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SOX2 Reprograms Resident Astrocytes into Neural Progenitors in the Adult Brain

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SUMMARY

Glial cells can be in vivo reprogrammed into functional neurons in the adult CNS; however, the process by which this reprogramming occurs is unclear. Here, we show that a distinct cellular sequence is involved in SOX2-driven in situ conversion of adult astrocytes to neurons. This includes ASCL1$^+$ neural progenitors and DCX$^+$ adult neuroblasts (iANBs) as intermediates. Importantly, ASCL1 is required, but not sufficient, for the robust generation of iANBs in the adult striatum. These progenitor-derived iANBs predominantly give rise to calretinin$^+$ interneurons when supplied with neurotrophic factors or the small-molecule valproic acid. Patch-clamp recordings from the induced neurons reveal subtype heterogeneity, though all are functionally mature, fire repetitive action potentials, and receive synaptic inputs. Together, these results show that SOX2-mediated in vivo reprogramming of astrocytes to neurons passes through proliferative intermediate progenitors, which may be exploited for regenerative medicine.

INTRODUCTION

Neurons in the CNS are particularly sensitive to injury and degenerative conditions that frequently result in cell death. Although adult neurogenesis persists in restricted brain areas (Gage, 2000; Hsieh, 2012; Kriegstein and Alvarez-Buylla, 2009; Lie et al., 2004), neurons do not regenerate in most regions of the adult CNS. An unmet challenge in neural injury and degeneration repair is how to replenish lost neurons for functional recovery.

Cell fate reprogramming provides new means for regenerating damaged or dead neurons (Arlotta and Berninger, 2014; Cherry and Daley, 2012; Matsui et al., 2014). Not only can cells in culture be reprogrammed into pluripotent stem cells (Takahashi and Yamanaka, 2006), lineage-restricted stem cells (Kim et al., 2011a; Ring et al., 2012), and different postmitotic cell fates (Heinrich et al., 2010, 2011; Karow et al., 2012; Liu et al., 2013; Vierbuchen et al., 2010), but they are also amenable to in vivo fate conversion (Guo et al., 2014; Heinrich et al., 2014; Niu et al., 2013; Qian et al., 2012; Song et al., 2012; Su et al., 2014a, b; Torper et al., 2013; Zhou et al., 2008). In regards to the CNS, resident glial cells have been directly or indirectly converted into functional neurons in the adult brain and spinal cord (Guo et al., 2014; Heinrich et al., 2014; Niu et al., 2013; Su et al., 2014a, b; Torper et al., 2013). Glial cells are broadly distributed and comprise nearly half of the cells in the mammalian CNS. These cells become reactive, proliferate, and form glial scars in response to neural injuries and degeneration (Karimi-Abdolrezaee and Billakanti, 2012; Sofroniew, 2009). These reactive responses are initially beneficial, restricting the spread of damage, but ultimately are deleterious, acting as both a physical and chemical barrier to neuronal regeneration (Karimi-Abdolrezaee and Billakanti, 2012; Sofroniew, 2009). Reprogramming some of these glial cells to functional neurons may constitute a novel therapeutic strategy for diseases associated with the CNS.

Through in vivo screens of candidate factors that are able to induce neurogenesis in non-neurogenic regions of the adult brain and spinal cord, we previously showed that the ectopic expression of SOX2 is sufficient to reprogram resident astrocytes to DCX$^+$ induced adult neuroblasts (iANBs) (Niu et al., 2013; Su et al., 2014a). These iANBs pass through a proliferative state and generate mature neurons when supplied with neurotrophic factors. This SOX2-driven in vivo reprogramming process sharply contrasts direct lineage conversion strategies (Guo et al., 2014; Qian et al., 2012; Song et al., 2012; Su et al., 2014b; Torper et al., 2013; Zhou et al., 2008), which change cell fate in a linear fashion without amplification of the induced cell population. However, the cellular mechanism underlying SOX2-dependent in vivo reprogramming of astrocytes was unclear. Furthermore, the subtypes of iANB-derived neurons were not well characterized. In this study, we reveal that SOX2-driven reprogramming of astrocytes transits through intermediate neural progenitor states before the adoption of a mature neuron fate. Immunohistochemistry and electrophysiology further show that induced

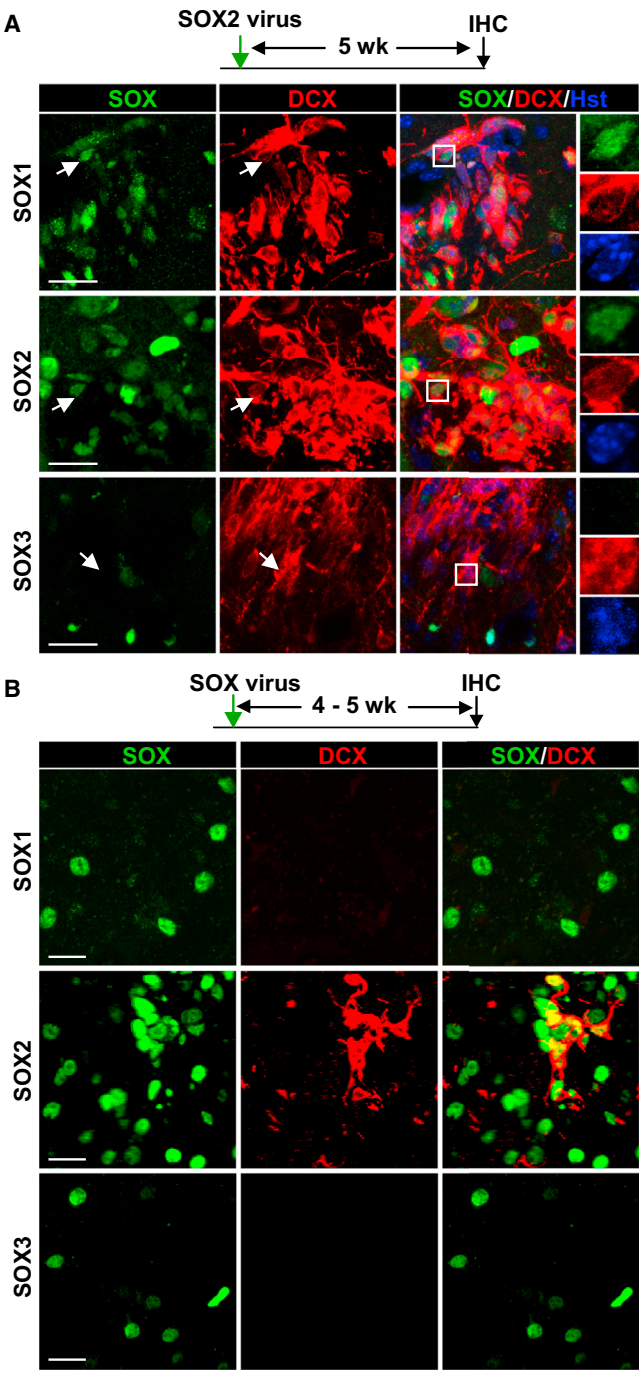

Figure 1. Expression and Reprogramming Ability of SOXB1 Factors

(A) Immunohistochemistry (IHC) showing expression of SOXB1 factors in striatal regions with iANBs at 5 weeks post-injection (wpi) of SOX2 virus. SOX1 and SOX2, but not SOX3, can be identified in some of the induced DCX$^+$ cells (right panels at higher magnifications). Hst, Hoechst 33342. The scale bars represent 20 μm.

(B) Ectopic SOX2, but not the other SOXB1 factors, induce striatal DCX$^+$ cells. Lentivirus expressing individual SOXB1 factors was injected into the adult mouse striatum and examined 4 or 5 weeks (wk) later. The scale bars represent 20 μm.

See also Figure S1.

neurons are functionally mature and predominantly express the marker calretinin.

RESULTS

Ectopic Expression of SOX2, but Not the Other SOXB1 Factors, Results in iANBs

SOX2 belongs to the SOXB1 subfamily of high-mobility group-box transcription factors, which also includes SOX1 and SOX3 (Sarkar and Hochedlinger, 2013). These factors are critical for specifying and maintaining the undifferentiated state of neural precursors. The expression of SOXB1 factors during SOX2-mediated in vivo reprogramming were investigated using immunohistochemistry (Niu et al., 2013). The adult mouse striatum was injected with lentivirus expressing SOX2 under the human *GFAP* promoter. When analyzed 4 weeks post-injection (wpi), DCX$^+$ iANBs were robustly detected in the virus-injected regions, confirming our previous results (Niu et al., 2013). Interestingly, SOX1 can also be detected in striatal regions with iANBs, especially in DCX$^+$ cell clusters (Figure 1A). However, SOX1 expression is lower in iANBs than neighboring DCX$^-$ cells, which is consistent with previous findings that the continued high-level expression of SOXB1 factors prohibits neuronal differentiation (Bylund et al., 2003; Niu et al., 2013). In contrast, SOX3 is only sporadically distributed in the adult striatum and is not detectable in DCX$^+$ iANBs (Figure 1A). Due to sequence and potential functional similarity (Bylund et al., 2003), we investigated whether the SOXB1 proteins can similarly induce DCX$^+$ cells. Lentiviruses expressing SOX1 or SOX3 under the *GFAP* promoter were individually injected into the adult striatum. However, when examined at 4 or 5 wpi, no DCX$^+$ cells were detected in striatal regions with ectopic SOX1 or SOX3 expression, in sharp contrast to areas injected with SOX2-expressing virus that contain DCX$^+$ cells (Figure 1B). Together, these data indicate that SOX2 has a unique property among the SOXB1 factors, enabling the reprogramming of resident astrocytes to iANBs.

SOX2-dependent iANBs in the adult striatum were further examined by injections of lentivirus expressing GFP-T2A-SOX2 under the *GFAP* promoter (Figure S1). The co-expressed stable GFP marked virus-transduced cells. Immunohistochemical analysis showed that about 23.2% ± 5.3% of GFP$^+$ cells (mean ± SD; n = 17,841 GFP$^+$ cells counted in sections from three mice) stained positive for DCX (Figure S1). This suggests a relatively efficient induction of iANBs from SOX2-expressing cells.