NEURAL REGENERATION RESEARCH
January 2016, Volume 11, Issue 1
www.nrronline.org

SPECIAL ISSUE

Direct reprogramming of somatic cells into neural stem cells or neurons for neurological disorders

Shaoping Hou[1], **Paul Lu**[2, 3, *]

1 Spinal Cord Research Center, Department of Neurobiology & Anatomy, Drexel University College of Medicine, Philadelphia, PA, USA
2 Veterans Administration Medical Center, San Diego, CA, USA
3 Department of Neurosciences, University of California at San Diego, La Jolla, CA, USA

How to cite this article: *Hou S, Lu P (2016) Direct reprogramming of somatic cells into neural stem cells or neurons for neurological disorders. Neural Regen Res 11(1):28-31.*

Funding: *This work was supported by the Veterans Administration, the Craig H. Neilsen Foundation (280072 to SH), the California Institute for Regenerative Medicine.*

Abstract

Direct reprogramming of somatic cells into neurons or neural stem cells is one of the most important frontier fields in current neuroscience research. Without undergoing the pluripotency stage, induced neurons or induced neural stem cells are a safer and timelier manner resource in comparison to those derived from induced pluripotent stem cells. In this prospective, we review the recent advances in generation of induced neurons and induced neural stem cells *in vitro* and *in vivo* and their potential treatments of neurological disorders.

Key Words: *neural cells; induced neural stem cells; induced neurons; pluripotent stem cells; neurological diseases*

***Correspondence to:**
Paul Lu, Ph.D., plu@ucsd.edu.

orcid:
0000-0001-7785-9620 (Paul Lu)

doi: *10.4103/1673-5374.169602*
http:*//www.nrronline.org/*

Accepted: *2015-04-28*

Introduction

Induced neural stem cells or induced neurons are ones artificially derived from somatic cells by epigenetic reprogramming techniques. Nobel Prize winner Shinya Yamanaka and colleagues firstly introduced the reprogramming of somatic cells using defined transcription factors (Takahashi and Yamanaka, 2006), in which fibroblasts were successfully reprogrammed into induced pluripotent stem cells. These cells were proved to differentiate into all types of cells when transplanted into the body. Although induced pluripotent stem cells can be differentiated into neural stem cells and further into neurons, they could form teratomas after transplanted into the host tissue due to the persistence of undifferentiated cells (Miura et al., 2009; Fong et al., 2010). In addition, recent studies have shown that initially silenced transgenes in induced pluripotent stem cells could be spontaneously reactivated and mediated a recovery to pluripotency, leading to a risk of tumor formation (Choi et al., 2014; Nori et al., 2015). To overcome these limitations, somatic cells, such as fibroblasts, are reprogrammed directly into induced neurons or self-renewing induced neural stem cells without the pluripotency stage (Vierbuchen et al., 2010; Pang et al., 2011; Han et al., 2012; Ring et al., 2012).

Despite definitive role of specific transcription factors, cultural condition is also important for the differentiation of induced neurons or induced neural stem cells *in vitro*. To determine the property of induced neurons or induced neural stem cells, several techniques are usually employed, such as morphology, molecular features, electrophysiology, and synaptic activity. To date there has been no report of tumor formation with grafting of these two cell types. Hence, the efficacious generation of induced neurons or induced neural stem cells from somatic cells provides a potentially unlimited source of neural cells, which may supply a novel and powerful system for studying cellular identity and plasticity, neurological disease modeling, drug discovery, and regenerative medicine.

Reprogramming of Induced Neurons *in vitro*

Expressing certain transcription factors can induce a neuronal fate in pluripotent cells or convert somatic non-neuronal cells to functional neurons. The latter is also termed as *transdifferentiation* (**Figure 1**).

Vierbuchen et al. (2010) screened a pool of 19 transcription factors by lentiviral transduction of embryonic fibroblasts from TauEGFP knock-in mice where EGFP expresses specifically in neurons. They eventually identified a neuronal-lineage-specific combination comprised of brain-2 (Brn2, also known as Pou3f2), achaete-scute complex-like 1 (Ascl1), and myelin transcription factor (Myt1l) (BAM). Forced expression of this combination rapidly converted mouse embryonic fibroblasts into functional neurons (Vierbuchen et al., 2010). Although the usage of single factor Ascl1 induced immature neuronal features, addition of Brn2 and Myt1l dramatically promoted the generation of induced neurons. It was further shown that the majority of induced neurons were excitatory and some were inhibitory. Moreover, these induced neurons exhibited functional neuronal activity, *i.e.*, action potential generation and synapse formation. The mechanism of this conversion was postulated to be that high expression levels of neural cell-fate-determining transcription factors activated salient features of neuronal transcriptional program. This could also lead to activation of downstream transcriptional regulators and adjustment

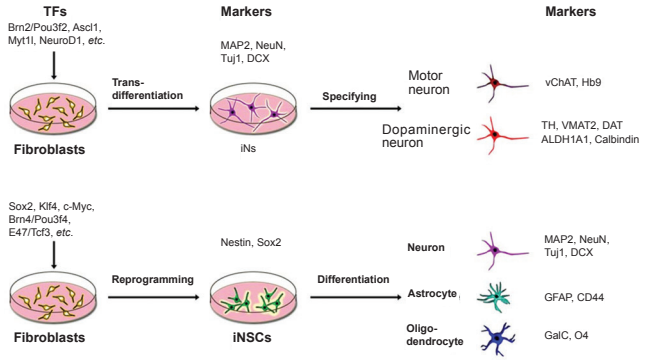

Figure 1 A diagram illustrates the reprogramming of somatic fibroblasts into induced neurons (iNs) or induced neural stem cells (iNSCs) *in vitro* by forced expression of transcription factors (TFs). Fibroblasts can be transdifferentiated into iNs expressing microtubule-associated protein 2 (MAP2), a neuronal specific nuclear protein (NeuN), neuron-specific class III beta-tubulin (Tuj1), and doublecortin (DCX) by transduction of transcription factors brain-2 (Brn2, also known as Pou3f2), achaete-scute complex-like 1 (Ascl1), myelin transcription factor (Myt1l), and NeuroD1. iNs can be further specified to become different phenotypes, such as motor neurons expressing vesicular acetylcholine transporter (vChAT) and homeobox Bb9 (Hb9), or dopamine neurons expressing tyrosine hydroxylase (TH), the vesicular monoamine transporter 2 (VMAT2), dopamine transporter (DAT), aldehyde dehydrogenase 1 A1 (ALDH1A1), and calbindin. Similarly, fibroblasts can be reprogrammed into iNSCs expressing nestin and sex determining region Y-box 2 (Sox2) by transduction of transcription factors Sox2, Kruppel-like factor 4 (Klf4), myelocytomatosis viral oncogene homolog (c-Myc), Brn4/Pou3f4, and transcription factor 3 (E47/Tcf3). iNSCs can be further differentiated into neurons expressing MAP2, NeuN, Tuj1 and DCX, astrocytes expressing glial fibrillary acidic protein (GFAP) and CD44, and oligodendrocytes expressing galactocerebroside (GalC) and oligodendrocyte marker O4.

of repressive and active epigenetic features, reinforcing the induced transcriptional program. Later, the same research group demonstrated that the same factor combination successfully converted fetal and postnatal human fibroblasts into induced neurons (Pang et al., 2011). With an additional factor NeuroD1, another basic helix-loop-helix transcription factor, the efficiency of conversion was improved two to threefolds. Compared to other two formulas including Brn2, Ascl1, NeuroD1 (BAN) and Brn2, Myt1l, NeuroD1 (BMN), this combination (BAMN) generated the most mature neuronal cells. By means of RT-PCR to measure mRNA levels of human endogenous and mouse exogenous BAMN, the four corresponding endogenous genes were activated even after the transgenes were shut down. This indicates the independence of induced neuron state on continuous exogenous gene expression. To determine the underlying mechanism of re-programming, a recent study used zinc finger nuclease (ZFN) technology to knock out *p53* in normal human primary fibroblasts to cause the conversion of fibroblasts into three lineages of neural cells (Zhou et al., 2014). The up-regulation of neurogenic transcription factors Ascl1, Brn2, and Neurod2 after deletion of *p53* suggests the critical role of this gene in cell reprogramming. Another research reported that the neural reprogramming genes were regulated by the neuron-specific microRNA, miR-124 (Lau et al., 2014).

If the transcription factors act on cells intrinsically rather than extrinsically, it would more precisely specify the exact property of induced neurons. A small set, including 3 reprogramming factors (BAM) and 4 committed motor neuron factors (Lhx3, Hb9, Isl1, and Ngn2), converted embryonic and adult fibroblasts into induced functional motor neurons. The induced motor neurons expressed pan-neuronal marker microtubule-associated protein 2 (MAP2) and motor neuronal specific markers vesicular acetylcholine transporter (vChAT) and homeobox Bb9 (Hb9), as well as the receptors and excitatory channels (Son et al., 2011). In combination with expression of BAM and two genes, Lmx1a and FoxA2, human fetal and neonatal fibroblasts were converted toward specific dopaminergic neurons (Pfisterer et al., 2011). Furthermore, three factors such as Ascl1, Nurr1, and Lmx1a can directly convert mouse and human fibroblasts into functional dopaminergic neurons without passing through the progenitor stage (Caiazzo et al., 2011). It has to be pointed out that marker expression alone is not sufficient to define a neuron. In fact, it was verified that induced neurons derived from Pax6-, Mash 1- and Ngn2-transduced mouse astroglial precursors are not functional in the absence of cortical neurons (Berninger et al., 2007).

Reprogramming of Induced Neural Stem Cells *in vitro*

Although transdifferentiation from one mature somatic cell type into neuronal cells can be achieved through overexpression of transcription factors, the population of induced neurons has very little or no proliferation potential, which restricts the efficiency and expandability for a large scale. In addition, mature induced neurons are not suitable to be transplanted *in vivo* due to their poor survivability. Derivation of neural stem cells would be desirable for transplantation in consideration of their capacity to proliferate and differentiate. By forcing expression of exogenous transcription factors, induced neural stem cells can be obtained from embryonic stem cells, fetal and adult somatic cells (**Figure 1**).

Many transcription factors play important roles in the maintenance of embryonic stem cell identity. Gene screening narrows transcription factors for inducing pluripotency, *i.e.*, Oct3/4, Sox2, Klf4, and c-Myc (Takahashi and Yamanaka, 2006; Yamanaka, 2009). c-Myc is ubiquitous stem cell marker expressing in pluripotent stem cells and neural stem cells. Activation or mutation of exogenous c-Myc gene integrated to the host cell genome usually results in tumor formation because of activation of many proliferation-related genes (Okita et al., 2007). Sox2 is expressed in neural stem or progenitor cells and its inactivation causes cell differentiation. Klf4 is a good indicator of mesenchymal stem cells that differentiate to skin, stomach, intestine, and skeletal muscle cells. Notably, Oct3/4 is so important and highly specific for pluripotent stem cells that expression of Oct3/4 alone can generate induced pluripotent stem cells from adult mouse neural stem cells (Kim et al., 2009). Neural stem cells endogenously express Sox2, Klf4, and c-Myc, but not Oct3/4. Thus, it is possible that suppressing Oct3/4 activity may transform somatic cells into neural stem cells.

Thier et al. (2012) transduced mouse embryonic fibroblasts using retroviruses constitutively expressing Sox2, Klf4, and c-Myc. Simultaneously, Oct4 activity was down-regulated by

doxycycline-controlled lentiviral infection. The curtailed reprogramming successfully yielded a population of neural stem cells, which were expanded for more than 50 passages and did not depend on sustained expression of the reprogramming factors. Microarray gene analysis showed some genes related to self-renewal or neural determination capacity, *e.g.*, Foxg1, Nes, Bmi1 and Olig2, were strongly upregulated in these induced neural stem cells, highly similar to their counterparts derived from mouse brain. In contrast, fibroblast-specific gene transcriptions, *e.g.*, Col1a1, Col3a1, Dkk3, and Thy1, were down-regulated. Immunolabeling illustrated that these induced neural stem cells were able to differentiate into the three main neural lineages: neurons, astrocytes, and oligodendrocytes. Expression of a different combination of transcription factors, including Brn4/Pou3f4, Sox2, Klf4, c-Myc, and E47/Tcf3, also directly transformed mouse fibroblasts into induced neural stem cells (Han et al., 2012). These cells exhibited cell morphology, epigenetic features, self-renewal capacity, and functionality similar to those of wild-type NSCs. Although the donor transcription profiles were gradually silenced over a period of time, the induced neural stem cells had the tripotential capability to differentiate into three neural lineages. In these two cases, the induced neural stem cells conserved some similar gene expression to mouse fibroblasts, indicating a certain residual fibroblast epigenetic memory, *e.g.*, Acta2 and Ctgf. Nevertheless, the newly established neural stem cells transcriptional network was dominant over that epigenetic memory. The induced neural stem cells can also be generated from monkey fibroblasts using Yamanaka (OSKM) factors in combination with chemically defined medium that favors for neural stem cell differentiation (Lu et al., 2013).

A single transcription factor can reprogram somatic cells to induced neural stem cells. Sox2 is a master regulator gene for neural stem cell identity and maintenance. With overexpression of Sox2 alone plus NSC-permissive culture conditions, Ring et al. (2012) transformed mouse and human fibroblasts to induced neural stem cells. The characteristics of these induced neural stem cells resembled to wild-type neural stem cells and could differentiate into mature neurons and glial cells. Because Sox2 and Nestin are neural stem cell markers, analysis of methylation patterns of the gene promoters revealed that both promoters were hypomethylated in reprogrammed induced neural stem cells, indicating their transcriptional activation. If one transcription factor is sufficient to induce multipotent state, a certain combination with others may be necessary for subtype-specific neuronal or glial progenitors.

Besides fibroblasts, multipotent neural stem cells can be induced from other types of adult somatic donor cells. Recently, Cassady et al. (2014) developed a genetic system to convert cells to tripotent neural stem cells. After transducing 13 transcription factors, including sex determining region Y-box 2 (Sox2), hes family bHLH transcription factor 1 (Hes1), Hes3, Brn2, Kruppel-like factor 4 (Klf4), regulatory factor X 4 (Rfx4), Zinc finger of the cerebellum 1 (Zic1), a dominant-negative RE-1 silencing transcription factor (DN-REST), notch intracellular domain (NICD), LIM homeobox 2 (Lhx2), pleiomorphic adenoma gene-like 1 (PLAGL1), myelocytomatosis viral oncogene homolog (Myc), and polycomb complex protein 1 (Bmi1), into embryonic fibroblasts, primary induced neural stem cell lines were successfully derived. By delivering retroviral induced pluripotent stem cell factors, induced neural stem cells were converted into induced pluripotent stem cells *in vitro* and then adult chimera mouse was generated from the iPSC lines. Adult liver and blood B cells were isolated from the chimera. When activating expression of 8 transcription factors, *i.e.*, Brn2, Hes1, Hes3, Klf4, Myc, NICD, PLAGL1, and Rfx4, these cultured somatic cells differentiated into secondary induced neural stem cells, which displayed similar property to the original induced neural stem cell lines and wild-type neural stem cells. Additionally, human hematopoietic cells can be converted to neural cells by expression of Sox2 and c-Myc (Castano et al., 2014).

Cell Reprogramming *in vivo*

The ultimate goal of cell reprogramming is to seek an innovative approach for cell therapy in human diseases. With understanding the property of induced cells *in vitro* and establishing transgenic reporter system, it is important to determine if the conversion is applicable *in vivo*. Recent studies transfected human fibroblasts and astrocytes with lentiviral tet-on system expressing regulatable neural reprogramming genes (BAM). After transplanted into the rat brain, these non-neuronal cells transdifferentiated into neurons when transgene expressions were turned on by doxycycline administration (Torper et al., 2013). Also, the same study reprogramed endogenous astrocytes into neurons using the same factors. This is the first study showing that direct neuronal conversion *in vivo* is achievable.

Glia in the central nervous system tissue themselves can be directly converted to neurons. It was demonstrated that a single transcription factor, Sox2 alone or together with Ascl1, converted $NG2^+$ glia into induced doublecortin $(DCX)^+$ neurons in the adult mouse cerebral cortex following brain injury (Heinrich et al., 2014). However, this did not happen in unlesioned cortex, indicating the necessity of signal from injury. Similarly, over-expression of a single transcription factor, Sox2, in the injured adult spinal cord, directly transformed resident astrocytes into DCX^+ neuroblasts (Su et al., 2014). Another single transcription factor, NeuroD1, is also able to convert reactive glial cells into functional neurons in the cortex of stab-injured or Alzheimer's disease mouse models (Guo et al., 2014). In this study, astrocytes were transdifferentiated into glutaminergic neurons whereas $NG2^+$ cells were reprogrammed into glutaminergic or GABAergic neurons. Besides residential glia in the central nervous system, cochlear non-sensory epithelial cells can be re-programmed into functional neurons using Ascl1 alone or Ascl1 and NeuroD1, which may be a good indicator for the regeneration of auditory neurons in the mammalian cochlea (Nishimura et al., 2014).

In vitro generated induced neural stem cells can be transplanted into the central nervous system for therapeutic purpose. A recent study transplanted induced neural stem cells into the contusion lesion site of rat spinal cord (Hong et al., 2014). It was reported that engrafted induced neural stem cells differentiated into all neural lineages, especially

several subtypes of mature neurons, suggesting that this strategy holds therapeutic potential for restoration of spinal cord injury. The induced neural stem cells were transplanted into the adult mouse brain and survived for up to 6 months without graft overgrowths (Hemmer et al., 2014). Although induced neural stem cells displayed a neural multi-lineage potential, the majority of grafted cells differentiated into glia. Likewise, graft-derived neurons formed synaptic connections and displayed neuronal electrophysiological properties, suggesting functional integration with the existing neuronal circuitry.

Altogether, reprogramming for conversion of somatic cell types into induced neurons or induced neural stem cells opens a door for studying cellular biology, disease processes, and cell-based therapeutic intervention. However, limitations still exist in the efficiency improvement, cell identity, and functional characterization. Researchers need novel approaches to overcome these hurdles before translational or clinical application for neurological disorders.

References

Berninger B, Costa MR, Koch U, Schroeder T, Sutor B, Grothe B, Gotz M (2007) Functional properties of neurons derived from in vitro reprogrammed postnatal astroglia. J Neurosci 27:8654-8664.

Caiazzo M, Dell'Anno MT, Dvoretskova E, Lazarevic D, Taverna S, Leo D, Sotnikova TD, Menegon A, Roncaglia P, Colciago G, Russo G, Carninci P, Pezzoli G, Gainetdinov RR, Gustincich S, Dityatev A, Broccoli V (2011) Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. Nature 476:224-227.

Cassady JP, D'Alessio AC, Sarkar S, Dani VS, Fan ZP, Ganz K, Roessler R, Sur M, Young RA, Jaenisch R (2014) Direct lineage conversion of adult mouse liver cells and B lymphocytes to neural stem cells. Stem Cell Reports 3:948-956.

Castano J, Menendez P, Bruzos-Cidon C, Straccia M, Sousa A, Zabaleta L, Vazquez N, Zubiarrain A, Sonntag KC, Ugedo L, Carvajal-Vergara X, Canals JM, Torrecilla M, Sanchez-Pernaute R, Giorgetti A (2014) Fast and efficient neural conversion of human hematopoietic cells. Stem Cell Reports 3:1118-1131.

Choi HW, Kim JS, Choi S, Hong YJ, Kim MJ, Seo HG, Do JT (2014) Neural stem cells differentiated from iPS cells spontaneously regain pluripotency. Stem Cells 32:2596-2604.

Fong CY, Gauthaman K, Bongso A (2010) Teratomas from pluripotent stem cells: A clinical hurdle. J Cell Biochem 111:769-781.

Guo Z, Zhang L, Wu Z, Chen Y, Wang F, Chen G (2014) In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell Stem Cell 14:188-202.

Han DW, Tapia N, Hermann A, Hemmer K, Hoing S, Arauzo-Bravo MJ, Zaehres H, Wu G, Frank S, Moritz S, Greber B, Yang JH, Lee HT, Schwamborn JC, Storch A, Scholer HR (2012) Direct reprogramming of fibroblasts into neural stem cells by defined factors. Cell Stem Cell 10:465-472.

Heinrich C, Bergami M, Gascon S, Lepier A, Vigano F, Dimou L, Sutor B, Berninger B, Gotz M (2014) Sox2-mediated conversion of NG2 glia into induced neurons in the injured adult cerebral cortex. Stem Cell Reports 3:1000-1014.

Hemmer K, Zhang M, van Wullen T, Sakalem M, Tapia N, Baumuratov A, Kaltschmidt C, Kaltschmidt B, Scholer HR, Zhang W, Schwamborn JC (2014) Induced neural stem cells achieve long-term survival and functional integration in the adult mouse brain. Stem Cell Reports 3:423-431.

Hong JY, Lee SH, Lee SC, Kim JW, Kim KP, Kim SM, Tapia N, Lim KT, Kim J, Ahn HS, Ko K, Shin CY, Lee HT, Scholer HR, Hyun JK, Han DW (2014) Therapeutic potential of induced neural stem cells for spinal cord injury. J Biol Chem 289:32512-32525.

Kim JB, Sebastiano V, Wu G, Arauzo-Bravo MJ, Sasse P, Gentile L, Ko K, Ruau D, Ehrich M, van den Boom D, Meyer J, Hubner K, Bernemann C, Ortmeier C, Zenke M, Fleischmann BK, Zaehres H, Scholer HR (2009) Oct4-induced pluripotency in adult neural stem cells. Cell 136:411-419.

Lau S, Rylander Ottosson D, Jakobsson J, Parmar M (2014) Direct neural conversion from human fibroblasts using self-regulating and nonintegrating viral vectors. Cell Rep 9:1673-1680.

Lu J, Liu H, Huang CT, Chen H, Du Z, Liu Y, Sherafat MA, Zhang SC (2013) Generation of integration-free and region-specific neural progenitors from primate fibroblasts. Cell Rep 3:1580-1591.

Miura K, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, Nakagawa M, Koyanagi M, Tanabe K, Ohnuki M, Ogawa D, Ikeda E, Okano H, Yamanaka S (2009) Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol 27:743-745.

Nishimura K, Weichert RM, Liu W, Davis RL, Dabdoub A (2014) Generation of induced neurons by direct reprogramming in the mammalian cochlea. Neuroscience 275:125-135.

Nori S, Okada Y, Nishimura S, Sasaki T, Itakura G, Kobayashi Y, Renault-Mihara F, Shimizu A, Koya I, Yoshida R, Kudoh J, Koike M, Uchiyama Y, Ikeda E, Toyama Y, Nakamura M, Okano H (2015) Long-term safety issues of iPSC-based cell therapy in a spinal cord injury model: Oncogenic transformation with epithelial-mesenchymal transition. Stem Cell Reports 4:360-373.

Okita K, Ichisaka T, Yamanaka S (2007) Generation of germline-competent induced pluripotent stem cells. Nature 448:313-317.

Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ, Citri A, Sebastiano V, Marro S, Sudhof TC, Wernig M (2011) Induction of human neuronal cells by defined transcription factors. Nature 476:220-223.

Pfisterer U, Kirkeby A, Torper O, Wood J, Nelander J, Dufour A, Bjorklund A, Lindvall O, Jakobsson J, Parmar M (2011) Direct conversion of human fibroblasts to dopaminergic neurons. Proc Natl Acad Sci U S A 108:10343-10348.

Ring KL, Tong LM, Balestra ME, Javier R, Andrews-Zwilling Y, Li G, Walker D, Zhang WR, Kreitzer AC, Huang Y (2012) Direct reprogramming of mouse and human fibroblasts into multipotent neural stem cells with a single factor. Cell Stem Cell 11:100-109.

Son EY, Ichida JK, Wainger BJ, Toma JS, Rafuse VF, Woolf CJ, Eggan K (2011) Conversion of mouse and human fibroblasts into functional spinal motor neurons. Cell Stem Cell 9:205-218.

Su Z, Niu W, Liu ML, Zou Y, Zhang CL (2014) In vivo conversion of astrocytes to neurons in the injured adult spinal cord. Nat Commun 5:3338.

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663-676.

Thier M, Worsdorfer P, Lakes YB, Gorris R, Herms S, Opitz T, Seiferling D, Quandel T, Hoffmann P, Nothen MM, Brustle O, Edenhofer F (2012) Direct conversion of fibroblasts into stably expandable neural stem cells. Cell Stem Cell 10:473-479.

Torper O, Pfisterer U, Wolf DA, Pereira M, Lau S, Jakobsson J, Bjorklund A, Grealish S, Parmar M (2013) Generation of induced neurons via direct conversion in vivo. Proc Natl Acad Sci U S A 110:7038-7043.

Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M (2010) Direct conversion of fibroblasts to functional neurons by defined factors. Nature 463:1035-1041.

Yamanaka S (2009) A fresh look at iPS cells. Cell 137:13-17.

Zhou D, Zhang Z, He LM, Du J, Zhang F, Sun CK, Zhou Y, Wang XW, Lin G, Song KM, Wu LG, Yang Q (2014) Conversion of fibroblasts to neural cells by p53 depletion. Cell Rep 9:2034-2042.