

## **Commentary to: Pharmacological Reprogramming of Fibroblasts into Neural Stem Cells by Signaling-Directed Transcriptional Activation**

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The unlimited proliferative and developmental potential harbored by stem cells promised to be the ideal source of unlimited testing and transplant material necessary to cure many human diseases. In 2006 induced pluripotent stem cells (iPSCs) eliminated host versus graft problems for stem cell therapy and provided new disease modeling avenues (1). Since then, a wide variety of articles on the differentiation of iPSCs towards specific cell lineages again have promised to provide the long-awaited host-specific models and cellular sources to study various human diseases (2, 3). However, iPSCs are routinely virally reprogrammed and highly variable (1, 4), which raises concerns about their tumorigenicity and reproducibility. Unlike viral reprogramming, small molecules interact with the pre-existing molecular machinery and so can bypass any dormant virus-related tumorigenicity. Also, small molecules could potentially reduce the variability of reprogramming and subsequent differentiation of iPSCs, given the robustness of their production and optimization.

Zhang and colleagues recently endeavored to obtain neural stem cells (NSCs) through pharmacological reprogramming (5). Their study begins with a very elegant and stringent selection of aged mouse embryonic fibroblasts (MEFs). Using aged fibroblasts, as opposed to the total fibroblast population, is a critical aspect of the study since it excludes the possibility of stem cell-like cells being carried over in the fibroblast cultures obscuring their results. Moreover, after a 15-day differentiation of these cultures, the authors did not detect any Tuj1+ cells, confirming the low neurogenic potential of their baseline cultures. Four previously described compounds that affect BMP and TGF inhibition of mesoderm and endoderm specification (6, 7) and GSK3 and bFGF promotion of neural development (8, 9) were used as a basal cocktail in a screen for additional compounds. An initial screen revealed Hh-Ag1.5, an agonist of the Smoothed (Smo) receptor that activates sonic hedgehog (Shh) signaling, and retinoic acid (RA) as potential candidates, whereas a second screen identified RG108, Parnate and SMER28 as critical components of the final compound cocktail, consistent with the importance of DNA methylation, histone modification and autophagy, respectively, in cell reprogramming (10, 11).

Chemically induced neural stem-like cells (ciNSLCs) generated with these compounds were characterized and compared to other mouse neural stem cell populations. The ciNSLCs expressed the neural markers Sox2, Nestin and Pax6, were highly proliferative, and did not form neural rosettes, indicating that they are similar to post-rosette proliferating neural progenitors. The changes that took place during reprogramming were analyzed by RNA-seq, which showed that fibroblasts underwent a gradual transition to NSCs with no non-neural lineage contamination. When spontaneously differentiated, the larger proportion of the cells differentiated into excitatory glutamatergic neurons, while few underwent GABAergic or glial

differentiation. The resulting neurons exhibited functional potassium and sodium channels, strong spontaneous synaptic network activity, and responses to activation of excitatory or inhibitory receptors. While addition of T3 to ciNSLCs was able to induce oligodendrocytic differentiation, BMP4 treatment was able to stimulate astrocytic differentiation. When injected into cortices, ciNSLCs were able to form functional neurons, astrocytes and oligodendrocytes that integrated into the existing cortex. The cells did not generate tumors for at least 4 weeks. However, longer-term studies will be needed to further assess the potential tumorigenicity of these cultures. As for reproducibility, Zhang et al show reproducible results across 4 different batches of MEFs. Furthermore, Sox2/Nestin+ cells were obtained from MEFs with different genetic backgrounds (129 x C57BL/6 and 129). The question remains however, if these ciNSLCs can give rise to the same “neuro-glial progeny” or have distinct phenotypes upon differentiation.

Interestingly, early passage ciNSLCs expressed forebrain markers, whereas late passage ciNSLCs expressed hindbrain markers, indicating a caudalizing effect of passaging. However, midbrain markers were not detected in the cultures and, most interestingly, could not be induced by addition of Fgf8 or Shh to hindbrain-primed ciNSLCs. The absence of midbrain markers could be related to a narrow temporal window for them during the process of passaging and/or due to the presence of RA, a known caudalizing agent (12), in the initial cultures. In any case, it would be highly interesting to investigate whether altering the initial compound cocktail could result in midbrain- or forebrain-primed ciNSLCs.

Hh and bFGF were found to be the most important components of the small molecule cocktail. It was therefore investigated whether there exist transcription factors downstream of these molecules that are important for reprogramming. The cocktail stabilized both Elk1 and Gli2 transcription factors and their overexpression increased the reprogramming efficiency of ciNSLCs, while silencing of these transcription factors dramatically reduced their reprogramming efficiency, directly implicating them in the reprogramming process. Moreover, in the presence of the small molecule cocktail, Elk1 and Gli2 were able to bind to the promoter of the neural master gene Sox2.

These results show for the first time that chemical reprogramming allows the differentiation of several neuro-glial lineages, opening a new avenue for modeling in the field. Many issues remain regarding pharmacological reprogramming that need to be addressed. What is the long-term tumorigenicity of these cells? How reproducible is the differentiation of adult neuro-glial populations from different cellular backgrounds? How do specific neuronal populations compare to those derived from virally-reprogrammed cells and to those in the adult brain? Can the compound identified also improve reprogramming of human fibroblasts? Hopefully, this and other studies to follow will answer these and many more questions and allow us to build strong disease models to help develop novel therapies.

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## **Footnote**

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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