In Vivo Reprogramming Neural Cell fate by Proneural Factors in the Postnatal Cerebral Cortex

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Direct lineage reprogramming of glial cells into induced neurons emerges as an experimental strategy towards brain repair (Leaman et al., 2022). Indeed, in an experimental model of epilepsy, glia-to-neuron conversion has exerted a disease modifying effect (Lentini et al., 2021). We are exploring the possibility of converting mouse astrocytes and oligodendrocyte progenitor cells into induced interneuron-like cells by over-expressing neuronal fate determinants such as the proneural gene achaete-scute complex like 1(Ascl1) in the mouse cerebral cortex *in vivo*.

Methods: C57Bl6/J or mGFAP-Cre (B6.Cg-Tg(Gfap-cre)77.6Mvs/2J, JAX024098) mouse pups received intracortical injections of retroviruses encoding reprogramming factors (wildtype Ascl1 or phospho-site mutant Ascl1SA6, w/o Bcl2) (UK Home Office, permits numbers PD025E9BC and PP8849003). Brain tissue was obtained at distinct stages following retroviral transduction (i.e., day 3, 12 and 28 post transduction) and analysed by immunohistochemistry and electrophysiology. In some experiments, animals received a single injection of 50 mg/kg at the time of retrovirus injection or 3 h prior to sacrifice.

Results: We found that retrovirus-mediated expression of wildtype Ascl1 causes selective activation of cell cycle activity in oligodendrocyte progenitor cells but not astrocytes in the early postnatal mouse cerebral cortex. In contrast, expression of the phospho-site mutant Ascl1SA6 (carrying replacement of six conserved serine residues by alanine) induced the conversion of astrocytes into interneuron-like cells exhibiting molecular and electrophysiological hallmarks of fast-spiking, Parvalbumin-positive interneurons.

Discussion: Our data indicate that cellular context and posttranslational modifications strongly modulate the outcome of proneural gene expression in cortical glia. We provide evidence that wildtype and mutant Ascl1 differentially impact neural fate decisions in cortical astrocytes and oligodendrocyte progenitor cells. This will inform our strategies towards modifying diseased brain circuits via *in vivo* reprogramming.

References: Leaman S., Marichal N., Berninger B. *Development* 149(4):dev200433 (2022). Lentini C., d'Orange M., Marichal N., Trottmann M.M., Vignoles R., Foucault L., Verrier C., Massera C., Raineteau O., Conzelmann K.K., Rival-Gervier S., Depaulis A., Berninger B, and Heinrich C. *Cell Stem Cell* 28(12):2104-2121.(2021).

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