In vivo astrocyte-to-neuron reprogramming for central nervous system regeneration: a narrative review

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Abstract

The inability of damaged neurons to regenerate within the mature central nervous system (CNS) is a significant neuroscientific challenge. Astrocytes are an essential component of the CNS and participate in many physiological processes including blood-brain barrier formation, axon growth, regulation, neuronal support, and higher cognitive functions such as memory. Recent reprogramming studies have confirmed that astrocytes in the mature CNS can be transformed into functional neurons. Building on in vitro work, many studies have demonstrated that astrocytes can be transformed into neurons in different disease models to replace damaged or lost cells. However, many findings in this field are controversial, as the source of new neurons has been questioned. This review summarizes progress in reprogramming astrocytes into neurons in vivo and animal models of spinal cord injury, Huntington’s disease, Parkinson’s disease, Alzheimer’s disease, and other neurodegenerative conditions.

Key Words: astrocyte; astrocyte-to-neuron; central nervous system; in vivo; nerve regeneration; neurological disorders; reprogramming; review

Introduction

Damaged neurons in the adult mammalian central nervous system (CNS) show an inability to regenerate and form new functional connections. As a result, most CNS injuries and diseases cause permanent disability (Curcio and Bradke, 2018). The ability of the CNS to undergo the same robust regeneration and axonal growth observed during embryonic development would allow for novel, more effective treatments of neurodegenerative conditions and CNS injuries. Stem cell transplantation was once thought to be the future of neural repair and was expected to promote production of neurons that could restore function. However, stem cell use is subject to ethical controversy, and immune rejection and the inability of differentiated cells to functionally integrate into the CNS have hindered use of this technology (Li and Chen, 2016). Therefore, focus has shifted to repairing neural function through endogenous cells.

Historically, glial cells in the CNS were believed to primarily support and protect neurons. Advances in molecular biology and genetics, and advances in characterization of morphology and physiology have provided a more in-depth understanding of glial cells, and significant progress has also been made in the field of neuronal transdifferentiation (Wang et al., 2015; Gascón et al., 2017; Yu et al., 2020). Astrocytes are the most widely distributed and largest glial cells in the mammalian nervous system. In addition to providing physical and metabolic support to neurons and isolating the CNS from the external environment, astrocytes participate in many physiological processes including blood-brain barrier formation, axon growth, regulation, neuronal support, and higher cognitive functions such as memory. Recent reprogramming studies have confirmed that astrocytes in the mature CNS can be transformed into functional neurons. Building on in vitro work, many studies have demonstrated that astrocytes can be transformed into neurons in different disease models to replace damaged or lost cells. However, many findings in this field are controversial, as the source of new neurons has been questioned. This review summarizes progress in reprogramming astrocytes into neurons in vivo and animal models of spinal cord injury, Huntington’s disease, Parkinson’s disease, Alzheimer’s disease, and other neurodegenerative conditions.

Search Strategy

We performed electronic searches using PubMed, Medline, and Google Scholar databases for literature published in English before March 2022. The keywords used for the literature search were: Astrocyte AND in vivo AND reprogramming OR transdifferentiation. The results were further screened by heading and abstract.

Astrocyte Functions and Reprogramming

The physiological functions of astrocytes

Glia cells are the second-most abundant component of the mammalian nervous system after neurons. Although they do not produce electrical impulses, they participate in CNS activities in a variety of ways. The four primary types of glial cells in the human CNS are astrocytes, oligodendrocytes, microglia, and ependymal cells. Each of these four cell types affects neurons in different ways. Glial cells regulate synapse formation (Farhy-Tselnicker and Allen, 2018), participate in physiological activities such as breathing (Beltrán-Castillo et al., 2017), remove dead neurons and pathogens (Clarke and Barres, 2013), and are involved in learning and other advanced cognitive functions (Fields et al., 2014). Astrocytes are the most widely-distributed type of glial cells in mammals, and are also the largest (Freeman and Rowitch, 2013). In addition to providing physical and metabolic support, and isolating the CNS from BBB formation, astrocytes are involved in synapse development, maturation, and pruning. As their list of known functions has grown, their potential for repairing the nervous system has received increased attention (Clarke and Barres, 2013).

When the CNS is damaged, glial cell activity increases, and glial scars form in a process called “reactive gliosis” to prevent further damage to adjacent tissues. Although glial cells can secrete substances that promote neural repair, glial scars create physical obstacles to axon regeneration (Pekny and Nilsson, 2005; Hara et al., 2017; Boghdadi et al., 2020). Therefore, proliferation of reactive glial cells proliferation is a double-edged sword for the CNS. Simply inhibiting glial proliferation slows recovery (Burdta and Sofroniew, 2014). Identification of mechanisms to promote nerve repair while eliminating scars would significantly improve CNS structural and functional recovery.

Discovery of astrocyte reprogramming potential

In 1999, Doetsch et al. reported the existence of neural stem cells with differentiation potential in the subventricular area of adult mammals. Astrocytes in this area can self-renew and act as stem cells to generate immature precursors and neuroblasts (Doetsch et al., 1999). These intriguing findings raised many questions. Why do glial cells in specific areas function as neural stem cells? Can this feature be leveraged to promote nerve injury repair?
repair? Researchers have attempted to induce migration of astrocytes in these regions to injured sites (Alvarez-Buylla and Garcia-Verdugo, 2002; Cayre et al., 2006; Mamber et al., 2010). However, due to the limited number of astrocytes in the subventricular area, and the difficulty in precisely regulating their migration, the efficacy of this method for treating serious diseases is unclear.

In the 21st century, researchers have moved beyond the subventricular area and hippocampus and begun to ask whether astrocytes elsewhere in the CNS could transform into neurons. In vivo and in vitro studies have shown that glial cells can differentiate into neurons through activation of transcription factors and through drug treatments (Li and Chen, 2016; Qin et al., 2017; Tai et al., 2020). In 2012, the Nobel Prize in Physiology or Medicine was awarded to Sir John B. Gurdon and Shinya Yamanaka for their discovery that mature cells can be reprogrammed to become pluripotent.

In addition, development of viral vector technologies such as adenovirus-associated viruses (AAVs) and retrovirus, and new theoretical and experimental methods, have enabled rapid advances in the field of cell reprogramming (Verdera et al., 2020; Muhuri et al., 2021). Breakthroughs have been made in treating neurological diseases by reprogramming cells and transforming them into neurons in vitro and in vivo.

**Astrocyte Reprogramming and Transdifferentiation into Neurons**

The main difference between reprogramming and transdifferentiation is whether protocols become pluripotent stem cells. During reprogramming, protocols dedifferentiate into pluripotent stem cells, then differentiate into specific cell types via retroviruses (Chen et al., 2017). AAV viruses (Chen et al., 2020), and small molecule drugs (Qin et al., 2017), in the process of transdifferentiation, protocols are directly induced into target cells without becoming pluripotent stem cells (Xie et al., 2017).

Animal studies of traumatic central nervous system injury and degenerative central nervous system disease have shown that regulating astrocyte fate through viral vector-mediated overexpression of crucial transcription factors can convert a subset of astrocytes into neurons in situ following CNS damage. These transformed neurons can connect with the existing neural network and produce spontaneous electrical activity (Figure 1 and Table 1).

![Figure 1](Image 526x758 to 559x787)

**Figure 1** | Schematic depicting the in vivo astrocyte conversion strategy. (A) The most widely used methods for astrocyte reprogramming involve injection of adenovirus-associated viruses or lentiviruses carrying different transcription factors using micro-injectors directly into the spinal cord, cortex, striatum, or other central nervous system tissues. (B) Adeno-associated viruses and lentiviruses are the most common vectors in the field of astrocyte in vivo reprogramming. (C) A variety of transcription factors have been implicated in astrocyte in vivo reprogramming, including NeuroD1, Sox2, Neurog2, Ascl1, SOX10, NURR1, and shPTB. (D) Astrocyte reprogramming begins when the viral vector containing the target gene is injected in situ. A certain proportion of astrocytes will be reprogrammed into neurons. (E) Some studies reported that neurons reprogrammed by astrocytes have biological activity, can form functional synaptic connections, and generate electrical activity. Created with BioRender.com.

**Astrocyte reprogramming after brain injury**

Non-specific neuronal death caused by external forces or ischemia and hemorrhage is the leading cause traumatic brain injury and stroke-induced dysfunction. The prevalence of TBI was 55.50 million in 2016, and caused 8.1 million years of life lived with disability (YLDs). In addition, stroke caused 6.1673 million all-aged death (GBD 2017 Causes of Death Collaborators, 2018; GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators, 2019). Replacement of dead neurons with reprogrammed astrocytes may be a novel therapeutic avenue.

The Sox (sex-determining region Y) family of transcription factors plays an essential role in mammalian embryo development, and Sox2 is necessary for maintaining embryonic stem cell pluripotency (Sarkar and Hochedlinger, 2013). Many studies have examined how the Sox family of transcription factors affects astrocyte reprogramming. Injection of lentivirus overexpressing Sox2 into mouse brains resulted in reprogramming of astrocytes in the stratum of the Parkinsonian autonomic nervous system. Further induction by brain-derived neurotrophic factor (BDNF)/BDNF noggin or histone deacetylase inhibitor resulted in transformation of DCX/Ascl1 neuronal progenitors into Neun+ neurons that persisted for as long as 32 weeks after injection (Niu et al., 2013, 2015).

NeuroD1 (neurogenic differentiation 1) is a neuronal transcription factor that participates in initiation of neural differentiation. It plays a vital role in embryonic brain development and maintenance of normal peripheral nervous system (PNS) and CNS functions (Cho and Tasi, 2004; Jahan et al., 2010; Lai et al., 2019). Several studies reported that NeuroD1 was closely related to astrocyte transdifferentiation (Chen et al., 2017, 2020; Puls et al., 2020; Tang et al., 2021). When NeuroD1 is overexpressed in the brains of mice with ischemic brain injury, the number of damaged neurons is reduced by one-third (in another one experiment, the damaged number was transformed by glial cells. After 2 months, the motor and cognitive functions of the treatment group were significantly improved compared with those of the control group (Chen et al., 2020). Similarly, Xiang et al. (2021) showed that NeuroD1 overexpression with a single injection of AAV vector transformed astrocytes into neurons in the cortices of mice with ischemic stroke. In this study, the investigators suggested avoiding high concentrations of injected virus to avoid “artifacts” (Xiang et al., 2021). A study using a rhesus monkey cerebral ischemia model showed that astrocytes were efficiently transformed into neurons by an AAV carrying NeuroD1 with a > 90% conversion efficiency (Ge et al., 2020). These positive results in primates are an important step toward clinical application.

**Astrocyte reprogramming in Huntington’s disease**

Huntington’s disease (HD) is caused by a gene mutation that results in loss of neuronal (A-17B)-tubulin (GABAergic)-releasing neurons, leading to motor, cognitive, and mental dysfunction. The population incidence of HD varies from 0.2 to 1 per 10,000 (Rikani et al., 2014; Rawlins et al., 2016). Transformation of astrocytes into GABAergic neurons to supplement lost neurons is a promising example of reprogramming to treat HD. When NeuroD1 and Dlx2 are overexpressed in the striatum of HD model mice, astrocytes can be reprogrammed into GABAergic neurons. These transformed neurons show similar electrophysiological characteristics as the original neurons and reduced dysfunction and prolonged the lives of model mice (Wu et al., 2020).

**Astrocyte reprogramming in Parkinson’s disease**

Parkinson’s disease (PD) is an autosomal recessive disease characterized by progressive loss of dopaminergic neurons in the striatum (Kalia and Lang, 2015). The goal of astrocyte reprogramming to treat PD is to transform astrocytes in situ into dopaminergic neurons in the striatum to replenish the lost neurons and prevent disease progression. Regulation of transcription factors and micro RNAs are typically used to replenish dopaminergic neurons (Wei and Shetty, 2021). Astrocytes can be reprogrammed into dopaminergic neurons using lentiviral vectors that overexpress NeuroD1, Ascl1, and Lmx1a, and microRNA218. The results from 2 weeks to 15 weeks after injection showed that these transformed neurons gradually matured and generated action potentials. Furthermore, these PD model mice showed a degree of improvement in motor function (Rivetì di Val Cervo et al., 2017). Another study showed that injection of a lentiviral vector carrying CRISPR/Cas9 knockdown polypyrimidine tract binding protein 1 (PTBP1) into the striatum of PD model mice induced transformation of astrocytes into functional dopaminergic neurons in the PD mice in this study (Zhou et al., 2020). Recently, Giehrl-Schwab et al. (2022) reported that an AAV-based intrin sp dcsa9 activator system (AAV DCAS) carrying different combinations of Ascl1, Lmx1a, NeuroD1, and miRNA218 (ALNe-218) could reprogram astrocytes into GABAergic neurons, resulting in improved motor function in mice. In addition to astrocytes, fibroblast reprogramming is also a major research hotspot in PD research (Yavarpour-Bali et al., 2020).

**Astrocyte reprogramming in Alzheimer’s disease**

Alzheimer’s disease (AD) is a common neurodegenerative disease characterized by neuronal and synaptic loss in the cerebral cortex and subcortical areas (Wenk, 2003). Overexpression of NeuroD1 in the cortices of AD model mice resulted in reorganization of retinovisual glial cells into functional glutamatergic neurons. These neurons produced spontaneous synaptic responses and induced postsynaptic reactions, which indicated that the resulting neurons were successfully integrated into the local neural network (Guo et al., 2014). That study also showed that NG2 cells were reprogrammed to glutamatergic and GABAergic neurons (Guo et al., 2014).

**Astrocyte reprogramming after spinal cord injury**

Spinal cord injury (SCI) interrupts communication between the CNS and the PNS. Axonal rupture and neuronal death caused by various physical and chemical factors are the fundamental causes of sensory, motor, and autonomic nervous system dysfunction (Zhang et al., 2021). The goal of astrocyte reprogramming in SCI is to replace dead neurons with transformed...
neurons and restore function by forming functional connections with the original system (McDonald and Sadowsky, 2002). Like other CNS injuries, SCI can induce glial scar formation through reactive gliosis mediated by astrocytes (Hara et al., 2017). Furthermore, astrocyte proliferation after SCI is more extensive than following brain injury, so glial cells may play a more critical role in the pathological process of SCI (Boghadi et al., 2020). For these reasons, glial cell reprogramming after SCI is a research hotspot (Schnell et al., 1999; Bradbury and Burnside, 2019).

When a lentiviral vector overexpressing NeuroD1 was delivered to the spinal cords of SCI rats, the numbers of neural stem cell marker-labeled cells, immature neurons, and mature neurons in the spinal cord all increased significantly (Chen et al., 2017). Similarly, when an AAV was used to overexpress NeuroD1 in the spinal cords of SCI model mice, astrocytes were transformed into neurons (Puls et al., 2020). Interestingly, when NeuroD1 alone was used for induction, most of the transformed neurons were glutamatergic. When Dlx2 was included with NeuroD1, most of the transformed neurons were GABAergic. Electrophysiological techniques were used to confirm that the transformed neurons participated in physiological evaluation of astrocyte-specific markers and lineage tracing confirmed that knockdown of polypyrimidine trace binding protein 1 (ptbp1) using gene in the brains of mice with cerebral ischemia resulted in reprogramming of astrocytes into functional neurons that responded to visual stimuli. Within 3–6 weeks after reprogramming, gradual maturation and functional integration of synapses of the transformed neurons was observed, and this process was similar to cortical circuit development (Tang et al., 2021).

Müller glial cells are the primary type of glial cell in the retina, and provide structural support for neurons. Reprogramming retinal Müller glial cells and oligodendrocytes through overexpression is a potential strategy for restoration of visual function (Seikyu and Matsuda, 2021). A study showed that knockdown of polyprymidine trace binding protein 1 (ptbp1) using an AAV vector carrying RNA targeted CRISPR/Cas9 resulted in efficient transformation from Müller glial cells to retinal ganglion cells, which then underwent functional integration. This resulted in reduced symptoms associated with RGC loss in a retinal injury mouse model (Zhou et al., 2020). The regulatory transcription factors Math5 and Bdn3b were able to reprogram Müller glia in mice to retinal ganglion cells that had typical neuronal electrophysiological characteristics and improved the visual function of the mice (Xiao et al., 2021). Interestingly, the AAV-specific promoter used in this study was GFAP (typically considered an astrocyte-specific promoter), but the neuralization of astrocyte-specific markers and lineage tracing confirmed that the induced cells were indeed Müller glia and not astrocytes (Xiao et al., 2021).

In addition, regeneration and axon formation of retinal ganglion cells can also be induced by pharmacological reprogramming (Mahato et al., 2020) and regulation of DNA methylation mediated by transcription factor overexpression (Lu et al., 2020). These findings may provide an essential theoretical basis for restoration of visual function through reprogramming glial cells.
Challenges and Perspectives

Identifying the source of newborn neurons

Although many studies have shown that astrocyte reprogramming can be accomplished using many different strategies (Lentini et al., 2021; Ma et al., 2021; Zhou et al., 2021), recent studies have questioned the source of regenerated neurons. Tai et al. reported that functional neurons could be regenerated over a period of time, but the source was not astrocytes, but NG2 glial cells (Tai et al., 2021). Another study reported extremely low transfection efficiency after viral vector treatment, especially in an aged animal model (Gresita et al., 2019).

NG2 glial cells are oligodendrocyte precursor cells that proliferate throughout the CNS and participate in neuronal information transmission (Dimou and Götz, 2014). The ability of NG2 cells to proliferate throughout their lifetime is a trait of stem and progenitor cells, and indicates that these cells may be attractive targets for reprogramming. A significant controversy in the field is the origin of the source of reprogrammed neurons is NG2 glial cells or astrocytes. According to Heinrich et al. (2014), DCX positive neuroblasts were obtained by lentiviral overexpression of Sox2 in cortical stab model mice, and the source was traced using fat genetic mapping. More than 60% of the cells were derived from NG2 cells, and only a small proportion of the reprogrammed cells were derived from reactive astrocytes.

The gold standard for direct reprogramming in vivo: Lineage tracing

In the past decade, direct induction of neurons to treat diseases has shown promise in various animal injury models. However, the primary problem remains the identification of the source of induced neurons and confirmation that they are functional and exert physiological functions. Multimodal evaluation of neuronal induction and morphology requires information transmission ability to be strictly defined as the “spectrum of neurons” (Yang et al., 2013). Therefore, regardless of the origin of the neurons, the possibility that the body’s internal regeneration mechanisms must be ruled out. The most serious challenge facing this field may be to clarify that “newborn neurons” generated by different regulatory means actually come from astrocytes, and eliminate other possible sources as the progenitors of the reprogrammed neurons. This problem is a primary focus in the field of astrocyte reprogramming.

Wang et al. (2021a) reported that neither AAV-induced NeuroD1 overexpression nor Ptbp1 gene knockdown induced transformation of astrocytes into neurons. Instead, the regenerated neurons were neurons that were already present, and leakage of the AAV likely caused the regeneration. These results were reported in previous studies, but the mechanism is unclear (Wang et al., 2021a). In that study, Wang et al. (2021a) found that the commonly used viral vector AAV, even with the commonly used astrocyte-specific promoter GFAP, had significant differences in the specificity of vector transfection for different serotypes. After 4 days of in vivo transfection, different serotypes mainly transacted astrocytes, but after 14 days, the commonly used serotypes 8, 9, and PHP.βE transfected significantly less astrocytes, and the expression of fluorescent protein began to appear in many pre-existing neurons (Wang et al., 2021a). Similarly, Chen et al. (2022) recently reported that pedigree tracking of expression of ptbp1 showed that reactive astrocytes did not transform into neurons in the brains of PD model mice. Furthermore, they previously reported newborn neurons may have been neurons originally.

In addition, some researchers have expressed concern that direct transformation of astrocytes may negatively impact the nervous system. At the same time, due to the problems of promoter leakage and specificity in astrocyte-based regulation systems, it may not be applicable in the proof of lineage (Swendsen and Sofroniev, 2022). Recently, Giehl Schwab et al. (2022) reported that multiple transcription factors were coexpressed simultaneously in PD model mice following treatment with an AAV-based inducible splice oractivator system (AAV DCAS9 activator system). This report may be consistent with the findings of other studies, demonstrating that AAV vector can eliminate ambiguity with regard to the source of newborn neurons. Strict lineage tracking has received increased attention in the field of direct astrocyte reprogramming in vivo (Wang and Zhang, 2022). Therefore, a more specific and safer delivery system, and well-designed experiments, are needed to clarify the source of newborn neurons.

Optimizing reprogramming strategies

Single agonists or blockers may not provide adequate therapeutic benefit due to differences in CNS disease pathogenesis, damaged cell types, and pathways of astrocyte reprogramming are needed, as identification of safe and efficacious strategies is thus the less than 10% of all the GNPs (with 1:1 (von Bartheld et al., 2016). Although the actual GNP is not known, GNP stability is essential for normal physiological function of the CNS. Prior to artificial manipulation of the GNP, high-quality research must be performed to demonstrate whether GNP manipulation will affect normal CNS function, including the integrity of the blood-brain barrier and accuracy of electrical activity.

In traumatic injuries of the CNS, the ideal time window for using cell reprogramming to repair damage may be after primary scar formation and injury inhibition by reactive glial cells. The purpose of reprogramming reactive glial cells is to functionally reprogram them so that they can differentiate into neurons and eventually recover lost neuronal function. In the early stage of damage and disease, the microenvironment will be in a relatively unstable state due to acute stress reactions such as inflammation (Ahuja et al., 2017). In the chronic stages, the entire CNS may have undergone adaptive changes. Therefore, it is necessary to determine the optimal treatment windows for different pathological states. In some degenerative diseases, harmful factors such as ROS and inflammatory cytokines may be present throughout the nervous system, and even neurons produced by reprogramming may degenerate (Wang et al., 2021b). Therefore, more high-quality research is needed on how to get the best therapeutic effect of reprogramming in different pathological environments.

Limitations

This review was subject to some limitations. First, we mainly searched PubMed/MEDLINE, and Google Scholar databases. Although these databases are widely used, some relevant literature may have been omitted. Second, we included manuscripts published up to March 2022. Therefore, ongoing research and new results were not included in this review. Finally, the manuscripts reviewed in this paper were only published in English language journals.

Conclusion

Astrocytes have been successfully reprogrammed into different types of neurons through pharmacological approaches and regulation of various transcription factors. These findings could be promising not only in improving animal models of disease. Future studies of the mechanisms and pathways of astrocyte reprogramming are needed, as identification of safe and efficacious strategies is thus the less than 10% of all the GNPs (with 1:1 (von Bartheld et al., 2016). We must carefully evaluate the long-term effects of transformed neurons before applying this technology to treat human diseases. Developing the potential of transforming glial cells into neurons changes our understanding of the CNS and has the potential to open a new research field. However, the road to clinically treat CNS diseases using this approach will be long. Therefore, it is necessary to further develop tools for identifying the source of reprogramming cells, obtaining safer and more efficient gene vectors, and optimizing reprogramming schemes for different diseases.
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