Endogenous Brain Repair: Overriding intrinsic lineage determinates through injury-induced micro-environmental signals

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ABSTRACT

Adult human neurogenesis has generated excitement over the last 2 decades with the idea that endogenous adult stem cells could act as a potential cell source for brain repair after injury. Indeed, many forms of experimentally induced brain injury including stroke and excitotoxic lesioning can promote proliferation from the subventricular zone and mobilise neuroblasts and oligodendrocyte progenitor cells to migrate through brain parenchyma to damaged regions. However the failure of neuroblasts to mature into appropriate neuronal subtypes for cell replacement has been an issue. Recent work by our group and others has indicated that micro-environmental signals released from areas of cell loss may be able to override intrinsic gene expression lineages and covert neuroblasts into oligodendrocyte progenitor cells. This commentary will discuss the enhanced fate plasticity of both adult neural progenitors and parenchymal NG2 cells after injury, and the importance of understanding brain-injury induced micro-environmental signals in the quest toward promoting endogenous regeneration after injury.

There has been much excitement over the years since adult neurogenesis was first discovered, and found to be widespread in many mammals including humans.1-3 It was thought that neurogenesis could provide a mechanism for endogenous repair of the brain after an injury or disease. Supporting this concept has been decades of research in rodents that has shown that endogenous regeneration from neurogenic regions does indeed occur after many forms of brain injury, including excitotoxic lesioning, stroke, traumatic brain injury and in models of neurodegenerative diseases including Parkinson and Huntington’s disease.4 In more recent years however the excitement has waned. It has become apparent that while brain injury or inflammation does cause an increase in proliferation from neurogenic regions, and the generation of neuroblasts that are capable of migrating long distances through brain parenchyma to damaged areas, the response is not large enough or complete enough to result in functional recovery.5,6 In addition to this, novel studies using 14C retrospective birth-dating of human neurons have found, that some of the neurogenic niches of the larger, more evolved human brain do not function the same way as in rodents.2,7

In both humans and rodents, the subventricular zone (SVZ) lining the lateral ventricles is one of the 2 ‘cannonical’ sites of adult neurogenesis, the other being the sub-granular zone of the dentate gyrus in the hippocampus. In the normal rodent SVZ, slow dividing Type B stem cells generate heterogeneous Type C cells (transiently amplifying precursors or TAPs) that express a range of proneural genes including Mash1, Gsh2, Dlx2, Pax6 and Olig2, and can generate both neuroblasts and oligodendrocytes. Large numbers of neuroblasts migrate daily along a specified path through the rostral migratory stream into the olfactory bulb when they differentiate into olfactory granule and periglomerular cells and contribute to odor discrimination.8 In the human adult brain, the proliferative niche of the SVZ also exists and neuroblasts have been observed in a lateral ventricular extension. However, few contribute to the adult olfactory bulb, perhaps reflecting our reduced dependence on our olfactory system.9 Instead it is thought human SVZ-derived neuroblasts may be contributing to neurogenesis in the striatum, as 14C dating of striatal neurons showed they were continuously generated
throughout life. This is an incredible finding, as the striatum is often the region damaged from stroke caused by MCAO, and is the region that degenerates in Huntington’s disease. This suggests a cell source for compensatory neurogenesis in humans does exist and may be able to be mobilized for therapy for human disease.

If endogenous repair is to be restorative after disease or injury, attraction of the appropriate cellular phenotype to repopulate and repair damaged areas of the brain is essential. However, neural stem cells of the SVZ are regionally specialized depending on what embryonic progenitor domain they developed from, and dispersed through regions throughout the SVZ-lateral ventricle system. This heterogeneity in progenitor domain they developed from, when compared with controls. We speculate this was a selective compensatory repair mechanism as Dlx2 is one of the key genes involved in the development of the ganglionic eminencies, which develop into the striatum and also a fate determinate of striatal medium spiny neurons.

The answer turns out to be not so simple. Supporting the intrinsic lineage work, studies using rodent hypoxic/ischemic and cortical stroke models have found redirected neuroblasts do remain on their original lineage program. Cells that migrated into damaged striatal or cortical regions expressed calretinin, the GABAergic granule cell fate, and not the DARPP32 or glutamatergic subtypes that were required for successful repair of the regions. With the rodent striatum being made up from <1% calretinin interneurons, even assuming redirected neuroblasts could integrate and become functional, the effect of this cell replacement would be minimal in contributing to repair. In contrast, early pioneering work using rat middle cerebral artery occlusion (MCAO) models found a sustained neuroblast migration from the SVZ into the damaged striatum, with cells maturing into both calbindin-+, and DARPP32+ neurons, the latter being the major cell type lost from injury. This showed promising endogenous repair, with a switching of cell fate from what the neuroblasts would normally have differentiated into had they remained migrating to the olfactory bulb. Further, work using the quinolinic acid (QA) excitotoxic lesion model of Huntington’s disease also showed increased proliferation in the SVZ after lesioning, with recruitment of both neuroblasts and Olig2+ oligodendrocyte progenitor cells into the lesioned striatum. Attempting to promote this effect, and reduce the glial response to injury, we overexpressed the proneural genes Pax6 or Dlx2 in TAPs in the SVZ. Interestingly, while both Pax6 and Dlx2 expressing progenitor cell types were recruited into the lesioned striatum, only the Dlx2 overexpressing cells demonstrated an induction to a neuronal fate when compared with controls. We speculate this was a selective compensatory repair mechanism as Dlx2 is one of the key genes involved in the development of the ganglionic eminencies, which develop into the striatum and also a fate determinate of striatal medium spiny neurons.

We were however surprised to observe the Pax6-overexpressing cells within the lesioned striatum downregulated the expression of ectopic Pax6 and were converted into NG2+ oligogial cells. This suggested that signals within the lesion microenvironment were overriding the intrinsic gene expression, resulting in an altered cell fate. This surprising effect was also observed in a model of white matter demyelination, where cells from the SVZ switched their identity from Pax6+ neuronal progenitors into Olig2+ and NG2+ oligodendrocyte progenitor cells, and matured into oligodendrocytes within lesioned white matter. The fate alteration was found to be controlled extrinsically by BMP antagonist Chordin. Intrinsic control over this process has also been linked to Brg1 expression in Pax6 SVZ progenitors. When Brg1 was experimentally knocked down, Pax6+ TAPs were converted into Olig2+ and NG2+ oligodendroglial progenitor cells within the RMS. It is likely then that extrinsic molecules play a role in altering Brg1 expression, and contribute to the fate switching we observed in the lesioned striatum. Therefore, while in the normal brain lineage appears to be intrinsic, plasticity is increased after injury, and fate is affected by microenvironmental signals released from injured tissue.

The brain microenvironment plays a major role in both the regulation of endogenous SVZ neurogenesis, and also in the recruitment and fate of progenitors in the injured brain. If we had a greater understanding of which chemokines/signals could attract not just
neuroblasts, but specific sub-types of SVZ TAPS or neuroblasts we would be better able to target recruitment of specific cell populations for brain repair. It is well reported that the chemokines SDF-1, MCP-1, MIP1α and GROα can recruit SVZ neuroblasts into the striatum after ischemic or QA-induced injury.22,23 We have also found the temporal profile of chemokine expression after QA lesioning is dynamic and reflects the time points when neuroblast migration is maximal.23 What is less well characterized is the effect chemokines have on cell fate determination; MCP-1 has been shown to promote neural differentiation, and the SDF-1 receptor CXCR4 to promote oligodendroglial fate after experimental demyelination. Further, injection of LPS into the striatum of rats was found to elicit a neurogenic response and migration of neuroblasts into the striatum comparable to that observed in a stroke model, indicating the significant effect inflammatory signals have on neurogenesis.24 Could these same chemokines be initially recruiting neuroblasts into lesioned regions, then converting their fate into oligoglial cells? Poor temporal characterization of inflammatory and other signals generated after injury is potentially one reason reports of subtypes of neurons/glia generated vary between studies.

Many groups including our own have focused on overexpression of proneural fate determinates or in vivo reprogramming to attempt to induce neural repair after injury, with varying degrees of success.16,25–27 Specifically, alterations in the expression of Gsh2, Mash1, Pax6, Ngn2, Dlx2 and/or Olig2 have been observed in the adult SVZ and parenchyma in various models of neural cell loss, consistent with their potential roles in the endogenous repair process.25–33 We recently showed that Dlx2 overexpression in SVZ progenitors could increase neuroblast recruitment and neuronal fate in the QA-lesioned striatum compared with controls.16 Others have generated replacement neuronal cells from reprogramming GFAP+ or NG2+ glia within brain parenchyma by overexpressing proneural genes including Dlx2, Sox2 and Mash1, or by downregulation of Olig2.25,26,34 Specifically, conversion of NG2+ glia into neurons through Sox2/Mash1 viral transduction only occurred in the cortex of stab wound injury, and never in controls.34 Particularly interesting is the result that NG2+ oligodendroglia and neuroblast fate may be reversible from signals released after injury,16,19 or through in vivo reprogramming.34,35 Other studies also report conversion of astrocytes into neuroblasts after injury, with endogenous re-expression of Sox2 and Mash1.31 This is exciting as is indicates multiple pools of cells may be able to contribute to endogenous regeneration, perhaps responding to similar micro-environmental signals to turn on latent genetic programmes.

The key to moving forward from here is to discover which injury-induced signals in the microenvironment around damaged areas are directing or inhibiting re-specification of neuronal fate, and in particular specification to subtypes of neuronal fate. This is important because even with successful gene overexpression or neuronal reprogramming, if the micro-environmental cues are not addressed to enable a more permissive environment for neuroblast migration, survival, maturation and integration, neural repair is not going to be fully restorative.

Addressing how to make a permissive environment for neuroblasts is difficult. Within the micro-environment surrounding areas of cell death there are a plethora of factors all working together that need to be untangled, in order for researchers to identify factors to increase endogenous neurogenic responses. In the future, perhaps a combined therapy will be required to target recruitment or reprogramming of specific progenitor pools in combination with neurotropic, pro- and anti-inflammatory or oligogial inhibitory factors. So while successful endogenous repair has not been achieved yet, there is still reason to be optimistic. More possibilities to promote neurogenesis and repair after injury are becoming apparent than just the traditional neurogenic zones, and with striatal neurogenesis now confirmed in humans, there is more reason than ever to continue to develop this as a potential strategy to treat human brain injury and disease.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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