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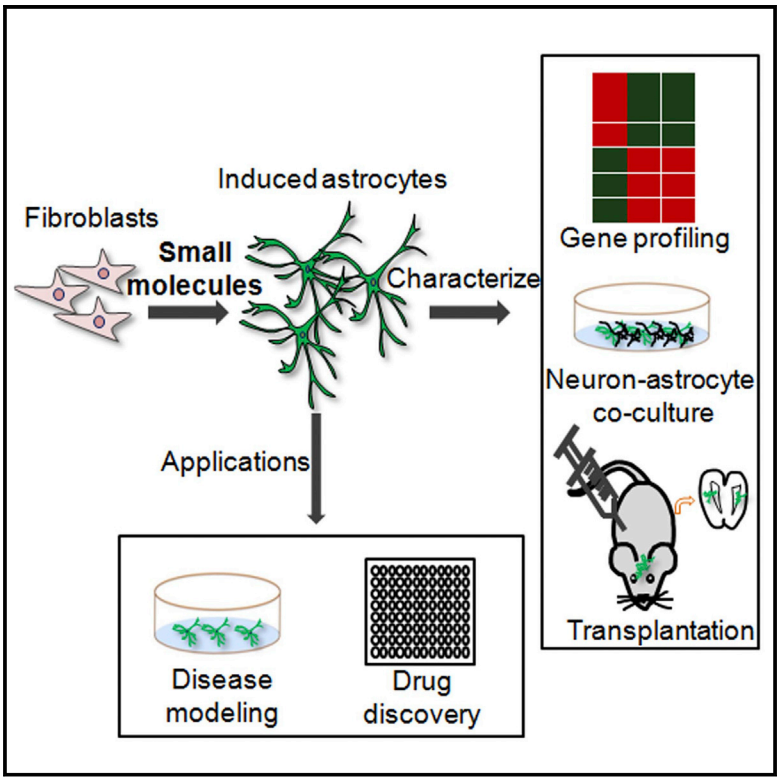
Cell Reports

# Small-Molecule-Based Lineage Reprogramming Creates Functional Astrocytes

## Graphical Abstract

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## In Brief

In this study, Tian et al. report that a small-molecule cocktail can directly convert mouse and human fibroblasts into mature, functional astrocytes without the use of transgenes. These chemically induced astrocytes resemble primary astrocytes in gene expression and function and can be used to study diseases of astrocyte dysfunction.

## Highlights

- Fibroblasts can be directly converted into astrocytes with a chemical cocktail
- Induced astrocytes resemble primary astrocytes in gene expression profile
- Induced astrocytes exhibit similar functional properties as primary astrocytes
- Induced astrocytes can be used for disease modeling and drug discovery

## Accession Numbers

GSE81927

Tian et al., 2016, Cell Reports *16*, 781–792
July 19, 2016 
http://dx.doi.org/10.1016/j.celrep.2016.06.042

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# Small-Molecule-Based Lineage Reprogramming Creates Functional Astrocytes

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## SUMMARY

**Growing evidence indicates important roles for astrocytes in neurodevelopment and diseases. However, astrocytes and their roles in these processes remain poorly understood. Despite recent progress in reprogramming somatic cells into different types of neural cells, reprogramming to astrocytes has lagged. Here, we show that functional astrocytes can be generated from mammalian fibroblasts using only small molecules. Induced mouse astrocytes resemble primary astrocytes in astrocytic gene expression and epigenomic status and exhibit functional properties in promoting neuronal maturation, glutamate uptake, and calcium signaling. Moreover, these cells can recapitulate the Alexander disease phenotype of protein aggregation when expressing Gfap with a disease-causing mutation. The same compounds can also reprogram human fibroblasts into astroglial progenitor cells that can further mature into functional astrocytes. These chemically induced astrocytes may provide cellular models to uncover roles of astrocytes in normal neurodevelopment and pathogenesis of neurological diseases.**

## INTRODUCTION

Astrocytes are glial cells that are located in all regions of the brain (Molofsky et al., 2012; Verkhratsky et al., 2012). They have long been held as the supporting components in neural tissues (Wang and Bordey, 2008; Sofroniew and Vinters, 2010). However, over the past decades, increasing evidence has established a variety of essential functions for astrocytes in neural development and in the pathogenesis of neurological diseases (Verkhratsky et al., 2012). Astrocytes play a critical role in neuronal maturation, synapse formation and plasticity, and glutamate clearance to reduce excitotoxicity (Banker, 1980; Song et al., 2002; Hama et al., 2004; Eroglu and Barres, 2010). Astrocyte dysfunction contributes to many neurodegenerative diseases and is the direct cause for some neurological disorders (Molofsky et al., 2012; Verkhratsky et al., 2012), such as Alexander disease (AxD) (Messing et al., 2012). Despite increasing data revealing new roles for astrocytes, our knowledge on astrocytes remains largely behind what we know about their neuronal counterpart. There is an urgent need to establish new cellular models for astrocytes to uncover their versatile roles in the nervous system.

Expression of lineage-specific factors has been shown to induce cell fate change, including reprogramming somatic cells to induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006) and converting one type of somatic cells to another (Davis et al., 1987). The latter is also called direct reprogramming or conversion. Extensive efforts have been devoted into converting somatic cells, like fibroblasts, into different types of neural cells, such as neural stem cells (Kim et al., 2011; Han et al., 2012; Lujan et al., 2012), neurons (Vierbuchen et al., 2010; Caiazzo et al., 2011; Pang et al., 2011; Yoo et al., 2011), and oligodendrocytes (Najm et al., 2013; Yang et al., 2013). However, direct reprogramming of somatic cells into astrocytes has just begun (Caiazzo et al., 2015).

Introducing exogenous factors in reprogramming has raised various concerns, including the risk of insertional mutagenesis and genetic alteration associated with retroviral delivery (Hawley, 2008) and low reprogramming efficiency associated with episomal transfection (Okita et al., 2008). During the course of this study, cocktails of small molecules were shown to convert mouse or human fibroblasts into neurons (Hu et al., 2015; Li et al., 2015). However, no chemical reprogramming has been reported to change fibroblasts, or any other mature cell types, to astrocytes yet. Here, we demonstrate that small molecules can be used to directly convert fibroblasts into functional astrocytes without transgenes.

## RESULTS

### A Compound Cocktail Induces the Conversion from MEFs to Astrocyte-like Cells

During our search for small molecules that can reprogram somatic cells into iPSCs, a chemical cocktail VC6TFZ was used to reprogram mouse embryonic fibroblasts (MEFs) into iPSCs (Hou et al., 2013). This compound combination includes the histone deacetylase inhibitor VPA (V), the GSK3β inhibitor