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New approaches for direct conversion of patient fibroblasts into neural cells

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Abstract

Recent landmark studies have demonstrated the production of disease-relevant human cell types by two different methods; differentiation of stem cells using external morphogens or lineage conversion using genetic factors. Directed differentiation changes embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) into a desired cell type by providing developmental cues in an *in vitro* environment. Direct reprogramming is achieved by the introduction of exogenous lineage specific transcription factors to convert any somatic cell type into another, thereby bypassing an intermediate pluripotent stage. A variety of somatic cell types such as blood, keratinocytes and fibroblasts can be used to derive iPSC cells. However, the process is time consuming, laborious, expensive and gives rise to cells with reported epigenetic heterogeneity even amongst different iPSC lines from same patient which could propagate phenotypic variability. A major concern with the use of pluripotent cells as starting material for cell replacement therapy is their incomplete differentiation and their propensity to form tumors following transplantation. In comparison, transcription factor mediated reprogramming offers a direct route to target cell types. This could allow for rapid comparison of large cohorts of patient and control samples at a given time for disease modeling. Additionally, transcription factors that drive maturation may yield more functionally mature cells than directed differentiation. Several studies have demonstrated the feasibility of generating of cell types such as cardiomyocytes, hepatocytes, and neurons from fibroblasts. Here, we will discuss recent advances and key challenges regarding direct reprogramming of somatic cell types into diverse neural cells.

Keywords

Induced neuron; reprogramming; direct conversion; lineage conversion; disease modeling; neurological disease

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Introduction

Cellular reprogramming, the ability to convert one cell type into another desired cell type, can be achieved by either directed differentiation of pluripotent stem cells or direct reprogramming of somatic cells. Directed differentiation changes embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) into a desired cell type by providing developmental cues in an *in vitro* environment. Direct reprogramming is achieved by the introduction of exogenous lineage specific transcription factors to convert any somatic cell type into another, bypassing an intermediate pluripotent stage. A variety of somatic cell types such as blood, keratinocytes and fibroblasts can be used to derive iPSCs (Aasen and Izpisua Belmonte, 2010; Su et al., 2013; Takahashi et al., 2007). However, the process is time-consuming, laborious, expensive and gives rise to cells with reported epigenetic heterogeneity even amongst different iPSC lines from same patient which could propagate phenotypic variability (Egawa et al., 2012; Israel et al., 2012). A major concern with the use of pluripotent cells as starting material for cell replacement therapy is their incomplete differentiation and their propensity to form tumors following transplantation (Kim et al., 2010; Miura et al., 2009). In comparison, transcription factor mediated direct reprogramming strategy offers a direct route to target cell types. The feasibility of direct reprogramming in other cell types such as cardiomyocytes, hepatocytes, and neurons from fibroblasts has been successfully demonstrated (Ieda et al., 2010; Sekiya and Suzuki, 2011; Son et al., 2011; Vierbuchen et al., 2010). Additionally, direct reprogramming yields more functionally mature cells than directed differentiation (Lujan and Wernig, 2013). This could allow for rapid comparison of large cohorts of patient and control samples at a given time for disease modeling. It is likely the target neural cell types derived from direct reprogramming preserve their genomic integrity in contrast to cells obtained through directed differentiation because of prolonged culturing of iPSCs, which might lead to higher chances of introducing mutations.

Direct reprogramming as a tool to derive functional neurons and neuronal cell types

Neurons

Many neurological disorders have specific subtypes of neurons that are affected. The earliest report of direct reprogrammed neurons described the use of three transcription factors *Ascl1*, *Brn2*, *Myt1L* to reprogram mouse fibroblasts into excitatory functional neurons. These induced neurons (iNs) could fire repetitive specific action potentials and exhibited glutamatergic and GABAergic phenotype (Vierbuchen et al., 2010). Addition of *NeuroD1* to the three factors could generate functional human induced neurons (Pang et al., 2011). Subsequently, several groups have successfully generated many clinically relevant neuronal subtypes such as dopamine neurons, motor neurons, medium spiny neurons, nociceptors and retinal ganglions from fibroblasts using direct reprogramming methods (Table 1) (Blanchard et al., 2015; Caiazzo et al., 2011; Hu et al., 2015; Kim et al., 2011b; Li et al., 2015; Liu et al., 2012; Meng et al., 2013; Pfisterer et al., 2011; Sheng et al., 2012a; Son et al., 2011; Victor et al., 2014; Wainger et al., 2015).

Neural stem cells

One of the earliest studies to induce a cell type with proliferative and progenitor like phenotype was the induction of neural progenitor cells from mouse fibroblasts (Kim et al., 2011a). In comparison to post-mitotic induced neurons which are directly converted from fibroblasts, induced neural progenitor cells (iNPCs) and/or neural stem cells (iNSCs) have the advantage of being expandable *in vitro* and have the ability to give rise to multiple neuronal subtypes and glial cells (Table 1)(Cheng et al., 2014; Han et al., 2012; Kim et al., 2011a; Lujan et al., 2012; Thier et al., 2012; Zhu et al., 2014). Transient induction of pluripotency factors (Oct4, Sox2, Klf4, and c-Myc (OKSM) in murine fibroblasts in the presence of appropriate signaling inputs can promote selective lineage conversion to induce neural stem cell state (Kim et al., 2011a). Since then, several reports have generated expandable multipotent murine NPCs with Sox2 alone or Sox2 in combination with either pluripotency related transcription factors such as c-Myc and KLF4 (Han et al., 2012; Ring et al., 2012; Thier et al., 2012), or transcription factors such as Brn4/Pou3f4, E47/Tcf3, FoxG1 (Han et al., 2012; Lujan et al., 2012). iNSCs derived in the above studies closely resemble native brain NSCs in morphology, gene expression patterns, self-renewal and differentiation potential, as well as *in vitro* and *in vivo* functionality. When transplanted in mouse neonatal brain, iNSCs committed to the neuronal lineage and had the ability to differentiate into neurons (GABAergic and dopaminergic neurons), astrocytes and oligodendrocytes and unlike iPSC-derived NSCs, do not generate tumors (Ring et al., 2012). Notably, self-maintaining tripotent proliferative neural cells can also be induced from non-ectodermal cells like sertoli cells, B-lymphocytes and adult liver cells by expressing a specific combination of transcription factors (Cassady et al., 2014; Sheng et al., 2012b). Recently, induced neural progenitor cells (iNPCs) from human neonatal and adult peripheral blood were generated using a single transcription factor OCT4 combined with SMAD+GSK-3 inhibition. Blood-derived iNPCs differentiated *in vivo* and responded to guided differentiation *in vitro* into glia (astrocytes and oligodendrocytes) and multiple neuronal subtypes, including dopaminergic and nociceptive neurons (Lee et al., 2015). Furthermore, nociceptive neurons obtained from differentiated iNPCs phenocopy chemotherapy-induced neurotoxicity in a system suitable for high-throughput drug screening and therefore harbor great utility for the study of clinically relevant neurological diseases directly from patient samples (Lee et al., 2015). Tian et al recently demonstrated the direct conversion of mouse fibroblasts into lineage restricted induced dopaminergic precursors by ectopic expression of Brn2, Sox2 and Foxa2 (Tian et al., 2015). Upon transplantation, the induced dopaminergic precursors differentiated into dopaminergic neurons, which functionally alleviated the motor deficits, and reduced the loss of striatal dopaminergic neuronal axonal termini in the MPTP-lesioned mouse model of Parkinson's disease.

Neural crest cells

Overexpression of a single transcription factor, Sox10, in combination with environmental cues including Wnt activation was able to induce direct reprogramming of human fibroblasts into induced neural crest (iNC) (Kim et al., 2014). The human induced neural crest cells exhibit capacity for multi-lineage differentiation into the four main NC lineages. The iNCs also migrated *in vivo* and demonstrate the feasibility of isolation of disease-specific iNCs

directly from patient fibroblasts. Purified iNCs from familial dysautonomia (FD) patient fibroblasts displayed previously known disease relevant defects in cellular migration, transcription and mRNA splicing, providing insights into FD pathogenesis (Kim et al., 2014). Direct generation of expandable multipotent neural stem cells and neural crest cells could serve as a renewable source of patient specific neurons for disease modeling and transplantation therapy. However, more studies are needed to validate if using neural stem cells are a better alternative to iPSCs or ESCs as a starting cell population, because much like use of pluripotent iPSCs or ESCs, the “stemness” of NSCs raises concerns of tumorigenicity.

Glial Cells

The non-neuronal glial cells mainly comprises of astrocytes, oligodendrocytes and microglia. Of these, astrocytes have been long known to play a role in brain homeostasis, providing support and protection to the resident neuron population. Recent discoveries such as the capability of postnatal astrocytes in promoting synaptogenesis and their active role in formation and maintenance of the blood-brain barrier have shed light on unique and important roles of astrocytes. Recently, Han et al. demonstrated that engrafting human astroglial progenitors in mouse forebrain results in enhanced synaptic plasticity and learning (Han et al., 2013). The emergence of the understanding that astrocyte dysfunction during development has profound non-cell autonomous effects on the surrounding tissues leading to disease pathology, is very critical to the future of designing therapeutic strategy for these diseases. Many neurodevelopmental diseases that fall in this spectrum including neuropsychiatric ailments (Rett syndrome, Fragile X mental retardation, Alexander’s disease, epilepsy, autism as well as ‘late-onset’ forms of psychiatric diseases like Schizophrenia and pathological depression) and neurodegenerative diseases (Huntington’s disease, ALS, and FTD) have been linked to astrocyte dysfunction and the resulting non-cell autonomous effect on the surrounding tissues, chiefly neurons (Molofsky et al., 2012). Astroglial cells can be derived from embryonic stem cells and induced pluripotent stem cells using directed differentiation (Krencik et al., 2011; Krencik and Zhang, 2011). However, this strategy essentially recapitulates the *in vivo* developmental stages on a dish in which the starting stem cell population undergoes transition from a pluripotent phase to a neuroepithelial cell, a neural progenitor cell to an astroglial progenitor cell to finally becoming a fully mature astrocyte. This process takes place over a period of several months which is both time- consuming and labor-intensive, suggesting the need for a more feasible and efficient process to generate these astrocytes to develop an accessible human cellular system for the study of these neurological disorders and their potential therapeutics.

Initial studies to derive induced astrocytes (i-astrocytes) were performed by differentiating induced neural progenitor cells (iNPCs), which were obtained by direct conversion of human fibroblasts (Meyer et al., 2014). In this study Kaspar and colleagues modeled ALS disease phenotype using i-astrocytes from healthy and ALS patients harboring SOD1 and C9ORF72 mutations by co-culturing them with healthy motor neurons to test the astrocyte induced non-cell autonomous toxicity. The study demonstrated that in contrast to astrocytes from healthy donors, the astrocytes from familial ALS patients harboring SOD1 and C9ORF72 mutations were equally toxic to the surrounding motor neuron survival when compared to

those derived from sporadic patients, where the cause is unknown, alluding towards the possibility of a common disease mechanism. More recently, Broccoli and colleagues used a minimal set of three transcription factors NFIA, NFIB and Sox9 to convert embryonic and postnatal mouse fibroblasts into astrocytes (Caiazzo et al., 2015). They further demonstrated that the induced astrocytes have a similar gene expression profile and are functionally comparable to native brain astrocytes. These induced astrocytes are also activated upon cytokine stimulation and the same cocktail was able to induce astrocyte-like cells from human fibroblasts. The two recent direct reprogramming studies provide an easy and high-throughput technique to make patient-derived astrocytes, which can be useful in modeling neurodegenerative and neurodevelopmental diseases, therapeutic drug screening and toxicity assays as well as studying the neuronal microenvironment under disease states.

Many neurological diseases ranging from multiple sclerosis, subcortical stroke, vascular dementia, childhood leukodystrophies and cerebral palsy involve some loss of oligodendrocytes, the myelin-producing cells of the brain (Goldman, 2013). Cellular replacement of oligodendrocyte precursor cells (OPCs) is a promising potential therapeutic strategy for such myelin disorders as well as spinal cord injuries. Partially overlapping transcription factor combinations containing Sox10, Olig2 with either Nkx6.2 or Zfp536, is sufficient to convert mouse fibroblasts to iOPCs that exhibit morphological and molecular features resembling primary OPCs (Najm et al., 2013; Yang et al., 2013). iOPCs derived from the two methods display slightly different lineage potentials, however, differentiate into multiprocessed oligodendrocytes *in vitro*. When transplanted *in vivo*, iOPCs give rise to mature oligodendrocytes that can integrate into the CNS and myelinate axons of congenitally dysmyelinated mice. When this strategy is translated to obtain human iOPCs, it can facilitate neurological disease modeling and autologous remyelinating therapies.

Strategies for direct reprogramming to neurons

Transcription factors

The earliest strategy for direct lineage conversion into neurons involved the over expression of transcription factors Ascl1, Brn2 and Myt1L (Vierbuchen et al., 2010) and NeuroD1 (Pang et al., 2011). Many of these factors have an important role in the neuronal lineage determination during *in vivo* development. Subsequently studies identified and utilized transcription factors that were highly expressed in subtype specific neurons and over expressed them with or without the addition to the pro neural transcription factors to derive many other subtypes of neurons (Table 1). Notably, repression of a single RNA binding protein PTB leads to expression of several neuronal lineage specific microRNAs and transcription factors resulting in transdifferentiation of diverse cell types into neuronal like cells and fibroblasts into functional neurons (Xue et al., 2013).

MicroRNAs

In addition to ectopic expression of transcription factors to mediate lineage conversion, other approaches such as microRNA mediated conversion and chemical reprogramming have been explored for various neuronal cell types. In contrast to complete induction of neuronal state when using transcription factors, overexpression of the neuronal-specific microRNAs

miR-9/9* and miR-124 alone in human fibroblasts could only induce cells that express the neuronal marker MAP2 (Ambasudhan et al., 2011; Pang et al., 2011; Yoo et al., 2011). Addition of other transcription factors was required for deriving fully functional neurons when overexpression neuronal microRNAs to induce lineage conversion (Table 1) (Ambasudhan et al., 2011; Pang et al., 2011; Yoo et al., 2011). Remarkably, the reprogramming into neuronal lineage induced by a single RNA binding protein PTB is achieved by the de-repression of a large array of neuronal genes, including microRNA miR-124 and multiple neuronal-specific transcription factors, in non-neuronal cells (Xue et al., 2013).

Small molecules/chemical reprogramming

Induction of cellular reprogramming using small molecules provides an attractive alternative to transcription factor mediated lineage reprogramming owing to many advantages such as cost effectiveness; cell permeability and temporal and spatial control (Li et al., 2013). Although the identification of small molecules that can completely replace ectopic transcriptional factors for direct reprogramming remains a major challenge, many studies have complemented the transcription factors with small molecules in deriving neuronal lineages (Table 1) (Cheng et al., 2014; Kim et al., 2014; Ladewig et al., 2012; Liu et al., 2013; Zhu et al., 2014). More recently, iNPCs were also generated using only small molecules with a chemical cocktail containing VPA, an inhibitor of HDACs; CHIR99021, an inhibitor of GSK-3 kinases and Repsox, an inhibitor of TGF- β pathways) under physiologically hypoxic conditions (Cheng et al., 2014). The chemically induced NPCs (ciNPCs) display proliferative and self-renewing abilities, gene expression profiles, and multipotency for different neuroectodermal lineages *in vitro* and *in vivo* and are similar to mouse brain-derived NPCs. More recently, neuronal cells were obtained directly from mouse and human fibroblasts using only a cocktail of small molecules (Hu et al., 2015; Li et al., 2015). Using a combination of four molecules (Forskolin; ISX9; CHIR99021, a GSK3 beta inhibitor, and I-BET151), Li et al., induced the reprogramming of 90% of fibroblasts into neurons. Hu et al., used a potent combination of seven molecules consisting of valproic acid, CHIR99021, Repsox, forskolin and other compounds known to induce neuronal differentiation such as SP600125 (JNK inhibitor), GO6983 (PKC inhibitor) and Y-27632 (ROCK inhibitor), which induced neuronal conversion 7 days after incubation. The chemically induced neurons resembled hiPSC-derived neurons and human iNs (hiNs) with respect to morphology, gene expression profiles, and electrophysiological properties. This approach was further applied to generate hciNs from familial Alzheimer's disease patients establishing this method as a transgene-free and chemical-only approach for direct reprogramming of human fibroblasts into neurons for modeling neurological diseases and for regenerative medicine (Hu et al., 2015).

Modeling neurological diseases using direct reprogrammed neurons

One of the first studies to provide evidence that neurons obtained from direct lineage conversion can survive *in vivo* in mice was performed with induced dopaminergic neurons (Caiazzo et al., 2011). Using three transcription factors - Ascl1, Nurr1 and Lmx1a, Caiazzo et al derived induced dopamine neurons that displayed several hallmarks of functional

dopamine neurons *in vitro*, including morphology, marker gene expression, electrophysiological and biochemical properties. The same transcription factors were also used to convert human cells from aged individuals suffering from Parkinson's disease. A combination of six factors containing Lmx1a, Nurr1, Foxa2, EN1, Ascl1, and Pitx3 was also able to reprogram mouse fibroblasts into induced dopaminergic neurons which displayed gene expression patterns and electrophysiological properties similar to primary midbrain dopaminergic neurons (Kim et al., 2011b). Both studies demonstrated that when transplanted into a mouse, induced dopaminergic neurons engrafted into the neural circuit and provided symptomatic relief in the mouse model of Parkinson's disease. Furthermore, the induced dopaminergic neurons maintain functional properties such as electrically excitable membranes, synaptic currents, and dopamine release after long-term engraftment in the brain tissue and lead to substantial reduction of motor symptoms in a Parkinson's disease animal model (Dell'Anno et al., 2014). While three factors were sufficient to generate the induced dopaminergic neurons from mouse cells, more factors were necessary to induce the reprogramming of human cells into dopaminergic neurons. The proneural factors Ascl1, Brn2 and Myt1L were used in addition to FoxA2 and Lmx1a to induce direct conversion of human fibroblasts to dopaminergic neurons (Pfisterer et al., 2011). Introduction of five transcription factors Ascl1, Ngn2, Sox2, Nurr1 and Pitx3 could also directly reprogram human fibroblasts to dopaminergic neurons that resembled morphologically and electrophysiologically to their *in vivo* counterparts, and could provide symptomatic relief in a rat model of Parkinson's disease (Liu et al., 2012). These studies suggest that induced dopaminergic neurons generated from fibroblasts by direct lineage reprogramming hold promise for modeling neurodegenerative disease and for cell-based therapies of Parkinson's disease.

One other neuronal subtype of clinical importance is motor neurons, which are affected in patients with spinal muscular atrophy and amyotrophic lateral sclerosis. Using a combination of seven transcriptional factors (Ascl1, Brn2, Myt1L, Ngn2, Isl1, Lhx3 and Hb9) that include the three induced neuron factors, Son et al obtained induced motor neurons (Son et al., 2011). Much like conversion of human fibroblasts into induced neurons, the conversion of iMNs from human fibroblasts also required the addition of NeuroD1 (Son et al., 2011). The reprogrammed iMNs established appropriate transcriptional program, exhibited the electrophysiological characteristics of motor neurons, and formed functional synapses with muscle *in vitro*. The iMNs also displayed *in vivo* engraftment capacity in chicken fetal spinal cord similar to ES-derived motor neurons. The iMNs derived from a mouse model of ALS containing the SOD1G93A mutation displayed impaired survival relative to control iMNs and control iMNs showed selective sensitivity to the toxic effect of mutant glia from SOD1G93a ALS mouse (Son et al., 2011). Addition of two small molecules (forskolin and dorsomorphin) with the transcription factor Neurogenin 2 (Ngn2) converted human fetal lung fibroblasts into motor neuron like cells with high purity and efficiency. These neurons were cholinergic and showed mature electrophysiological properties and exhibit motor neuron-like features, including morphology, gene expression and the formation of functional neuromuscular junctions *in vitro* (Liu et al., 2013). Inclusion of an additional transcription factor, Sox11, improved the conversion of postnatal and adult skin fibroblasts from controls and human patients with spinal muscular atrophy or ALS to

cholinergic neurons (Liu et al., 2013). More recently, iMNs obtained by direct reprogramming were used to model the disease phenotype associated with expanded GGGGCC (G4C2) nucleotide repeats within the C9ORF72 gene, the most common genetic mutation associated with both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (Wen et al., 2014). Induced motor neurons derived from human C9orf2-ALS-patient exhibited intranuclear aggregates of the dipeptides (proline-arginine (PR)) from the expanded repeats than control cultures, much similar to the aggregates found in human spinal cord tissues from C9ORF72 ALS/FTD patients. In addition, the human C9orf2 ALS patient derived neurons showed significantly lower survival over extended *in vitro* culture periods suggesting that they undergo accelerated neurodegeneration (Wen et al., 2014). The evidence from the above studies indicates that the direct reprogrammed iMNs exhibit both cell-autonomous and non cell-autonomous phenotypes of diseases states making them a good model for use in *in vitro* disease modeling of motor neuron disorders, like spinal muscular atrophy and amyotrophic lateral sclerosis (Son et al., 2011).

Modeling the biology and physiology of pain has long been a major challenge in the identification of successful pain medications. Two recent studies have brought the area of pain biology closer to modeling pain and peripheral neuropathy *in vitro* (Blanchard et al., 2015; Wainger et al., 2015). Transient expression of BRN3A and Ngn1 or BRN3A and Ngn2 in mouse and human embryonic fibroblasts resulted in induction of three major classes of peripheral sensory neurons – nociceptors/pruritoceptors, mechanoreceptors, and proprioceptors (Blanchard et al., 2015). Similarly, a combination of five transcription factors Ascl1, Myt1l, Ngn1, ISL2 and KLF7 specifically induced nociceptors (as opposed to the broad class of sensory neurons) from mouse and human fibroblasts (Wainger et al., 2015). In both studies, the induced neurons expressed specific functional receptors and channels, displayed morphology and electrophysiological properties resembling sensory neurons (Blanchard et al., 2015; Wainger et al., 2015). The mouse and human induced sensory neurons were selectively responsive to known ligands that mimic itch and pain. Furthermore, the induced nociceptors could be used to model inflammatory pain hypersensitivity and painful chemotherapy-induced neuropathy *in vitro* (Wainger et al., 2015). Moreover, induced nociceptors from fibroblasts taken from individuals with familial dysautonomia (hereditary sensory and autonomic neuropathy type III), showed impaired neurite growth and a reduced number of branches per cell, compared with induced nociceptors from healthy controls, indicating a previously unknown aspect of the disease pathophysiology (Wainger et al., 2015). Given that other classes of peripheral sensory neurons such as mechanoreceptors and proprioceptors could also be generated with direct reprogramming, they could serve as an *in vitro* model for degenerative diseases such as Friedreich's ataxia in which the peripheral sensory neurons are particularly susceptible to neurodegeneration (Blanchard et al., 2015).

Coexpression of miR-9/9*-124 with transcription factors enriched in the developing striatum, BCL11B (also known as CTIP2), DLX1, DLX2, and Myt1l, can reprogram human fibroblasts into an enriched population of neurons similar to striatal medium spiny neurons (MSNs), the primary cell type affected in Huntington's disease (Victor et al., 2014). The reprogrammed MSNs have a gene expression profile comparable to the primary striatal neurons in the human brain that were collected by laser capture microdissection (LCM). When transplanted in the mouse brain, the reprogrammed human cells persisted in situ for

over 6 months, exhibited membrane properties equivalent to native MSNs, and functionally integrated into the local circuit and extended projections to the correct anatomical targets of MSNs (Victor et al., 2014). Notably, the induced neurons obtained by direct conversion of fibroblasts from a mouse containing autism-associated R704C substitution in *neurologin-3*, recapitulated the synaptic phenotype observed in R704C-mutant endogenous neurons indicating that broader target type of iN cells can be suitable for studying complex neurological diseases such as autism (Chanda et al., 2013).

Emergence of 3D organoids made from patient-specific induced pluripotent stem cells gives an opportunity to test neurological disease model in the context of their microenvironment. Recently, Lancaster et al. established a novel approach of developing cerebral organoids via *in vitro* culture system with human pluripotent stem cells (Lancaster et al., 2013). This could help recapitulate features of mammalian neurodevelopment. Additionally it could also help discern the pathogenesis of complex genetic neurological disorders, especially, when using patient-derived cells for making these “mini-organs” thus providing a window into differential developmental patterning between healthy and diseased states such as microcephaly. In future we could wield this organoid technology with more functionally mature directly reprogrammed cells along with providing means of vasculature for proper oxygen supply extending their size, viability and homogeneity. This can help us fully harness the potential of these cerebral organoids for disease modeling and drug screening (Lancaster and Knoblich, 2014).

***In vivo* reprogramming**

A major goal of regenerative medicine is to convert resident tissue-specific cells into the target cell types to replace diseased cells by a process called *in vivo* lineage reprogramming for *in vivo* or in situ regeneration and repair (Heinrich et al., 2015). In neurological diseases, the approach of converting the resident glial cells may be a viable strategy because transplantation can be highly invasive and the accessibility and availability of native progenitor cell types might be limited (Heinrich et al., 2015; Robel et al., 2011). Additionally, the *in vivo* microenvironment can provide an appropriate three-dimensional setting for functional maturation of the target cell types (Nizzardo et al., 2013). Several studies have successfully converted brain-resident cells such as astrocytes and NG2 glia into functional neurons *in vitro* using transcription factors (Heinrich et al., 2014; Heinrich et al., 2010; Heinrich et al., 2011; Heinrich et al., 2012; Karow et al., 2012). Extending a similar strategy, reprogramming of resident glial cells also shows promise for *in vivo* brain repair. Over-expression of Sox2 in astrocytes converts them into neuroblasts *in vivo* (Niu et al., 2013). In the presence of suitable conditions the induced neuroblasts could give rise to many neural cell types, electrophysiologically mature neurons, which functionally integrate into the local neural network (Niu et al., 2013). Overexpression of Sox2 can also induce the conversion of astrocytes and NG2 cells into neurons in an injury dependent manner (Heinrich et al., 2014; Su et al., 2014). Transplanted human fibroblasts and human astrocytes, which are engineered to express inducible forms of neural reprogramming genes (Ascl1, Brn2 and Myt1L), can convert into neurons upon activation of the neural reprogramming genes (Torper et al., 2013). Overexpression of NeuroD1 *in vivo* can directly reprogram proliferating astrocytes and NG2 glia to functional glutamatergic and GABAergic

neurons, which integrate into the host's neural circuitry, in mouse models of both injury and Alzheimer's disease (Guo et al., 2014). The induced neurons receive synaptic inputs from neurons neighboring the injury site. Recently, neuroblasts were generated directly from adult human and mouse astrocytes by miR-302/367-driven induction (Ghasemi-Kasman et al., 2015). Taken together, the in vivo reprogramming approach is promising for converting glial scar cells into neurons in a wide range of neurological diseases.

Challenges and new directions in deriving direct reprogrammed neurons

There are myriad ways of generating functional neuronal subtypes from somatic cells. Emergence of novel therapeutic approaches has contributed towards the innovation in the field of regenerative medicine. Nonetheless, each of these methods has its own set of limitations that needs to be addressed to improve the quality of induced neuronal cell types for better disease modeling (Fig. 1).

Transgene load

Defined factor reprogramming is a stochastic process requiring over-expression of multiple genes (Hu, 2014). Conventional reprogramming uses retroviral integration of transgenes, which is often maintained beyond the reprogramming stage with unknown consequences. The use of viral transgenes raises concerns of insertional mutagenesis, residual expression, uncontrolled silencing of transgenes, re-activation of reprogramming factors (Okita et al., 2007), immunogenicity (Zhao et al., 2011), senescence and cell death, and lastly variable vector tropism depending on starting cell types. Numerous alternate routes for effective gene delivery have evolved to alleviate these problems. These latest methods include transient transfection, non-integrating viral vectors, Cre-loxP excision of transgenes, excisable transposon, protein transduction, RNA transfection, microRNA transfection, RNA virion, RNA replicon, non-integrating replicating episomal plasmids, minicircles, polycistron, and preintegration of inducible reprogramming factors (Hu, 2014). Some of the transgene related concerns could also be alleviated by using small molecule mediated reprogramming or by using an inducible transgene expression. Several groups have used small-molecule mediated conversion for obtaining pluripotent cells as well as neuronal cell types (Hou et al., 2013; Li et al., 2013; Liu et al., 2013; Zhu et al., 2014; Zhu et al., 2010). A common shortcoming of these methods is lower conversion efficiency. Recently many studies have tried exploiting some of these techniques to obtain optimal stoichiometry of the desired reprogramming factors (Carey et al., 2011) and thus acquiring high conversion efficiency and functionally mature target cell type. A drug-inducible vector system could also be used to control the load of transgene expression in the resultant reprogrammed cells. Many reports have demonstrated successfully that an inducible single factor mediated reprogramming generates highly efficient conversion (Chanda et al., 2014; Kim et al., 2014; Lujan et al., 2012). Other studies have also utilized the inducible transgene expression when using multiple factors (Cassady et al., 2014; Mazzoni et al., 2013), generating neuronal cells from human fibroblasts with inducible microRNAs (Lau et al., 2014) and induced dopaminergic neurons from mouse fibroblasts (Kim et al., 2011b).

Transcriptional regulation

Molecular mechanisms of how the lineage specific transcription program, which are often epigenetically inactive in the initial cell population are activated during direct lineage reprogramming remain elusive. Few studies have used directly reprogrammed neurons as a tool in understanding the molecular mechanisms underlying reprogramming and for identifying barriers to reprogramming (Cahan et al., 2014; Mazzoni et al., 2013; Morris et al., 2014; Wapinski et al., 2013). Recent studies have provided insights into possible mechanisms occurring during the derivation of induced neurons generated from the overexpression of *Ascl1*, *Brn2* and *Myt1L* (Wapinski et al., 2013). Wapinski et al determined that the genomic binding patterns of *Ascl1* are similar between fibroblasts and neural progenitor cells. They further demonstrated that exogenous *Ascl1* binds to its *bona fide* neuronal target genes in fibroblasts during very early states of neuronal state induction and facilitates the subsequent recruitment of other exogenous factors, such as *Brn2* and *Myt1L*. This study proposes a hierarchical mechanism for the BAM factor induced neuronal conversion demonstrating that *Ascl1* acts as a pioneering during iN cell induction and can bind to its lineage-specific genomic targets and the pioneering role of *Ascl1* was dependent of the trivalent histone modification signature at its target sites (Wapinski et al., 2013). An emerging paradigm in the area of direct reprogramming of neuronal cells is to induce cell fate transitions using a single transcription factors such as the bHLH factors *Ascl1* and *Ngn2* in human and mouse ES or iPS cells (Chanda et al., 2014; Kim et al., 2014; Thoma et al., 2012; Zhang et al., 2013) which results in reproducible efficiencies and homogeneous target populations that are have similar properties independent of the ES or iPS cell line used. The induced neurons obtained by single factor induced reprogrammed displayed mature neuronal markers, exhibited typical passive and active intrinsic membrane properties, and formed functional pre- and postsynaptic structures. The ability of a single gene to determine cell fate decisions of uncommitted pluripotent stem cells provides insights into the role of key developmental genes during lineage commitment and raises interesting questions about the role of these lineage-determining factors in neurogenesis from different starting cell types (Chanda et al., 2014; Thoma et al., 2012; Zhang et al., 2013). Epigenetic context also plays a role in *in vivo* reprogramming. Regional differences and injury conditions and the resulting starting epigenetic configuration of target regions in the starting cell type have significant influence on the efficacy of reprogramming and subsequent survival of the newly generated neurons in the adult rodent brain (Grande et al., 2013). An understanding of the factor induced reprogramming will help identifying better conditions or combinations of transcription factors for neuronal subtype specification to develop clinically useful samples. Furthermore, if the hierarchical model holds true, it would be important of identify the starting epigenetic state of the cells that are being converted to overcome barriers that impede the reprogramming. Additionally, fewer transcriptional factors may be necessary for neuronal conversion when starting from an uncommitted cell state.

Molecular characterization of reprogrammed cells

A critical concern in lineage reprogramming is evaluating the molecular similarity between the converted cells and the primary neuronal subtypes. Several measures such as the analysis of global and lineage specific gene expression and epigenetic patterns, the silencing of exogenous factors have been used for evaluating the converted cells (Cohen and Melton,

2011). Currently, only few studies have included the primary isolated samples of target neuronal cell type as the positive control for a genome wide assessment of gene expression patterns (Caiazzo et al., 2011; Cassady et al., 2014; Han et al., 2012; Marro et al., 2011; Yang et al., 2013), which makes it relatively difficult to make an overall comparison of the function of directly converted neuronal cells generated from different studies. Most recently, using a network biology platform referred to as CellNet, the gene regulatory networks for several target cell types derived from directed differentiation and direct lineage conversion were compared to their native counterparts (Cahan et al., 2014). This study concurs with previous studies performed for the induced neurons in that although the reprogrammed neurons express many target specific genes, they fail to completely silence expression programs of the starting cell population (Cahan et al., 2014; Caiazzo et al., 2011; Cassady et al., 2014; Han et al., 2012; Marro et al., 2011; Yang et al., 2013). Moreover, Cahan et al. indicated that cells derived from directed differentiation had a more similar target cell type gene regulatory networks in comparison to the cells obtained from direct reprogramming. However, the Cellnet analysis was performed on data from bulk populations and in many cases cannot distinguish the target cell subtypes or reveal the heterogeneity in extent of reprogramming. Characterization of the functional and molecular properties of the reprogrammed cells at the single cell level would provide additional information on variations in gene expression patterns in the induced neuronal cells and enable evaluation of cell maturation of reprogrammed neurons. Although the residual transcriptional program might suggest an immature or incomplete phenotype, studies have indicated that the residual starting cell programs get silenced over time and do not tend to affect the functionality of the desired cell type (Caiazzo et al., 2011; Han et al., 2012; Marro et al., 2011; Yang et al., 2013). A comprehensive knowledge of the molecular mechanisms underlying the gene regulatory network, including the role of microRNAs and non-coding RNAs may enable the remodeling of their epigenome and will be of interest for both *in vitro* and *in vivo* reprogramming. Nevertheless, Cellnet is a valuable tool in identifying ways to improve maturation and functionality of reprogrammed neurons and could be used for identifying aspects required to reinforce upon the transcriptional network via locus specific epigenetic activation or repression of key functional genes or barriers for reprogramming (Morris et al., 2014; Xu et al., 2015).

Epigenomic profiling

Direct lineage conversion also involves ‘re-programming’ the epigenome of the starting cell type to match that of the desired target cells. The changes should be permanently etched into the epigenome of these reprogrammed cells so that it is reflected in their transcriptional profiles when compared to primary cells later on. Common pathways targeted by direct reprogramming strategies (transcription factors, microRNAs and chemical reprogramming) include a combination of robust activation of neural genes and repression of fibroblasts genes brought about by chromatin remodeling and resetting of the epigenetic configuration (Fig. 2). Epigenetic features such as lineage specific enhancers play an important role in cellular function. A critical concern in direct lineage reprogramming is to evaluate the similarity between the converted cells and the target cell type (Cohen and Melton, 2011). Although several studies have evaluated the gene expression profiles of the reprogrammed neurons, few very studies have characterized their epigenetic features and mechanisms

driving direct reprogramming into neuronal cells (Wapinski et al., 2013). The conversion of fibroblasts to neuronal fates, including diverse induced neuronal subtypes like motor and dopaminergic neurons, strongly suggests that core neuronal lineage-specific transcription factors initiate direct reprogramming and reconstitute the target transcriptional network activity. Interestingly, the conversion to many neuronal subtypes share common inducers, such as Ascl1 or Ngn2, implying that direct reprogramming to a neuronal cell fate generally occurs through common mechanisms. Existing technologies such as ChIP-seq for active enhancers (histone H3 lysine 27 acetylation) and recent methodologies such as assay for transposase accessible chromatin (ATAC-seq) could help unravel the similarities of the reprogrammed neurons by comparing their epigenetic profile of the primary neurons. Recently, Masserdotti et al. utilized an inducible activation system during neuronal conversion of astrocytes to dissect the role of Ascl1 and Ngn2 in neuronal reprogramming. Earlier the same group had identified that when expressed in astrocytes obtained from postnatal murine cerebral cortex, Ascl1 results in GABAergic neurons, while Neurog2 gives rise to glutamatergic neurons (Berninger et al., 2007; Heinrich et al., 2010). In their recent study, Masserdotti et al. identified that Ascl1 and Ngn2 rapidly elicited distinct neurogenic transcription programs with only a small subset of shared target genes including NeuroD4 (Masserdotti et al., 2015). Their results showed similarities in gene regulation during *in vivo* neuronal development and *in vitro* direct neuronal reprogramming, suggesting that transcriptional and epigenetic analysis of direct reprogramming might be useful for understanding the mechanisms underlying direct reprogramming of other neuronal subtypes.

Optimizing reprogramming efficiency

The efficiency of direct reprogramming into neurons is generally not very high based on fibroblast to neuron conversion (Son et al., 2011; Vierbuchen et al., 2010). Functional neurons being post-mitotic, the ones derived from direct lineage conversion are not expandable, which is also a barrier for disease modeling, drug development, and cell therapy that require large pool of cell. Although the current conversion efficiencies may be sufficient for *in vitro* studies, it might not represent a sufficient amount of cells for large-scale therapeutic screening and clinical analysis of neurotoxicity. Therefore, it is necessary to optimize and develop reprogramming techniques to allow for the robust and efficient generation of target desired cells for practical application. Recently, many proliferative stem cells such as neural stem cells or progenitors have been induced by lineage reprogramming resulting in an expandable system for generating the desired cell type (Cassady et al., 2014; Han et al., 2012; Kim et al., 2011a; Kim et al., 2014; Lujan et al., 2012; Ring et al., 2012; Zhu et al., 2010). The induced neural stem cells have also demonstrated that they can efficiently engraft and integrate into the *in vivo* microenvironment making them a potential renewable alternative to direct reprogrammed neurons. Nonetheless there still remains a dire need to improve the reprogramming efficiency, for generating large numbers of diverse neural subtypes for disease modeling and therapeutic purposes, by augmenting culture conditions, maybe by using signaling factors and small molecule cocktail.

Cellular microenvironment and functional maturation

Obtaining functionally mature neurons is crucial for disease modeling, drug development, and cell replacement therapy. Generating a homogenous population of reprogrammed and

functional mature neurons remains a major challenge. The inherent heterogeneity in reprogramming efficiencies of individual cells could play a role in determining the maturity of the reprogrammed neurons. Functional maturity of reprogrammed cells could be improved by identifying and adding transcription factors which induce functional maturation in addition to lineage determining factors (Xu et al., 2015). Transcription factors or epigenetic changes required for improving maturation properties could be identified by analyzing gene regulatory networks of functionally mature primary target cell types (Cahan et al., 2014; Morris et al., 2014). Maturation could also be improved by providing the optimal cellular microenvironment to the induced reprogrammed cells. In case of neuronal cells co-culturing them with glial cells or generating three-dimensional “mini-organs” is an attractive alternative as opposed to growing them on plastic dishes for effective reprogramming (Lancaster et al., 2013). It has been recently shown that co-culturing with glia provide necessary trophic support to increase neuronal maturity of these induced neurons (Chanda et al., 2014). Additionally, co-culturing healthy motor neurons with familial and sporadic ALS patient-specific induced astrocytes harboring mutations in SOD1 or C9ORF72 genes have shown motor neuron toxicity proving the glial dysfunction aspect of this neurodegenerative disease (Meyer et al., 2014). Studies like these can also help discern the sporadic conditions of ALS where the disease cause is unknown. This co-culture technique will serve as a more complete model for such complex neurological disorders like ALS, which could be an improved platform for testing drug responsiveness. A slew of recent studies have gone beyond the traditional two-dimensional co-culture techniques and incorporated extracellular 3D matrices with inherent self-organizing potential to create cerebral, retinal and inner ear organoids (Eiraku et al., 2011; Koehler et al., 2013; Nakano et al., 2012). It will be imperative to assimilate similar culture conditions while doing direct conversion into neural lineages using defined factor cocktails to induce *bona fide* functionally mature target cells.

In vivo functional assays

A general limitation in using *in vitro* reprogrammed neurons for transplantation *in vivo* is the lack of extensive characterization of the reprogrammed cells. *In vivo* functional analysis of reprogrammed neurons is the most stringent test to evaluate their function and maturation. Reprogrammed cells that are fully functional and should be able to survive and integrate into the existing tissue. Assessing the survival of *in vitro* reprogrammed neurons after transplantation and proper engraftment and axon projection into existing tissue, and formation of functional synapses *in vivo* are necessary to fully characterize the functionality of the reprogrammed neurons. Although many mouse induced neurons have been characterized *in vivo* by transplantation into the mouse brain, only a few induced human neurons have been transplanted *in vivo* and have shown the ability of surviving and integrating into the *in vivo* environment (Caiazzo et al., 2011; Cassady et al., 2014; Han et al., 2012; Kim et al., 2011a; Kim et al., 2011b; Kim et al., 2014; Najm et al., 2013; Torper et al., 2013; Yang et al., 2013). Reversion of motor symptoms or attenuation of atrophy in animal models of neurological diseases will emphasize the clinical suitability of the grafted reprogrammed neurons.

Conclusions and perspectives

Direct conversion to generate patient-specific cells, combined with genome editing technologies is a powerful tool for developing assays and conducting novel research, and could eventually allow the development of autologous target cell type transplantation upon gene correction. However, further studies in preclinical model systems remain to be addressed to improve this technology for expanding applications in clinical studies. Further work elucidating the mechanisms by which transcription factors promote neuronal cell type-specific conversion with distinct phenotypes is required. Further studies are required in identifying methods to introduce the transcription factor in a safe, efficient and specific manner to the desired resident cell type for *in situ* reprogramming. Assays to assess functional properties of reprogrammed cells and measure improvement upon cell transplantation and potential side effects must be validated. Knowledge gained from these studies would enable conversion patient specific somatic cells into neuronal subtypes for therapy in an injury- or neurological disease such as ALS, Alzheimer's and Parkinson's disease.

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Figure 1. Different approaches for somatic cell reprogramming into neuronal lineages

Novel strategies are required to successfully increase the yield of homogeneous population of target cells and efficiency of reprogramming. Different aspects of reprogramming can be manipulated to obtain that goal like defined transcription factor cocktail, small molecules, non-coding RNAs and/or epigenetic modulation of gene regulatory network. While most neural lineages (neural stem/progenitor cells, different subtypes of neurons, glial cells like astrocytes and oligodendrocytes) has been successfully generated from patient fibroblasts using one or a combination of these methods, some like microglial cells are yet to be made.

Figure 2. Common pathways targeted by different cellular reprogramming approaches

Most methods that dictate the cellular conversion of somatic cells into neural cells have a mechanistic convergence. Diverse approaches like transcription factor cocktail, small molecules, miRNAs, siRNA or shRNA knockdown and/or protein repression usually result in effective chromatin remodeling through robust activation of neural genes and repression of fibroblastic genes. These are the areas that can be exploited for improving reprogramming efficiency.

Table 1

List of neural cells generated by lineage conversion of somatic cells

Initial Cell Population	Target Cell Type	Morphogens or Small molecules	Reprogramming factors	References
Mouse in vitro lineage conversion				
Fibroblasts	Astrocyte		Nfia, Nfib, Sox9	(Caiazzo et al., 2015)
Fibroblasts	Neural progenitor cells		Sox2, Klf4, c-Myc, Oct4	(Kim et al., 2011a)
Fibroblasts	Neural precursor cells		Brn2, Sox2, FoxG1	(Lujan et al., 2012)
Fibroblasts	Neural progenitor cells	VPA, CHIR99021, RepSox under hypoxia		(Cheng et al., 2014)
Fibroblasts	Neural stem cells		Brn4, Sox2, Klf4, c-Myc, E47	(Han et al., 2012)
Fibroblasts	Neural stem cells		Sox2, Klf4, c-Myc, Oct4 (limiting activity at initial stage)	(Thier et al., 2012)
Fibroblasts	Neural stem cells		Sox2	(Ring et al., 2012)
Sertoli cells	Neural stem cells		Ascl1, Ngn2, Hes1, Id1, Pax6, Brn2, Sox2, c-Myc, Klf4	(Sheng et al., 2012b)
Adult liver cells	Neural stem cells		Brn2, Hes1, Hes3, Klf4, Myc, Notch1, (NICD), PLAGL1, and Rfx4	(Cassady et al., 2014)
B-lymphocytes	Neural stem cells		Brn2, Hes1, Hes3, Klf4, Myc, Notch1, (NICD), PLAGL1, and Rfx4	(Cassady et al., 2014)
Astrocytes	Neuroblasts		Sox2	(Niu et al., 2013)
Hepatocytes	Neurons		Ascl1, Brn2, Myt1l	(Marro et al., 2011)
Fibroblasts	Neurons		PTB repression	(Xue et al., 2013)
Fibroblasts	Neurons		ASCL1	(Chanda et al., 2014)
Astrocytes	Neurons		Ascl1, Brn2, Myt1l	(Torper et al., 2013)
Astrocytes	Neurons (glutamatergic)		NeuroD1	(Guo et al., 2014)
Fibroblasts	Neurons (dopaminergic)		Ascl1, Pitx3, Lmx1a, Nurr1, Foxa2, EN1	(Kim et al., 2011b)
Fibroblasts	Neurons (dopaminergic)		Ascl1, Lmx1a, Nurr1	(Caiazzo et al., 2011)
Fibroblasts	Neurons (dopaminergic)		Lmx1a, Foxa2, Ascl1, Brn2 or Lmx1b, Otx2, Nurr1, Ascl1, Brn2	(Sheng et al., 2012a)
Fibroblasts	Neurons (dopaminergic)		Brn2, Foxa2, Sox2	(Tian et al., 2015)
Astrocytes	Neurons (GABAergic)		Ascl1, Dlx2	(Heinrich et al., 2010)
Fibroblasts	Neurons (glutamatergic)		Ascl1, Brn2, Myt1l	(Vierbuchen et al., 2010)

Initial Cell Population	Target Cell Type	Morphogens or Small molecules	Reprogramming factors	References
Fibroblasts	Nociceptor, Mechanoreceptor, Proprioceptor neurons		Brn3a, Ngn1 or Ngn2	(Blanchard et al., 2015)
Fibroblasts	Nociceptor Neurons		ASCL1, MYT1L, ISL2, KLF7, NGN1	(Wainger et al., 2015)
Astrocytes	Neurons (glutamatergic)		Ngn2	(Heinrich et al., 2010)
Fibroblasts	Neurons (motor)		Brn2, Ascl1, Myt1l, Lhx3, Hb9, Isl1, Ngn2	(Son et al., 2011)
Fibroblasts	Oligodendrocyte progenitor cells		Olig1, Olig2, Nkx2.2, Nkx6.2, Sox10, ST18, Gm98, Myt1l	(Najm et al., 2013)
Fibroblasts	Oligodendrocyte progenitor cells		Sox10, Olig2, Zfp536	(Yang et al., 2013)
Astrocytes	Neuroblasts		miR-302/367	(Ghasemi-Kasman et al., 2015)
Mouse in vivo reprogramming				
NG2 cells	Neurons (Glutamatergic and GABAergic)		NeuroD1	(Guo et al., 2014)
Astrocytes	Neurons		Ascl1, Brn2, Myt1l	(Torper et al., 2013)
Astrocytes	Neurons (glutamatergic)		NeuroD1	(Guo et al., 2014)
Astrocytes	Neuroblasts		Sox2	(Niu et al., 2013)
Astrocytes	Neuroblasts		miR-302/367	(Ghasemi-Kasman et al., 2015)
Human lineage conversion				
Fibroblasts	Neural crest cells		SOX10	(Kim et al., 2014)
Fibroblasts	Neural stem cells		SOX2	(Ring et al., 2012)
Fibroblasts	Neurons	CHIR99021, SB431542	ASCL1, NGN2	(Ladewig et al., 2012)
Fibroblasts	Neurons		ASCL1	(Chanda et al., 2014)
Pericyte-derived cells	Neurons		SOX2, ASCL1	(Karow et al., 2012)
Fibroblasts	Dopaminergic Neurons		ASCL1, BRN2, MYT1L, LMX1A, FOXA2	(Pfisterer et al., 2011)
Fibroblasts	Dopaminergic Neurons		ASCL1, LMX1A, NURR1	(Caiazzo et al., 2011)
Fibroblasts	Dopaminergic Neurons		ASCL1, NGN2, SOX2, NURR1, PITX3	(Liu et al., 2012)
Fibroblasts	Glutamatergic Neurons		ASCL1, BRN2, MYT1L, NEUROD1	(Pang et al., 2011)
Fibroblasts	Glutamatergic Neurons		BRN2, MYT1L, miR-124	(Ambasudhan et al., 2011)
Fibroblasts	Glutamatergic Neurons	Forskolin, Dorsomorphin	NGN2	(Liu et al., 2013)
Fibroblasts	Glutamatergic and GABAergic Neurons		ASCL1, MYT1L, NEUROD2, miR-9/9*, miR-124	(Yoo et al., 2011)

Initial Cell Population	Target Cell Type	Morphogens or Small molecules	Reprogramming factors	References
Fibroblasts	Medium Spiny Neurons		DLX1, DLX2, MYT1L, CTIP2, miR-9/9*, miR-124	(Victor et al., 2014)
Fibroblasts	Nociceptor, Mechanoreceptor, Proprioceptor Neurons		Brn3a, Ngn1 or Ngn2	(Blanchard et al., 2015)
Fibroblasts	Nociceptor Neurons		ASCL1, MYT1L, ISL2, KLF7, NGN1	(Wainger et al., 2015)
Fibroblasts	Spinal Motor Neurons		Brn2, Ascl1, Myt1l, Lhx3, Hb9, Isl1, Ngn2, NEUROD1	(Son et al., 2011)
Fibroblasts	Astrocytes		Nfia, Nfib, Sox9	(Caiazzo et al., 2015)
Fibroblasts	Neurons		ASCL1	(Chanda et al., 2014)
Fibroblasts	Motor neurons	Forskolin, ISX9, CHIR99021, I-BET151		(Li et al., 2015)
Fibroblasts	Motor neurons	Valproic acid, CHIR99021, Repsox, forskolin, SP600125 (JNK inhibitor), G06983 (PKC inhibitor), Y-27632 (ROCK inhibitor), Dorsomorphin	(Hu et al., 2015)	
Neonatal cord blood and Adult peripheral blood cells	Neural progenitor cells	SMAD+GSK-3 inhibitor	Oct 4	(Lee et al., 2015)
Astrocytes (in vitro and in vivo)	Neuroblasts		miR-302/367	(Ghasemi-Kasman et al., 2015)