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Commentary

Emerging cues mediating astroglia lineage restriction of progenitor cells in the injured/diseased adult CNS

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ABSTRACT

Other than specific neurogenic regions, the adult central nervous system (CNS) is not conducive for neuronal regeneration and neurogenesis, particularly at sites of injury or neurodegeneration. Engraftment of neural stem/progenitor cells into non-neurogenic regions or sites of injury/disease invariably results mainly in astroglia differentiation. The reasons for such a lineage restriction have not been well defined. Recent findings have brought to light some underlying novel mechanistic basis for this preferential differentiation into astroglia. The more oxidized state of pathological brain tissue leads to upregulation of the protein deacetylase sirtuin 1 (Sirt1). Sirt1 appears to stabilize a co-repressor complex of *Hairy/enhancer of split* (*Hes*)1, thereby suppressing expression of the proneuronal transcription factor Mash1, and directs progenitor cell differentiation towards the glia lineage. Sirt1 upregulated by CNS inflammation may also inhibit neuronal differentiation. Myelin-associated inhibitors such as Nogo, acting through the Nogo-66 receptor (NgR), also appear to promote neural stem/progenitor cell differentiation into astrocytes. Understanding the molecular basis of glia lineage restriction of neural progenitors in the injured or diseased CNS would provide handles to improving the success of stem cell-based transplantation therapy.

1. Introduction

Other than restricted regions such as the hippocampal dentate gyrus (Eriksson et al., 1998) and the subventricular zone (SVZ) (Doetsch et al., 1999), the mammalian adult central nervous system (CNS) is apparently not conducive for neurogenesis. Endogenous neural stem/progenitor cells (NPCs) do exist in the adult CNS (Gage, 2000). CNS injury could in fact lead to increase in proliferation of these endogenous NPCs. Upon migration of these cells to injury sites, however, they differentiate mainly into astrocytes (Johansson et al., 1999). NPCs transplanted into neurogenic regions of the brain could potentially result in significant neuronal differentiation (Fricker et al., 1999; Shihabuddin et al., 2000). However, engraftments into non-neurogenic regions of the CNS give rise mainly to astrocytes and not neurons (Fricker et al., 1999; Cao et al., 2001, 2002; Yang et al., 2002; Seidenfaden et al., 2006). Thus, glial fate specification appears to occur in most regions of intact mammalian adult CNS. This restriction of neural progenitors to astroglia cell fate in the adult CNS represents a tremendous hurdle in developing stem cell-based therapies (whether through the activation of endogenous adult neural stem cells or via transplantation of neural progenitors to lesion sites) for brain pathologies, particularly in cases where functional neuronal replacement is critical.

Broadly speaking, there are several possible explanations as to why NPCs tend to differentiate along the astroglia lineage in adult brain. Firstly, the *in vivo* differentiation potential of these cells may be somewhat limited and neuronal differentiation may be intrinsically difficult. However, the neurogenic potential of NPCs isolated from both fetal and adult sources that were subsequently transplanted into neurogenic regions has been demonstrated. In fact, the activation of endogenous neural progenitors, augmented by infusion of growth factors, could aid regeneration of hippocampal pyramidal neurons after ischemic brain injury (Nakatomi et al., 2002). Retroviral-mediated expression of neurogenic transcription factors, in combination with growth factors, could also enhance neurogenesis by endogenous neural progenitors in injured adult spinal cord (Ohori et al., 2006). It would appear that cues at the local CNS environment may play a more prominent role in glia cell fate promotion. In this regard, it is worth noting that transplantation experiments are usually accompanied by immunosuppressive measures, particularly for cross-species heterologous grafting. A recent report involving the grafting of a human CNS stem cell line (hCNS-SCns) into a spinal cord injury (SCI) model of immune-compromised NOD-*scid* mice claimed that the experiment resulted in differentiation into

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largely oligodendrocytes and neurons, with minimal astrocytic differentiation (Cummings et al., 2005). An immune-compromised CNS environment induced by drugs or resulting from genetic manipulations of the immune system may therefore be more conducive for neurogenesis than a normal CNS.

The nature of glia differentiation restrictive cues acting on neural progenitors in a diseased or injured non-neurogenic part of the adult CNS was unclear. In general, these cues could be either the ones that actively induce differentiation along glia lineages or the ones that suppress differentiation into neurons. Notch signaling appears to play a key role in instructive determination of neural progenitor cells to an astroglial fate during development (Yamamoto et al., 2001; Tanigaki et al., 2001; Gaiano and Fishell, 2002; Grandbarbe et al., 2003), and the astrogliogenic response of the SVZ to injury has been shown to be accompanied by activation of the Notch pathway (Givogri et al., 2006). Notch signaling regulates the expression of members of the *Hairy/enhancer of split* (*Hes*) genes, which encode basic helix–loop–helix transcription factors that maintain neural progenitors and suppress neuronal differentiation (Kageyama et al., 2005; Shimojo et al., 2008). *Hes* genes antagonize the activator-type genes such as *Mash1*, *Math* and *Neurogranin*, also of the basic helix–loop–helix transcription factor family (Kageyama et al., 2005), which promote neuronal differentiation.

The bone morphogenetic proteins (BMPs) have been shown to promote astroglia lineage commitment of NPCs (Gross et al., 1996; Nakashima et al., 2001), an activity that is antagonized by noggin (McMahon et al., 1998; Lim et al., 2000; Hampton et al., 2007). CNS injuries are known to induce the expression of certain BMPs (Setoguchi et al., 2001, 2004; Hampton et al., 2007). Exogenous noggin expression has been shown to alter the fate of neural stem cells transplanted into injured spinal cord (Setoguchi et al., 2004), although this may not necessarily result in a better outcome at the lesion site (Enzmann et al., 2005). BMP signaling in adult NPCs could in fact be important for initiating the neurogenic lineage in the adult mouse subependymal zone, as both noggin infusion and conditional knockout of downstream Smad4 decreased neurogenesis and increased oligodendroglial differentiation (Colak et al., 2008). CNS lesioning also results in an elevation of the levels of proinflammatory neuropoietic cytokines (Bauer et al., 2007) such as leukemia inhibitory factor (LIF) (Banner et al., 1997; Kerr and Patterson, 2004) and ciliary neurotrophic factor (CNTF), as well as CNTF receptors (Oyesiku et al., 1997; Kirsch et al., 1998). These could potentially drive astrogliogenesis via the activation of the Janus kinase/signal transducers and activators of transcription (JAK–STAT) pathway (Bonni et al., 1997; Rajan and McKay, 1998; Aberg et al., 2001; Xia et al., 2002).

The pathological adult CNS is understandably complex in terms of environmental factors that might influence the survival and proliferative capacities, as well as the fate of NPCs. Recent findings have revealed several novel clues as to the nature of astroglia lineage restrictive cues in the pathological adult CNS. We discuss below these recent findings in the light of what is currently known about the CNS function of these cues.

2. Redox-dependent fate of neural progenitors in the pathological adult brain

Cells in the injured or diseased brain usually suffer from varying degrees of oxidative stress (Chong et al., 2005; Bayir et al., 2006; Slemmer et al., 2008). Severe oxidative stress would of course result in impairment of survival and cell death (Mazur-Kolecka et al., 2006). However, even subtle or non-lethal alteration of redox state may influence the proliferation and differentiation of NPCs. This was aptly illustrated in a recent study by Prozorovski et al. (2008).

The authors showed that pro-oxidants reduced the proliferation of embryonic mouse cortical NPCs in culture, whereas reducing agents had the opposite effect. These NPCs are multipotent and could differentiate into neurons, astrocytes or oligodendrocytes when manipulated by growth factor (basic fibroblast growth factor (bFGF)) withdrawal in culture. However, when pro-oxidants were added, there was a significant increase in astroglia differentiation with a corresponding decrease in neuronal differentiation. The authors noticed that, while levels of the general neural transcription factors such as NeuroD, Hes1, Hes5, Olig1 and Olig2 did not change significantly with pro-oxidant treatment, there was a marked upregulation of the histone deacetylase Sirt1. Sirt1 is the closest mammalian homologue to yeast silent information regulator (Sir)-2, a member of the sirtuin family of protein deacetylases, which have gained much attention as a mediator of longevity associated with caloric restriction in several model organisms (Haigis and Guarente, 2006; Michan and Sinclair, 2007; Tang and Chua, 2008). Sirt1 upregulation is apparently responsible for the astrocyte differentiation bias induced by pro-oxidants, as activation of Sirt1 by resveratrol mimicked, while Sirt1 silencing attenuated this bias.

How does Sirt1 induction by pro-oxidants result in astroglia lineage restriction? Sirt1 immunoprecipitation analysis revealed that the pro-oxidative conditions induced the activation of a Sirt1–Hes1 complex and deacetylation of the lysine 9 residue of histone H3, a putative target of Sirt1. Both pro-oxidants and the Sirt1 activator, resveratrol, markedly repressed the expression of the neurogenic transcription factor Mash1. The Sirt1–Hes1 complex was involved in this repression, as repression was abolished by the silencing of either Sirt1 or Hes1, and also by Sirt1 inhibitors, and did not occur in *Sirt1*$^{-/-}$ progenitor cells. Chromatin immunoprecipitation studies revealed that oxidative conditions increased Sirt1 interaction with *Mash1*'s promoter region and reduced H3K9 acetylation rather specifically at this promoter. *Mash1*'s expression upon exposure of NPCs to neurogenic factors such as platelet-derived growth factor (PDGF) involves the dissociation of the co-repressor transducin-like enhancer of split 1 (TLE1) (Ghosh et al., 2007) and the assembly of a histone acetyltransferase-containing co-activator complex containing the CREB-binding protein (CBP) at the *Mash1* promoter. Under oxidative conditions, Sirt1 appeared to stabilize the TLE1-containing co-repressor complex and prevents the formation of the CBP-containing co-activator complex.

Prozorovski et al. had extended their findings *in vivo* using mouse models. Sirt1-positive cells, like Mash1-positive cells, could be found at the neurogenic region SVZ and along the rostral migratory stream to the olfactory bulb, but very few cells were positive for both Sirt1 and Mash1. Pups that received pro-oxidants had elevated Sirt1 at the SVZ, which were mainly positive for the astrocyte marker glial fibrillary acidic protein (GFAP). This was accompanied by a reduction in Mash1-positive cells and a general decrease in cell proliferation. Antioxidants also reduced proliferating cells that expressed the neuroblast marker doublecortin in the rostral migratory stream. Again, silencing of either Sirt1 or Hes1 by *in utero* electroporation of shRNAs attenuated the effect of the pro-oxidants. Importantly, Sirt1 silencing *in utero,* or subcutaneous administration of a Sirt1 inhibitor, appeared to increase the proportion of Mash1-positive cells. In experimental autoimmune encephalomyelitis (EAE) (mouse model for the human disease multiple sclerosis) brain, inflammation and oxidative stress resulting from the generation of reactive oxygen species (ROS) by infiltrating macrophages are prevalent (Gilgun-Sherki et al., 2004). Sirt1 levels were specifically elevated in the nuclei of GFAP-positive cells in brain inflammation regions. Resveratrol