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Novel Direct Conversion of Microglia to Neurons

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Abstract

Direct cell reprogramming, the process by which a somatic cell is converted to another cell type, can potentially circumvent epigenetic changes and proliferative stages resulting from de-differentiation. Recently, Matsuda *et al.* (Pioneer-factor NeuroD1 rearranges transcriptional and epigenetic profiles to execute microglia-neuron conversion; *Neuronin* in press) demonstrated that expression of transcription factor NeuroD1 can convert mouse microglia to neurons, both *in vitro* and *in vivo*.

The generation of neurons from human induced pluripotent stem cells (hiPSCs) represents a breakthrough for transplantation paradigms and for studying mechanisms of neurodegenerative diseases; it also provides a novel system for drug development [1]. However, the reprogramming process requires an intermediate proliferative stage and is very time intensive and laborious. Moreover, the process of reprogramming to generate hiPSCs removes the epigenetic changes that reflect cell aging. Recent advances in cell reprogramming have generated methods for direct reprogramming (also termed direct conversion or transdifferentiation) of fibroblasts to neurons using defined transcription factors (TFs) and/or microRNAs [2–5]. Direct reprogramming of glial cells to neurons may have therapeutic implications, allowing cell replacement *in vivo* [6]. While direct conversion of astrocytes to neurons has been reported previously [7], using this approach as a potential treatment for neurodegenerative disorders `raises some concerns that these astrocytes may be gliotic, meaning reactive, hypertrophic, or dysfunctional. To provide another option, Matsuda *et al.* [8] endeavor to convert microglia directly to neurons, and their new approach is the first study to accomplish this, albeit in mouse.

Matsuda *et al.* [8] tested various TFs, using an unbiased approach, and found that the TF NeuroD1 (ND1), which has been previously used to convert astrocytes to neurons [9], had the highest efficiency of converting microglia to neurons (Figure 1). The previous report of direct reprogramming of astrocytes used retroviral expression of ND1 under an astrocyte-specific promoter, whereas Matsuda *et al.* [8] expressed ND1 using lentiviral expression under a microglia-specific promoter. Matsuda *et al.* [8] report a conversion efficiency of about 25–35%, which is considered a reasonably high rate for direct conversion. By

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immunostaining, the resulting cells were positive for the neuronal markers βIII-tubulin-and Map2ab, representing well-established markers for neurons. By additional immunocytochemical criteria, most of the cells were excitatory (~75%), but there was a large percentage of inhibitory neurons as well (~25%). Further characterization of neuronal cells types will be important to determine how cell-type distribution will affect attempts at cell replacement therapy for various brain regions. *In vitro*, the authors also demonstrated that the cells were functional by calcium imaging and electrophysiological analysis. Global gene expression analysis showed that microglial-derived neurons displayed a similar gene expression profile to that of primary neurons by principal component analysis.

To understand how ND1 acts on the genome to convert microglia to neurons, Matsuda *et al.* [8] performed ChIP-seq analysis and demonstrated that ND1 occupied genes that are associated with neuronal development and differentiation. Next, using ChIP-seq and whole-genome bisulfite sequencing, the authors studied epigenetic changes, including DNA methylation and histone modification, and reported that ND1 preferentially bound unmethylated CpG-rich regions. However, whether this contributes to changes in gene expression is not clear as there was no observed difference between upregulated and unchanged genes. Further analysis showed that ND1 accessed closed chromatin with bivalent modifications and induced the expression of neuronal development-and differentiation-related genes. The observed histone modifications in the induced neurons were similar to those observed in primary neurons but less frequent, suggesting that the conversion time may need to be extended to reach complete epigenetic change to more closely simulate primary neurons. The authors also identified 20 TFs that bear a bivalent domain that contains both H3K4 and H3K27 methylations, two modifications that have opposing effects, positively or negatively regulating transcription, respectively [10]. Matsuda *et al.* [8] demonstrated that three of these TFs, namely, Bhlhe22, Prdm8, and Myt1l, are individually sufficient to convert microglia to neurons. In addition, the authors identified several microglial TFs that are downregulated following ND1 expression and showed that ND1 suppressed the expression of two of these TFs, Mafb and Lyl1, which are known to regulate microglial cell identity.

To show that direct conversion of microglia to neurons can also be achieved *in vivo*, Matsuda *et al.* [8] expressed ND1 in the adult mouse brain under the microglial-specific CD68 promoter using a lentiviral vector injected into the striatum. Two weeks post-transduction, 33–50% of the transduced cells displayed the neuronal markers βIII-tubulin and Map2ab. Four weeks post-transduction 75% of the transduced cells were positive for DARPP32, a marker for striatal projection neurons.

Cell therapy in general and neuronal replacement in particular are important avenues for future therapy for neurodegenerative diseases. Matsuda *et al.* [8] provide an efficient process to use existing microglia, which may also be increased in number due to recruitment, activation, and proliferation [11] during the neurodegenerative process, to replace neuronal populations. Further work needs to address the functionality of these converted neuronal cells *in vivo* and improve the conversion efficiency in mouse models of neurodegeneration. It will be important to compare the yield of microglia from healthy versus diseased mouse brains as well as potential functional differences of the converted cells under these disparate

circumstances. Critically, it will be important to examine whether these microglial-converted neurons can become functional neurons and integrate into neural networks using calcium imaging and patch-clamp recording methods in 3D cerebral organoid models as well as *in vivo* models. Moreover, future research should aim to validate this work in a human context and investigate whether human microglia can also undergo direct conversion to functional neurons. Altogether, Matsuda *et al.* [8] demonstrate that direct conversion of microglia to neurons is feasible with a single transcription factor, thus contributing to the approach of direct conversion and cell replacement therapy. Along these lines, many exciting studies remain to be performed.

References

1. Takahashi K et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872 [PubMed: 18035408]
2. Vierbuchen T et al. (2010) Direct conversion of fibroblasts to functional neurons by defined factors. Nature 463, 1035–1041 [PubMed: 20107439]
3. Ambasudhan R et al. (2011) Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. Cell Stem Cell 9, 113–118 [PubMed: 21802386]
4. Pang ZP et al. (2011) Induction of human neuronal cells by defined transcription factors. Nature 476, 220–223 [PubMed: 21617644]
5. Yoo AS et al. (2011) MicroRNA-mediated conversion of human fibroblasts to neurons. Nature 476, 228–231 [PubMed: 21753754]
6. Kim J et al. (2012) Direct lineage reprogramming to neural cells. Curr. Opin. Neurobiol. 22, 778–784 [PubMed: 22652035]
7. Heinrich C et al. (2010) Directing astroglia from the cerebral cortex into subtype specific functional neurons. PLoS Biol. 8, e1000373 [PubMed: 20502524]
8. Matsuda T et al. Pioneer factor NeuroD1 rearranges transcriptional and epigenetic profiles to execute microglia-neuron conversion. Neuron (in press)
9. Guo Z et al. (2014) *In vivo* direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell Stem Cell 14, 188–202 [PubMed: 24360883]
10. Bernstein BE et al. (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125, 315–326 [PubMed: 16630819]
11. Perry VH and Holmes C (2014) Microglial priming in neurodegenerative disease. Nat. Rev. Neurol. 10, 217–224 [PubMed: 24638131]

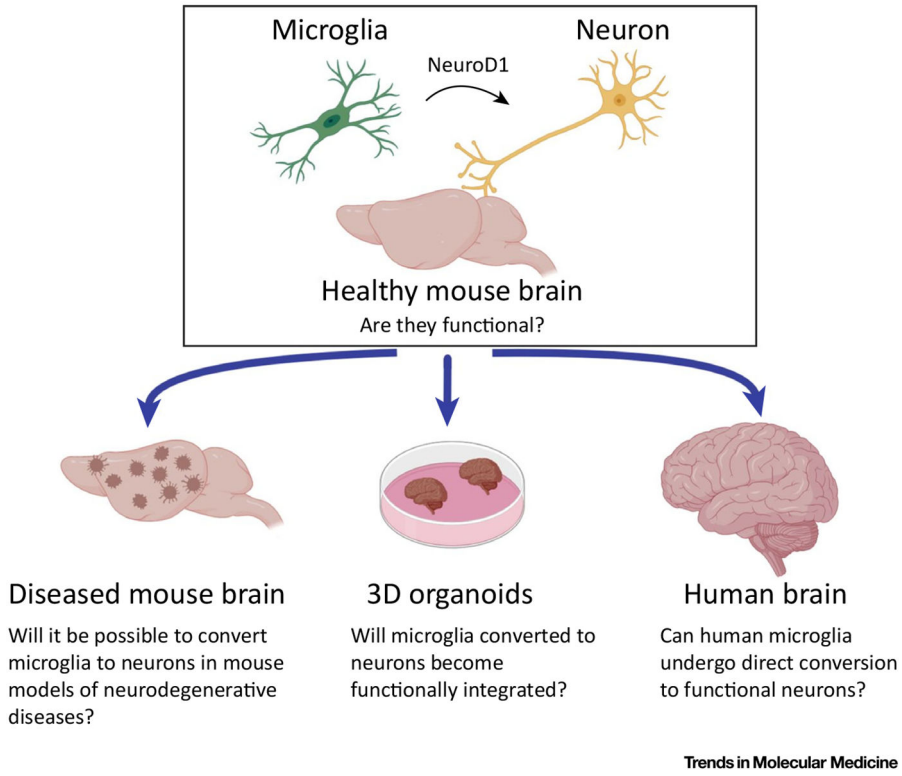

Figure 1. Direct Conversion of Microglia to Neurons.

Forced expression of NeuroD1 in mouse microglia directly converted the cells to neurons, as evidenced by immunostaining for βIII-tubulin, Map2ab, and other neuronal markers, and downregulated microglial markers. Moreover, *in vitro* the directly converted neurons displayed action potentials and synaptic currents by patch-clamp recording. Questions for the future are outlined at the bottom of the figure.