● PERSPECTIVE

Adult neurogenesis and *in vivo* reprogramming: combining strategies for endogenous brain repair

Functional recovery of the human brain after injury, or slowing of a neurodegenerative disease is the ultimate goal of brain research. Many promising studies have identified key genes involved in the generation of neuroblasts and oligodendrocytes from adult neurogenic niches and determined their involvement in endogenous regeneration after injury. Interestingly, some of the same genes have been found to be able to generate neuroblasts through *in vivo* cell reprogramming strategies, offering an alternative mechanism to regenerate the brain after injury. However, appropriate neuronal sub-type generation and functional integration is still lacking in many injury models. Key molecules must be identified from within the injury-induced micro-environment that can promote correct subtype maturation and integration before brain repair after injury can become a functional reality.

In the neurogenic niche of the subventricular zone lining the lateral ventricles of the rodent brain, GFAP+ stem cells generate rapidly dividing transit amplifying precursor cells (TAPs), which express combinations of neuronal or oligodendroglial lineage genes including (but not limited to) *Ascl1* (Mash1), *Dlx2*, *Pax6*, and *Olig2*. TAPs themselves can then generate neuroblasts which migrate down the rostral migratory stream into the olfactory bulb when they differentiate into olfactory granule and periglomerular cells and contribute to odour discrimination, or oligodendrocyte progenitor cells (NG2+ glia) that migrate locally into white matter tracts (Connor et al., 2011). Neural stem and progenitor cell genes *Sox2*, *Ascl1*, *Dlx2* and *Pax6* have been found to be important for both adult neurogenesis, neuronal sub-type specification and also for *in vivo* reprogramming to generate neuroblasts after an injury (Heinrich et al., 2010, 2014; Magnusson et al., 2014; Nato et al., 2015; Jones and Connor, 2016). Experimental stroke through a middle cerebral artery occlusion (MCAO), excitotoxic injury, stab wound or demyelination can all stimulate endogenous progenitors from the subventricular zone of the lateral ventricles to increase their proliferation, and redirect newborn neuroblasts towards the areas of damage and cell loss. Large numbers of neuroblasts can be recruited to damaged areas, travelling long distances through brain parenchyma, and this recruitment can persistent over a number of months (Jablonska et al., 2010; Connor et al., 2011). In addition, recent work has found that glial cells within the striatal parenchyma can also undergo endogenous neurogenesis after stroke or excitotoxic injury. GFAP+ astrocytes have be shown to upregulate *Ascl1* and generate neuroblasts locally within the striatum over a number of months following injury (Magnusson et al., 2014; Nato et al., 2015).

Interestingly, in comparison to endogenous neurogenesis, advances in the cell reprogramming field have shown that viral overexpression of neural stem or progenitor genes including *Sox2*, *Ascl1* or *NeuroD1* can reprogram parenchymal GFAP+ or NG2+ glia to generate neuroblasts. This can occur within both the normal and damaged striatum but only in the injured cortex, indicating increased plasticity of fate after injury within the

cortex. In the striatum, *Sox2 in vivo* reprogramming was also found to pass through a proliferative intermediate cell type that resembled the *Ascl1*+ TAPs found in the adult subventricular zone (SVZ) niche, linking the processes of endogenous neurogenesis and neuronal reprogramming (Heinrich et al., 2014; Niu et al., 2015). This process can be likened to generating an induced neural progenitor cell within the parenchyma. Using a retrovirus expressing *NeuroD1*, Guo et al. (2014) directly reprogrammed parenchymal GFAP+ and NG2+ glia into functional neurons after a cortical stab wound, and in a rodent model of Alzheimer's disease. This strategy was more comparable to directly generating induced neurons, as no proliferative intermediate was observed, and *Ascl1* expression was not described (Guo et al., 2014).

With multiple ways of generating adult born neuroblasts, through both endogenous and exogenous means, one may think that neural repair after injury is close to becoming a reality. However, for repair to be successful newly generated neuroblasts must mature into the neuronal subtype appropriate for the region of cell damage or loss. They must also integrate into the host circuitry and signal appropriately. For neuroblasts that are recruited from the adult SVZ after injury, there has been no consensus on what drives their subtype specification when they reach the site of injury. Indeed after striatal cell loss following MCAO, some groups have shown SVZ-derived neuroblasts can generate DARPP32+ neurons, the appropriate cell type for striatal repair. but others found they matured into SP8+ (a positional gene found in lateral/caudal ganglionic eminence-derived interneurons) calretinin expressing neurons, which would be unable to repair the striatum (Inta and Gass, 2015). Similarly, quinolinic acid (QA)-induced neurogenesis from striatal astrocytes generated neuroblasts that again expressed SP8, but no DARPP32 expression was reported (Nato et al., 2015). Attempts to promote correct subtype specification have been tested using retroviruses expressing proneural genes (Heinrich et al., 2010). *Dlx2* overexpression in SVZ progenitors in a QA lesion model both enhanced neuronal fate in the lesioned striatum and prolonged the migratory response to the lesion (Jones and Connor, 2016). However, the response was still acute and not large enough for complete brain repair.

In general, lineage specification of neuroblasts from the SVZ in the normal brain is thought to be intrinsic, however injury to the brain appears to allow increased plasticity and subtype alterations (Jablonska et al., 2010; Inta and Gass, 2015). The cues for a neuroblast to mature appropriately must come from micro-environmental signals released from the injured area. In fact lesion-induced signals have been found to not only influence neuronal subtype, but are able to convert neural progenitors from the SVZ into oligodendroglial cells. In a model of white matter demyelination, Chordin, a bone morphogenetic protein (BMP) antagonist was found to convert SVZ derived PAX6+ neural progenitors into OLIG2+ NG2+ oligodendroglia within the white matter (Jablonska et al., 2010). In this case the lineage conversation was appropriate, but a similar effect was also observed following excitotoxic injury to the striatum. *Pax6*-GFP expressing cells from the SVZ were recruited into the lesioned striatum, but the proneural gene expression was lost and cells converted to a NG2+ oligodendroglia fate (Jones and Connor, 2016). In this case the conversion was not appropriate, as regeneration of the DARPP32+ neuronal population was required. These results indicate that signals released in areas of cell loss can influence both plasticity of cells and their differen-

tiation potential within damaged areas. A better understanding of these processes is critical if we are to direct specific neuronal subtypes for appropriate repair.

Also critical is the ability of newly recruited neurons to become functionally integrated into the local circuitry. Very few endogenous regeneration studies have demonstrated this to date, and those that have do not show appropriate neuronal subtype differentiation for neural repair (Ardelt et al., 2013). In contrast, using viral directed *in vivo* reprogramming, multiple groups have shown that GFAP$^+$ or NG2$^+$ glia that are reprogrammed to generate neurons that are electrophysiologically functional can integrate into the endogenous circuitry of either the normal or damaged brain (Kronenberg et al., 2010; Heinrich et al., 2014; Niu et al., 2015). Interestingly, in the normal brain Niu et al. (2015) additional signalling molecules were required to promote maturation of their reprogrammed neuroblasts. Noggin, BDNF and valproic acid was used and cells matured into functional caltretinin$^+$ neurons. The finding that both recruited SVZ cells and GFAP$^+$ reprogrammed neurons both preferentially generate calretinin$^+$ neurons is important, because to the majority of the striatal population lost through MCAO or the neurodegenerative disease Huntington's disease are DARPP32$^+$ medium spiny striatal neurons, not the calretinin+ interneuron population. In the lesioned cortex, many newborn neuroblasts also remained immature, perhaps because the micro-environment surrounding the areas of damage was either inhibiting or lacking the appropriate signals for neuronal maturation (Heinrich et al., 2014).

The intrinsic gene programmes that direct neurogenesis are now well characterised, but what are the all-important micro-environmental signals that are over-riding the neuronal programmes in recruited cells, or inhibiting subtype specification and maturation of neuroblasts? It is likely that there are a multitude of factors working in combination, but factors from major signalling families have been implicated to date. BMP signal antagonism by chordin was shown to drive the neuronal to oligodendroglial fate change after demyelination, conversely, inhibition by NOGGIN promoted maturation of reprogrammed neuroblasts in the striatum (Jablonska et al., 2010; Niu et al., 2015). Alterations in BMP signal pathway molecules were also found within the SVZ after QA damage of the striatum, with both *Noggin* significantly increased three days post injury, and *Inhibin βA* (a putative BMP antagonist) and *Bmp2* significantly downregulated for 7 days following injury (unpublished data, Jones and Connor) (Jones and Connor, 2016). These contrasting effects from BMP signalling indicate how understanding injury- and time-dependent signalling is crucial for determining which molecules are important for each model. Further, inhibition of Notch signalling has been shown to be crucial for stimulating endogenous neurogenesis in striatal astrocytes, and downregulation of *Notch* ligands were also identified in the SVZ after QA lesioning (Magnusson et al., 2014; Jones and Connor, 2016). Chemokines also play a large role in recruitment of neuroblasts from endogenous neurogenic regions, they can influence neuronal-oligodendroglial fate specification and modulate synaptic transmission (Connor et al., 2011; Ardelt et al., 2013). With the ability to promote neurogenesis after injury using multiple endogenous and exogenous methods, the focus on finding key molecules to promote these processes to enable functional recovery is the next big step. There is much work to be done, but it is an exciting time to be researching neural regeneration.

Kathryn S. Jones[*], Bronwen Connor

Department of Pharmacology and Clinical Pharmacology, Centre for Brain Research, School of Medical Sciences, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand

*****Correspondence to:** *Kathryn S. Jones, Ph.D.,
ks.jones@auckland.ac.nz.*

References

Ardelt AA, Bhattacharyya BJ, Belmadani A, Ren D, Miller RJ (2013) Stromal derived growth factor-1 (CXCL12) modulates synaptic transmission to immature neurons during post-ischemic cerebral repair. Exp Neurol 248:246-253.

Connor B, Gordon RJ, Jones KS, Maucksch C (2011) Deviating from the well travelled path: Precursor cell migration in the pathological adult mammalian brain. J Cell Biochem 112:1467-1474.

Guo Z, Zhang L, Wu Z, Chen Y, Wang F, Chen G (2014) In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell Stem Cell 14:188-202.

Heinrich C, Bergami M, Gascon S, Lepier A, Vigano F, Dimou L, Sutor B, Berninger B, Gotz M (2014) Sox2-mediated conversion of NG2 glia into induced neurons in the injured adult cerebral cortex. Stem Cell Rep 3:1000-1014.

Heinrich C, Blum R, Gascon S, Masserdotti G, Tripathi P, Sanchez R, Tiedt S, Schroeder T, Gotz M, Berninger B (2010) Directing astroglia from the cerebral cortex into subtype specific functional neurons. PLoS Biol 8:e1000373.

Inta D, Gass P (2015) Is forebrain neurogenesis a potential repair mechanism after stroke? J Cereb Blood Flow Metab 35:1220-1221.

Jablonska B, Aguirre A, Raymond M, Szabo G, Kitabatake Y, Sailor KA, Ming GL, Song H, Gallo V (2010) Chordin-induced lineage plasticity of adult SVZ neuroblasts after demyelination. Nat Neurosci 13:541-550.

Jones KS, Connor BJ (2016) The effect of pro-neurogenic gene expression on adult subventricular zone precursor cell recruitment and fate determination after excitotoxic brain injury. J Stem Cells Regen Med 12:25-35.

Kronenberg G, Gertz K, Cheung G, Buffo A, Kettenmann H, Gotz M, Endres M (2010) Modulation of fate determinants Olig2 and Pax6 in resident glia evokes spiking neuroblasts in a model of mild brain ischemia. Stroke 41:2944-2949.

Magnusson JP, Goritz C, Tatarishvili J, Dias DO, Smith EM, Lindvall O, Kokaia Z, Frisen J (2014) A latent neurogenic program in astrocytes regulated by Notch signaling in the mouse. Science 346:237-241.

Nato G, Caramello A, Trova S, Avataneo V, Rolando C, Taylor V, Buffo A, Peretto P, Luzzati F (2015) Striatal astrocytes produce neuroblasts in an excitotoxic model of Huntington's disease. Development 142:840-845.

Niu W, Zang T, Smith DK, Vue TY, Zou Y, Bachoo R, Johnson JE, Zhang CL (2015) SOX2 reprograms resident astrocytes into neural progenitors in the adult brain. Stem Cell Rep 4:780-794.