In vivo direct lineage reprogramming as a therapy for neurodegeneration

This semester, we learned about cellular reprogramming to iPSCs with Yamanaka factors. Moreover, we learned that it's possible to directly convert one type of cell into another, skipping any pluripotent stage in between. I was particularly interested in the therapeutic applications of these reprogramming techniques, and direct lineage conversion was uniquely fascinating to me for its therapeutic potential, since direct reprogramming allows us to bypass transitioning a cell into a potentially tumorigenic intermediate pluripotent stage. As I read papers, discussed ideas, and listened to lectures, I wondered about how our knowledge of direct lineage conversion and its potential applications have grown since the method was first discovered. In this paper, I discuss some of the research into this topic that I've found particularly compelling.

Direct lineage reprogramming is possible between many different cell types (Ruzittu, Willnow and Spagnoli, 2020), and here I'm specifically interested in direct lineage reprogramming in the brain. As stated in Amamoto & Arlotta's 2013 review (Amamoto and Arlotta, 2013) of nervous system direct lineage conversion, one of the first studies into the topic took place in 2002 and found that "Pax6 expression instructs neurogenesis even in astrocytes from postnatal cortex *in vitro*." The researchers in that study hypothesized that Pax6 reprogramming would be possible by looking at expression patterns during development: Pax6 is expressed in radial glial cells which drive neurogenesis during development. (Heins *et al.*, 2002) This seems to be a trend for direct lineage conversion experiments: as Ninkovic and Gotz say in their 2018 review, "The choice [of genes utilized for reprogramming] is mostly for TFs that are very potent during development" (although, as they go on to say, these genes might not actually be the best candidates) (Ninkovic and Götz, 2018). Since then, direct lineage

conversion has come a long way. *In vitro* direct lineage conversion followed by transplantation can reproduce cell types lost in neurodegenerative disorders and transplanted cells can, in fact, re-integrate into brain circuitry. *In vivo* direct lineage conversion has been studied since at least 2013 (Torper *et al.*, 2013) and involves the delivery of "conversion genes" to a target brain region to create an overexpression of the TFs that facilitate the cell-type conversion. If all goes well, the reprogrammed cells can then form new connections and circuits with neighboring cells in the brain (Vierbuchen *et al.*, 2010), and ultimately function as non-converted cells would.

The therapeutic applications of *in vivo* direct lineage reprogramming have long been suggested and theorized, and new studies seem to be moving in an exciting therapeutic direction: *in vivo* direct lineage reprogramming as a treatment for brain injury and neurodegeneration. As was mentioned in class, Parkinson's Disease mice showed cognitive improvement after *in vivo* direct lineage conversion to create induced dopaminergic as early as 2017 (in this study, the conversion was actually facilitated by the use of electromagnetized gold nanoparticles and a specific EMF frequency which increased Brd2 activation, notable because the procedure is safe and noninvasive) (Yoo *et al.*, 2017).

A 2020 study discusses a gene-therapy approach to *in vivo* astrocyte-to-neuron conversion for "functional brain repair." Using an AAV to deliver the TF NeuroD1, the researchers convert reactive astrocytes into neurons in large quantities, a task that had proved challenging in previous *in vivo* direct lineage conversion studies. They were ultimately able to "regenerate 30%–40% of lost neurons in the motor cortex of adult mice." Moreover, the researchers measured cognitive improvement after the neuronal loss from the injury: "behavioral tests indicate that NeuroD1-treatment significantly rescues both motor and fear memory deficits after ischemic injury in rodent animals." The neurons converted

from astrocytes matured in a matter of weeks, the converted neurons intermingled with non-converted (pre-existing) neurons, possibly aiding in the post-stroke recovery of the preexisting neurons, and formed long-range connections with other brain regions (Chen *et al.*, 2020).

I found these results fascinating. In high school, while working in a lab studying Alzheimer's Disease (AD), I found myself wondering if brain regions experiencing neuronal death during neurodegeneration could be "grown back." Yoo et al.'s results suggest that *in vivo* reprogramming can be used to restore regions of the brain in neurodegenerative disorders, and Chen et al.'s results suggest that after a brain injury, *in vivo* reprogramming could not just restore (some) functionality but also "regenerate" the neurons in the injured regions.

In AD, tau and amyloid-beta pathology might complicate the matter of "brain regeneration" since, as opposed to a nervous system injury like a stroke, there are upstream factors that might cause the neurodegeneration. However, as Yavarpour-Bali et al. discuss in their review of cellular reprogramming as a potential therapy for AD (Yavarpour-Bali, Ghasemi-Kasman and Shojaei, 2020), the results have been promising so far. For example, in 2014, Guo et al. used NeuroD1 (the same TF used by Chen et al. to restore brain regions after a stroke) to convert reactive glial cells in an AD mouse model into neurons *in vivo* and observed that the neurons integrated into new circuits (Guo *et al.*, 2014). And four years later, in 2018, Ghasemi-Kasman et al. *in vivo* reprogrammed reactive glial cells into neurons in an AD mouse model via a microRNA injection, and not only detected new induced neurons after 6 months but also observed improvements in the mice's spatial memories (Ghasemi-Kasman *et al.*, 2018). The future of *in vivo* cell reprogramming for therapy is so exciting, and I'm looking forward to seeing what comes next — and perhaps even playing a role in it.

References

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