Reprogram Astrocytes into Neurons by Small Molecules

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Abstract: Regeneration of functional neurons in neurodegenerative diseases or after brain injury holds great promise for future therapeutic strategies but remain a major challenge in the field. Current efforts are largely focused on cell replacement using exogenous cells derived from embryonic stem (ES) or induced pluripotent stem (iPS) cells. Despite great potential, such approaches still face huge hurdles such as potential immunorejection, tumorigenesis and differentiation uncertainty in vivo. We have recently demonstrated that reactive glial cells in the mouse brain can be directly reprogrammed into functional neurons by a single proneural transcription factor NeuroD1. Here we report that a combination of small molecules can replace NeuroD1 to reprogram human astrocytes in culture into fully functional neurons. After testing many small molecules in a variety of combinations, we found that a sequential exposure to a cocktail of small molecules can successfully reprogram human astroglial cells into neuron-like cells in 8 days. These human astrocyte-converted neurons can survive for more than 3 months in culture and form functional synaptic network, demonstrated by robust synchronous burst activities among the converted neurons. Extensive time-lapse imaging experiments showed that majority of the converted neurons were directly converted from astrocytes, in line with immune-staining results showing that there are no detectable neural progenitors or pluripotent cells in our human astrocytes. Moreover, after injected into the lateral ventricle of the mouse brain, the small molecule-reprogrammed human neurons can survive for at least one month and migrate out to integrate into local neural circuits. Our studies
open a new avenue using small molecules to reprogram reactive glial cells into functional neurons for brain repair.

**Key Words:** Human Primary Astrocyte; Chemical Conversion; Brain Repair; Brain Injury; Neurodegenerative Disease