Stem Cell Reports
Previews
ISSCR
OPEN ACCESS

Quick Access to Human Astrocytic Software that Drives Neuronal Hardware

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https://doi.org/10.1016/j.stemcr.2018.09.009

Astrocytes have important functions in the brain and their deregulation may cause disease. Current ways to derive astrocytes from pluripotent stem cells are labor, time, and resource intensive, but in this issue of *Stem Cell Reports*, Li et al. present a faster method to produce functional astrocytes using transcription factors.

Astrocytes were for a long time regarded as a substance filling up the brain, and the name "glia cell" comes from the German word for nerve glue coined by Virchow in 1856 (Kettenmann and Verkhratsky, 2008). This limited view of astrocytes has, however, changed over recent years and they are now widely recognized for their importance in many functions in the brain. Astrocytes represent a subgroup of glia cells in which microglia and oligodendrocytes are the other two main classes; each subgroup has distinct functions (Zheng et al., 2018). Astrocytes are important for regulating extracellular neurotransmitter levels, supply energy to neurons, maintain the blood-brain barrier, control blood flow, and regulate formation and elimination of synapses (Zheng et al., 2018). These are all important functions that when de-regulated may cause diseases like ALS, schizophrenia, Rett syndrome, and Alzheimer's disease (Chandrasekaran et al., 2016). With the growing interest in studying astrocytes, researchers have run into the problem of access to cells from the healthy or diseased human brain. The use of transcription factors to reprogram cells from one identity to another through transdifferentiation and, most importantly, the ground-breaking finding of Yamanaka that somatic cells can be reprogrammed to induced pluripotent stem cells (iPSCs) opened up many possibilities to derive unlimited numbers of cell types of choice (Takahashi and Yamanaka, 2006), provided appropriate protocols are available. For astrocytes, protocols for differentiating pluripotent stem cells are continuously improving, the main goals currently being to simplify and shorten the process, standardize the culture conditions, and validate their functionality as reflecting that of their *in vivo* counterparts (Zheng et al., 2018). Differentiation protocols generally build on environmental cues that recapitulate developmental paths where pluripotent stem cells differentiate into neural stem cells that first give rise to neurons and later astrocytes. However, long differentiation times are for obvious reasons intrinsic to mimicking development. An alternative route to guide cell fate specification and differentiation is to use key transcription factors that bypass developmental steps.

In this issue of *Stem Cell Reports*, Li et al. present a rapid method for generating astrocytes of different subtypes from human pluripotent stem cells using key transcription factors (Li et al., 2018) (Figure 1). They explored the literature on transcription factors important for gliogenesis and selected NFIA and SOX9, both of which are important and key for glia development (Kang et al., 2012). By using the CRISPR/Cas9 technique to target NFIA and/or SOX9 to the Adeno-Associated Virus Integration Site 1 locus of human pluripotent stem cells, they found that NFIA alone facilitated astrocyte differentiation but that both transcription factors together enhanced the process. The induced astrocytes (iAstros) showed molecular and functional signatures of astrocytes, which were maintained also after the expression of the exogenous factors NFIA and SOX9 were switched off. The iAstros displayed a wide range of typical astrocyte features, including GFAP promoter demethylation that resulted in robust GFAP expression, expression profile clustering with primary astrocytes, and the ability to support elaborate neurite outgrowth when co-cultured with neurons. The authors further showed that iAstro cells were capable of propagating calcium waves and expressed glia-associated glutamate transporter *SLC1A3* (*EAAT1*, *Glast*) with glutamate uptake similar to that of primary astrocytes. Both functions are important tasks that astrocytes of the brain should master and that iAstros replicated. Transplantation of iAstros into the brain of adult mice confirmed that the iAstro cells still maintained astrocyte features as indicated by morphology and astrocytic marker expression. Morphologically, human astrocytes were larger and had more elaborate processes compared to mouse astrocytes.

Interesting times are ahead of us with these new opportunities to elucidate the mystery of the human brain and its evolution. Astrocytes have received much less attention than neurons as being the cells responsible for the evolution, intelligence, and higher cognition in humans. However, recent studies show that the anatomy of the human astrocyte is

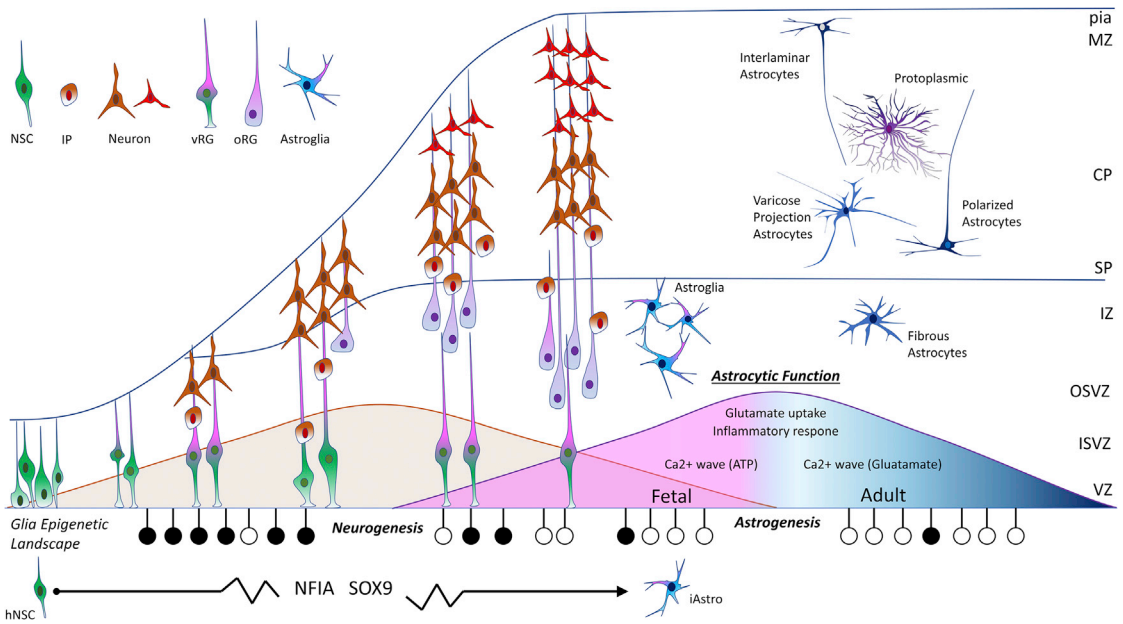

Figure 1. Factor Expression Speeds Up Human Astrocytic Cell Fate

Neurogenesis precedes astrogenesis during brain development. Progenitor populations switch from being neurogenic to being gliogenic during brain development, a phenomenon under heavy investigation. In contrast to their mouse counterparts, human astrocytes can be categorized into several subtypes, albeit with very limited information about their molecular and functional diversity (Oberheim et al., 2012). As shown in Li et al., overexpression of NFIA and SOX9 in parallel with morphogen direction is enough to fast-track an astrocytic cell fate of a human neural stem cell. The iAstros possess well-known functional properties of astrocytes and have the potential to start answering questions about astrocyte evolution and their biology in humans.

NSC, neural stem cells; IP, intermediate progenitor; vRG, ventrical radial glia; oRG, outer radial glia; iAstro, induced astrocytes; VZ, ventricular zone; ISVZ, inner subventricular zone; OSVZ, outer subventricular zone; IZ, intermediate zone; SP, subplate; CP, cortical plate; MZ, marginal zone.

fairly different from that of other animals: the human astrocyte in the neocortex is 27-fold larger and has 10 times more processes which are 2.6-fold longer compared to those in rodents (summarized in Robertson, 2014). Functionally, human astrocytes outcompete their rodent counterparts as shown in an elegant study where human glia progenitors were transplanted into mouse forebrains. The human glia progenitor cells migrated, integrated, and matured to astrocytes resulting in a mouse with improved long-term potentiation (LTP) and learning compared to mice in which murine progenitors were grafted (Han et al., 2013). Moreover, Caizzano et al. showed that human fibroblasts are less efficiently converted to astrocytes by NFIA/B and SOX9 overexpression than mouse fibroblasts are, demonstrating that the regulatory mechanism of glia cell fate differs between human and rodent (Caiazzo et al., 2015).

Thus, there are multiple limitations in using rodent models for studying human astrocytes, making the method presented by Li et al. an important step toward understanding the human brain and its evolution.

Li et al. show that SOX9 and NFIA do not work as sole master regulators in human glia development as these factors cannot completely change the epigenetic landscape and drive glia cellular fate from pluripotent stem cells. Further, SOX9 and NFIA only drove gliogenesis after the neural stem cell state had been achieved, providing valuable insights into details of human brain development and highlighting the critical aspect of working with human cells when connecting astrocyte biology and human diseases.

Astrocytes are like neurons in that they are regionally patterned dependent on where in the brain they reside. Li et al. were able to regionally pattern iAstros using morphogens to acquire dorsal or ventral forebrain and spinal cord fate (Li et al., 2018). This enables studies of possible regional functionality and specific micromilieus combining neuronal cultures of the same brain regions.

The field of studying human astrocytes and their importance for maintaining our brains as smart and healthy started with the realization that astrocytes execute instrumental

functions in the brain and have been massively expanded during human evolution. Since then, many protocols for producing human astrocytes have been developed and are constantly improving, with the latest addition being the use of transcription factors to direct astrocyte fate (Li et al., 2018; Canals et al., 2018). Now, the field is in a position where we can study the unique human astrocyte function in co-cultures with neurons in cellular 2D and 3D models that resemble *in vivo* counterparts very closely indeed.

DECLARATION OF INTERESTS

Anders Lundin is employed by AstraZeneca.

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Regenerative Medicine: Taming the Chimaera

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https://doi.org/10.1016/j.stemcr.2018.09.003

In this issue of *Stem Cell Reports*, Hamanaka et al. (2018) describe the generation of chimeric mice with all vascular endothelial cells derived from pluripotent stem cells. This approach is desirable to prevent immune rejection when human stem cells are combined with animal embryos to grow human organs in animals.

One of the main goals of the stem cell and regenerative medicine fields is the generation of functional tissues or organs to be used for transplantation to the human body. Although the information needed to form all tissue types and how these need to interact is present in the genomes of almost all cells, the differentiation and *in vitro* culture of complex three-dimensional structures with different tissue types, such as organs, has proven to be rather difficult indeed (Peloso et al., 2015). One way to approach this formidable task is to carefully observe nature and in particular embryology: identifying the signaling pathways, processes, and structures active in developing embryos during organ formation. This knowledge can subsequently be used to mimic the processes *in vitro* from stem cells (Gilmour et al., 2017). The activation of the various pathways and interactions between the cells is of such an overwhelming complexity that it seems an arduous task to replicate this *in vitro*. On the other hand, in developing embryos these processes are carried out seemingly effortlessly.

When two embryos at an early stage of development are combined they can develop to form one single organism. The animal formed is called a chimera, in reference to the fearsome fire-breathing creature of Homer's Iliad with the head of a lion, a