injury, never, or in the case of brain ischemia, barely (18) leads to endogenous neuronal cell birth.

Direct *in vivo* reprogramming into the desired phenotype

'Direct reprogramming' has become a popular approach to induce a neuronal fate. Direct reprogramming of somatic cells into pluripotent stem cells *in vitro* (19) has opened up entirely new possibilities in regeneration research. Successful direct *in vivo* reprogramming of desired tissue-specific cells was achieved in the pancreas (20) and myocardium (21). Recent research has refined technologies to induce neurogenesis using gene therapy. In the adult brain, gene delivery and lineage tracing approaches were used to create a neurogenic niche and to drive cell fate *in vivo*. Using one of the several factors studied [e.g., BDNF, Sox2, Pax6, Olig2, achaete-scute complex homolog-like 1 (Ascl1), Bcl-2], cell genesis or enlargement of the progenitor cell pool was accomplished in neurogenic niches and following stroke. It has been shown that selectively modifying transcriptional cues is sufficient to induce a cell fate switch in the dentate gyrus neurogenic niche and striatum: e.g., conversion of neural stem/progenitor cells into oligodendrocytes by Ascl1, also known as Mash1 (22); enlargement of the pool of glia cells that may support a cell-replacement strategy aimed at remyelination after injury (23); reprogramming of striatal astrocytes into neuroblasts by viral-mediated expression of Sox2 (24); and retroviral delivery of the lineage-instruction factors Olig2 and Pax6 altering the neurogenesis-gliogenesis ratio (25). Although retroviral overexpression of the pro-neuronal factor Pax6 pushes precursor cells in the dentate gyrus into a neuronal phenotype, survival of these engineered cells is significantly reduced (25). In models of mild cerebral ischemia, induced cell death was delayed by altering cell cycle regulatory mechanisms (26), and gene delivery of fate determinants induced the birth of immature neurons in the non-neurogenic injured striatum (18). Newly generated cells after brain injury or stroke were identified by co-expression of the thymidine analog bromodeoxyuridine (BrdU) and doublecortin. Known as a marker of immature neurons in neurogenic regions, doublecortin has distinctive characteristics dependent on the niche environment (27).

Altogether, only few (typically immature) cells are generated upon viral gene delivery of fate determinants. Furthermore, bona fide neurons commonly identified by co-expression of BrdU and the neuronal nuclear marker NeuN may possibly represent an artifact (28). Indeed, aberrant DNA synthesis may lead to incorporation of BrdU and, thus, may indicate the induction of cell death rather than the birth of a new neuron (26,28). Another challenge besides ectopic induction of neurogenesis is to generate neurons that become functionally integrated. So far, these efforts have met with limited success, although resident glia cells have been shown to differentiate into spiking neuroblasts in models of stroke (18,29). Cleary, outside the canonical neurogenic brain region, newly generated immature neurons lack crucial signals within the non-neurogenic and injured region. The niche environment therefore needs to be primed as well for neuronal fate conversion and subsequent neuronal differentiation, which, in turn, requires coordinated and cell type-specific transcription of the genes involved alongside the release of neurotrophic factors to foster a pro-neurogenic milieu.

Taken together, the existing literature suggest that driving neurogenesis *de novo* from stem/progenitor cells *in vivo* has its downsides as it often leads to cell death, disruption of homeostasis within the niche, and not to the desired recovery of the region. In their study, Magdalena Goetz and co-workers define cell death as the 'main limiting factor of neuronal reprogramming' and study coordinated metabolic reprogramming at the time of cell fate transition. At this 'metabolic checkpoint', key neuronal genes are rapidly regulated leading to either cell death or survival. Gascón *et al*. made use of the highly advanced method of

continuous single-cell imaging to track fate conversion of (I) *Ascl1*-transfected astrocytes and (II) after brain injury *in vivo*. Thereby, the focus of their study was on metabolic features common to all cell types that may improve cellular reprogramming (1).

Redox homeostasis in neuronal reprogramming

Reactive oxygen species (ROS) production, lipid peroxidation, or cell death-regulating proteins such as Bcl-2 are emerging as potent contributors to cell death or survival decisions. Redox homeostasis, the interplay of oxidative stress and antioxidant signaling (30), is a biological feature common to all cell types. It marks a metabolic interface for intrinsic *vs*. environmental signals and can command cell death programs. At neuronal fate conversion, reprogramming from glycolytic to oxidative metabolism formidably challenges redox homeostasis. High oxidation levels lead to apoptosis or ferroptosis (an oxidative stress-dependent/caspase-independent form of cell death characterized by accumulation of lipid peroxidation products), thereby limiting the number of cells adopting a neuronal phenotype. This finding may not come as a big surprise to researchers at the bench, as we already know from daily cell culture work that neuronal progenitors almost literally hate oxidation. In the study by Gascón and co-workers, treatment with liproxstatin-1, a ferroptosis inhibitor, was shown to not only prevent cell death but also to improve reprogramming efficiency. This was accompanied by suppression of ROS production and lipid peroxidation, leading to increased survival of new neurons. Another efficient molecule to reduce lipid peroxidation is forskolin. When added to *Ascl1*-transduced astrocytes, a cell type-independent increase in fate transition was observed which Nrf2 anti-oxidative pathways supported (1).

Yet another regulator of the metabolic switch is the anti-apoptotic protein Bcl-2 which improves metabolic conversion at the time of direct neuronal reprogramming. In their report, Magdalena Goetz and coworkers demonstrate that Bcl-2 co-expression in *Ascl1*-transfected astroglia cells facilitates neuronal conversion in tandem with increased cell survival via the non-canonical pathway (Bcl-2 inhibits apoptosis by sequestering Bax/Bak when phosphorylated at serine 69/70) (1). Thereby, Bcl-2 improves maturation uncoupled from cell proliferation leading to successful neuronal transition: up to 80% of *Ascl1*-transduced astrocytes turned into neurons upon Bcl-2 (1). Furthermore, both Bcl-2 and forskolin together enhanced conversion efficiency in the presence of neuronal fate determinants (e.g., Neurog2) (1).

So far, the replenishment of neuronal populations in the injured brain has fallen short both in quantity and in quality. The oxidative stress response along with lipid peroxidation plays a key role in directed reprogramming as shown *in vitro* (1). *In situ*, injury profoundly affects the niche environment inducing inflammation and thereby shifting redox homeostasis. Previous *in vivo* work by the same group had already provided invaluable insights into initiation of adult neurogenesis in the injured brain (16,17). Here, the authors followed up on their previous experiments and tested whether Bcl-2 and forskolin are capable of enhancing neuronal transition after acute stab wound injury. Excitingly, retroviral gene delivery and singe-cell imaging revealed that direct reprogramming of resident cortical progenitor cells with Bcl-2 (in the presence of the fate determinant Neurog2) increased neuronal conversion and accelerated neuronal maturation at 10 days post-injection. Importantly, two-thirds of transduced cells *in vivo* were shown to express mature neuronal marker NeuN (1).

Intriguingly, co-transfection with another anti-oxidant and 'top transcriptional regulator', calcitriol receptor (a.k.a., vitamin D receptor), further improved neuron transition and complexity. Calcitriol-dependent anti-oxidant signaling protected against lipid peroxidation by a mechanism that was independent of mitochondria (1). This exciting discovery raises questions on whether vitamin D itself may also increase cell survival or whether it acts indirectly, for example, via inhibition of the immune system.

In conclusion, the new findings by Gascón and co-workers will have a profound impact on regeneration research by taking the field a huge step forward toward increasing the pool of newly generated neurons. Firm control of metabolic reprogramming in concert with refined timing of genetic factors involved in neuronal fate choice offers great promise for the repair of the injured brain.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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