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Induced neuronal reprogramming

Cheen Euong Ang[1,2] and **Marius Wernig**[1]

[1]Institute for Stem Cell Biology and Regenerative Medicine and Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

[2]Department of Bioengineering, Stanford University School of Medicine, Stanford, CA 94305

Abstract

Cellular differentiation processes during normal embryonic development are guided by extracellular soluble factors such as morphogen gradients and cell contact signals eventually resulting in induction of specific combinations of lineage determining transcription factors. The young field of epigenetic reprogramming takes advantage of this knowledge and uses cell fate determination factors to convert one lineage into another such as the conversion of fibroblasts into pluripotent stem cells or neurons. These induced cell fate conversions open up new avenues for studying disease processes, generating cell material for therapeutic intervention such as drug screening and potentially also for cell-based therapies. However, there are still limitations that need to be overcome to fulfill these promises centering on reprogramming efficiencies, cell identity and maturation. In this review, we will discuss the discovery of induced neuronal reprogramming, ways to improve the conversion process and finally how to proper define the identity of those converted neuronal cells.

2. Introduction

Early somatic cell nuclear transfer experiments in amphibians by Briggs, King and Gurdon have heralded the notion that every somatic cell type can be reprogrammed to pluripotency [1, 2]. Later cell fusion experiments by Blau and colleagues illustrated cell conversions between somatic cell types [1, 2]. Finally, transcription factors have been identified that are sufficient to interconvert closely related cell types such as fibroblasts to muscle cells with MyoD or B-cells into macrophages with CEBPα ([3] and references therein). In 2006, Takahashi and Yamanaka have demonstrated that induced pluripotent stem (iPS) cells can be directly derived from fibroblasts by a combination of just 4 transcription factors Oct4, Sox2, Klf4 and cMyc [4, 5]. The induction of pluripotency in somatic cells is a very dramatic change in cell fates unlike the other cell interconversions reported before. This begged the question whether such dramatic cell lineage changes can also be induced between distantly related somatic cell types. In 2010, we have shown that fibroblasts can be reprogrammed into induced neuronal (iN) cells illustrating that direct lineage conversion is possible even between cell types representing different germ layers [6]. Following our work more examples were added such as the induction of cardiomyocytes, blood cells and hepatocytes from fibroblasts [7–10]. In this review, we will focus on several aspects of iN

Correspondence: Marius Wernig (wernig@stanford.edu).

cell reprogramming and discuss methods of improving reprogramming efficiency and criteria to functionally characterize those iN cells.

3. Induced neuronal cells

3.1. The beginning: induced neuronal cells from developmentally related cells

The first example of direct conversion to cells of neural lineages using transcription factors is the effort led by Goetz and coworkers [11]. Followed by the observation that Pax6 transduction increased neurogenesis in embryonic cortical precursor cells, they examined whether forced expression of Pax6 in Pax6-negative astrocytes can instruct them to neuronal fate. The authors found that in mere seven days almost half of the Pax6 infected astrocytes were converted into β-tubulin-III-, NeuN-positive neurons, some of which expressed markers for excitatory neurons others for inhibitory neurons. Similarly in a stab wound model, Buffo et al. [12] showed that Olig2 positive cells formed near stab wound lesions can be converted into Dcx+ positive migrating neuroblasts via transduction of a dominant negative form of Olig2 (Olig2-VP16). Olig2-VP16 transduction has been shown to derepress Pax6 which could mechanistically contribute to the instruction of neurogenesis. However, conclusions should be made cautiously when marker expression alone is used as the only criterion to define a neuron. In fact, it was later shown that neurons derived from Pax6, Mash1 and Ngn2 transduced astroglial precursors do not have any spontaneous synaptic events in the absence of cortical neurons and are therefore not fully functional. Given that neurons devoid of any synaptic events might be suggestive of an absence of a functional presynaptic and/or postsynaptic compartment, the authors attempted co-culturing the transduced cells with E16 cortical neurons and showed that Pax6 transduced astrocytes might have incomplete assembling of the presynaptic compartment [13]. The presynaptic incompetence of those Ngn2 transduced neurons was later solved by the same group [14] by simply changing the original long terminal repeat driven Moloney Murine Leukemia Virus retroviral vector into a self-inactivating retroviral vector driving gene expression by a stronger chicken beta-actin promoter which has a persistent expression in adult mouse brain. This early work demonstrated already that absolute levels and the dynamic expression of reprogramming factors are of critical importance. With Ngn2 and Dlx2 in the new retroviral construct, the authors could demonstrate that forced expression of those two transcription factors individually in postnatal cortical astroglial cells give rise to synaptically competent glutamatergic and GABAergic neurons respectively. The same group also later proved that cultured Ng2+ and GFAP+ glia cells from postnatal rat cortex can be converted to glutamatergic neurons by mere Ngn2 transduction [15]. More recently, de-novo *in vivo* generation of cells expressing neuronal markers were demonstrated by a number of groups, however due to the limitations of transgenic reporter systems the exact cell of origin remains elusive in these cases and functionality of newly induced cells has not been shown [16–20].

3.2. Crossing major lineage boundaries: mesodermal to ectodermal conversion

Our group found that functional neurons can be generated from a non-ectodermal cell type demonstrating that direct reprogramming is possible between two different germ layers [6]. Starting from a pool of 19 candidate transcription factors, systematic enriched iterations of combinations narrowed the candidates to just three active transcription factors: Brn2, Ascl1,

and Myt1l (the “BAM” pool of factors). Those iN cells exhibited stereotypical neuronal morphology, expressed multiple pan-neuronal and subtype specific markers such as b-III-tubulin, Map2, NeuN, Tau, vGluT1, Tbr1, and synaptic markers when cultured for longer time periods. Moreover, the cells also exhibited the two functional principal properties of neurons: the ability to fire action potentials and synapse formation. We demonstrated that fibroblast-derived iN cells could form synapses within themselves, and could also integrate into pre-existing cortical neuronal networks where they exhibited short term synaptic facilitation and depression [6]. We and several other groups extended these findings to human fibroblasts [21–23]. Since the BAM combination of factors yielded only immature-looking iN cells, we screened an additional set of 20 factors on top of the BAM factors to improve neuronal reprogramming [21]. We found that the addition of NeuroD1 greatly facilitated the neuronal induction in human fibroblasts. Those human iN cells expressed multiple pan neuronal marks, exhibit spontaneous action potentials and can be integrated into existing mouse cortical neuronal networks [21]. Yoo et al examined whether brain specific microRNAs (miR-9/9* and 124) could have instructive roles in induced neuron reprogramming [23]. Remarkably, the authors showed that overexpression of miR-9/9* and miR-124 are sufficient to generate MAP2-positive iN cells, however co-introduction of the three transcription factors NeuroD2, Myt1l and Ascl1 was required to generate functional iN cells. Albeit rare, postsynaptic activity could be detected even in the absence of co-cultured glial cells. Along the same vein, Ambasudhan et. al. reported miR-124 together with two other transcription factors, Brn2 and Myt1l, in defined conditions are enough to convert human postnatal and adult fibroblasts into mostly excitatory neuronal cells [22]. Remarkably, in their transcription factor combination, the authors did not use any of the neurogenic basic-helix-loop-helix transcription factors reported by our group and others [21, 23] although this family of transcription factors has the most pervasive role in neuronal development and are therefore considered the strongest drivers of reprogramming [24]. Indeed, our recent molecular insights into the reprogramming process revealed the critical contributions of Ascl1 and we could demonstrate that this bHLH factor alone is sufficient to induce fully functional iN cells from fibroblasts [25, 26]. Future studies will show whether the Brn2, Myt1l, miR-124 factor combination induces endogenous expression of bHLH transcription factors in fibroblasts and whether this induction is essential for reprogramming. Transfection of miR-124 in P19 cells led to markedly increased expression of the two bHLH transcription factors Ngn2 and NeuroD1 [27]. More recently another study claimed the generation of iN cells from embryonic fibroblasts without use of bHLH factors by simple knock-down of the splicing factor PTB [28]. Those mouse iN cells surprisingly exhibited both spontaneous glutamatergic and GABAergic postsynaptic events in the absence of primary neuronal co-culture. This finding implicates the presence of glutamatergic and GABAergic subtypes of iN cells. Since only one single gene was knocked-down in this experiment, it appears that the subtype specification was not controlled by this genetic manipulation and was stochastic. This conclusion would have important mechanistic implications on how neuronal subtypes actually develop under normal differentiation conditions.

In addition to inhibitory neurons there are several other clinically relevant neuronal subtypes which are the focus of intense investigation. One of those cell types are dopamine neurons

of the ventral midbrain type. Given the cell type's importance a large body of literature has characterized the molecular pathways and transcription factors involved in specifying the dopaminergic lineage [29]. Pfisterer and coworkers combined several dopamine-specific transcription factors with the BAM factors and found that the addition of Lmx1a and FoxA2 is sufficient to generate induced dopaminergic neuronal (iDaN) cells from human fetal fibroblasts[30]. Those iDaN cells are tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AADC) positive, the two enzymes critical for dopamine synthesis. Those iDaN cells also exhibit spontaneous and pacemaking like action potential unique to dopaminergic neurons. These findings were exciting and promising because they demonstrated the principle that methods can be designed to induce iN cells of specific neuronal subtypes. However, some bottlenecks remained such as (a) only up to 25% of the total iN cells were TH positive and the culture remained rather heterogeneous, (b) it is unknown whether the protocols will work on adult human fibroblasts and (c) it was left unexplored whether these iN cells can release dopamine upon stimulation. In addition, (d) the exact phenotypic characterization as midbrain-type neurons remained unclear. Another study by Caiazzo et al., published around the same time, reported that transduction of Ascl1, Nurr1 and Lmx1a (ANL) is sufficient to generate functional dopaminergic neurons in mouse and human fibroblasts [31]. This study showed that the three factors can give rise to vesicular monoamine transporter 2 (VMAT2), dopamine transporter (DAT), aldehyde dehydrogenase 1a1 (ALDH1A1) and calbindin positive neurons in TH-GFP knock in mouse embryonic fibroblasts (MEF). Those iDaN cells also have spontaneous pacemaking properties and released dopamine as confirmed by amperometric and dopamine HPLC measurements. The same transcription factor combination was shown to also convert human fetal and adult fibroblasts into iDaN cells albeit with a much lower efficiency. Similar to mouse iDaN cells, human induced dopaminergic neurons are also positive for multiple dopaminergic neuronal markers (VMAT2, DAT and ALDH1A1), actively spiking and released dopamine. Despite the higher purity of TH positive neurons (reported fractions of 85% and 60% in mouse and human fetal fibroblasts, respectively), there are still clear differences between the gene expression profile of iDaN cells and that of bona fide midbrain dopaminergic neurons. Encouraging of course was the observation that the overall transcriptome of induced cells were more similar to dopamine neurons than to fibroblasts. Importantly, cell transplantation experiments were conducted into neonatal mouse brains and it was shown that the ANL transduced iDaN cells could functionally integrate into the mouse brain. The cells were not yet tested though for the ability to restore dopamine deficiency *in vivo* in animal models for Parkinson's disease. To address this question, Kim and colleagues subsequently used tail tip fibroblasts from Pitx3-GFP knock-in mice and screened for factors that induce GFP expression[32]. Pitx3 is a member of the RIEG/PITX homeobox family and is important for the early development and survival of midbrain dopaminergic neurons [33–35]. Using those knock-in GFP reporter fibroblasts, the authors managed to narrow 11 transcription factors to only two: Ascl1 and Pitx3. But from gene expression analyses, the two factor iDaN cells only partially upregulated other midbrain dopaminergic markers. In search for ways to improve the midbrain character of the induced cells, the authors added more transcription factors (Lmx1a, Nurr1, Foxa2, and En1) and midbrain patterning factors (Sonic hedgehog, SHH, and fibroblast growth factor 8, FGF8). These combinations yielded about 9.1% Pitx3-GFP-positive cells by day 18 post infection.

Not only did these iDaN cells exhibit the expected marker expressions (TH, DAT, AADC and VMAT), they also released dopamine *in vitro* and *in vivo* when they were transplanted into the 6-hydroxydopamine lesioned striatum model of Parkinson's disease and showed electrophysiological properties similar to that of primary dopaminergic neurons. While this optimized protocol yielded iDaN cells that are able to restore dopamine function *in vivo*, the gene expression profile of key midbrain dopamine marker genes was still different from endogenous Pitx3-EGFP midbrain dopamine neurons and the degree of functional restoration was significantly lower than when primary dopamine neurons were transplanted.

A second critical neuronal subtype is spinal motor neurons. Son and coworkers used seven factors (BAM factors plus Lhx3, Hb9, Isl1, and Ngn2) for mouse fibroblasts to generate cells similar to motor neurons termed induced motor neuronal (iMN) cells. A seventh factor NeuroD1 needed to be added to convert human fibroblast-like cells derived from human embryonic stem (ES) cells [36]. Mouse iMN cells shared similar electrophysiological and gene expression properties with motor neurons derived from ES cells and were able to innervate cultured myotubes in a curare-dependent manner, a specific blocker of acetylcholine receptors known to mediate synaptic transmission at the neuronal-muscular junction. The iMN cells were also shown to phenocopy disease traits. Cells expressing the SOD1G93A mutant form of SOD1, a mutation causing amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, displayed similar survival defects as primary motor neurons. Similarly, wild type iMN cells co-cultured with mutant glia were less viable compared to co-cultures with wild type glia as expected.

More recently, yet another neuronal subtype was generated from fibroblasts. Remarkably, the overexpression of Ascl1, Brn3b and Ngn2 reportedly could convert MEFs and adult ear fibroblasts into induced retinal ganglion-like cells [37]. Since Ascl1 and Ngn2 are highly abundant general pro-neural transcription factors, the subtype specification is likely to be driven by the third factor of the pool Brn3b, a more restricted Pou-domain transcription factor. Although the induced retinal ganglion-like cells expressed the retinal ganglion cells markers RPF-1, Ath5 and Thy1.2, the cells remained electrophysiologically immature.

Finally, should reprogrammed cells be considered for clinical (i.e. transplantation) use, the viral gene delivery would have to be replaced with safer methods. It is therefore important to demonstrate that iN cells can be generated using non-integrative gene delivery methods. Adler and coworkers transfected the BAM factors into MEFs using linear poly amido amine and obtained up to 7.6% Tuj1-positive cells with five serial transfections after 10 days [38]. In addition, Meng et al. used adenoviral Brn2, Ascl1 and Ngn2 and obtained a modest 2.9% of reprogramming efficiency [39]. These studies have established that non-integrative gene delivery methods are possible. They also highlight the need for further improvement of the gene delivery systems in order to make the study of iN cells useful.

3.3. Endodermal to ectodermal conversion

Most reports have used fibroblasts as donor cells for reprogramming experiments. Fibroblasts, however, are ill-defined and heterogeneous cell preparations. Thus, in those experiments, the exact cells undergoing fate conversion remained unclear. In a quest to better define the cell of origin for iN cell reprogramming, we explored better defined and

genetically tractable cell types. At the same time, we were curious whether a cell lineage derived from the endodermal germ layer could be converted into neurons. Using a defined genetic labeling and cell lineage tracing system, we unequivocally demonstrated that endodermal cells can be converted directly into iN cells (representing ectoderm) using the same BAM pool of transcription factors [40]. This suggested that – unlike predominantly assumed in the field – the degree of developmental relationship might not be the most important parameter whether cell fate conversions are feasible. The defined cell of origin also allowed us to address the question of the degree of reprogramming achievable in iN cells. We particularly focused on how well the donor cell transcriptional program is silenced in resulting iN cells. Using both bulk and single cell transcription profiling methods we concluded that the donor cell program is efficiently silenced while low levels of some hepatic transcripts were still detectable in iN cells 3 weeks after the induction of the reprogramming factors. It may be likely that further optimization and extension of the culture period would result in even less expression or even extinction of this “epigenetic memory”.

4. Methods to improve reprogramming

The reprogramming efficiency to iN cells varies substantially between cell types [21, 22, 30, 31, 36]. In particular human cells appear to be more resistant to iN cell reprogramming than their mouse counterparts [6, 28, 31, 36]. For example, the efficiencies of MEFs are around 20% whereas adult human fibroblasts are below 1%. There is also a developmental component with embryonic being more efficient than neonatal and neonatal being more efficient than adult fibroblasts. From a pragmatic point of view, skin-derived fibroblasts and fresh blood cells would represent ideal donor cell populations for reprogramming given the ease of access. Unfortunately, human blood cells appear to be even more refractory to iN cell reprogramming than adult dermal fibroblasts. Therefore, to improve iN cell reprogramming efficiencies of resistant cell types is one of the most critical issues to overcome before this technology can be used for translational use such as disease modeling of brain diseases using patient-derived cells. Similar efforts have been made for the iPS cell technology but their success is not so critical because typically only few iPS cell lines are required per patient [41–43]. So far, two approaches have been taken to optimize iN cell reprogramming which are discussed in the following.

Small molecules

Small molecule inhibitors or agonists can help the direct conversion process by simultaneously accelerating neuronal fate commitment and pruning unwanted side differentiations. For example, Ladewig and coworkers reported up to 200% and 80% in neuronal yield and purity respectively by dual inhibition of GSK-3β and SMAD signaling [44]. Ambasudhan [22] and Yoo [45] used bFGF and dibutyrl cyclic AMP/Forskolin to increase neuronal yield. These reports suggest three pathways critical for iN cell reprogramming: TGFβ/BMP inhibition, FGF activation and increasing the level of intracellular cyclic AMP. Given the conserved roles of BMP inhibition and FGF activation during neural induction [46], it is not surprising that modulating those two pathways leads to an enhancement of iN cell reprogramming. Future studies will have to show whether those

two pathways enhance iN cell reprogramming via a similar mechanism as early neural induction.

Oxygen tension

With the partial pressure of oxygen in common lab incubators consistently higher than that in the brain [47], it is almost tempting to hypothesize that lowering oxygen tension would imitate low oxygen milieus in the brain enhancing iN cell reprogramming. Similar to the observations made for iPS reprogramming [42], our lab recently found beneficial effects on iN cell reprogramming by acute reduction of environmental oxygen [48]. The quality and maturity of resulting iN cells were comparable to standard oxygen levels as determined by electrophysiological parameters. Overall, we observed an average 2.5-fold increase in the generation of iN cells from human fibroblasts.

5. Challenges to generate authentic neuronal subtypes?

There are now many papers reporting the generation of various types of neurons using permutations of diverse factors such as transcription factors, miRNAs, and small molecules. As discussed earlier some attempts have been made to generate specific neuronal subtypes with variable success. It has become more important than ever to properly define and characterize those iN cells. We have previously outlined gradual pan-neuronal parameters helping identify the maturation level of a given iN cell population [49]. Unlike other tissue systems that are made up of relatively homogenous populations of cells, the nervous system is bestowed with different neuronal subtypes formed by precise orchestrations of different morphogen gradients and transcription factors after early neural induction. Later in development, those different neuronal subtypes are further regionalized by transcription factors, morphogens and Hox gene expressions. All of these culminate into a complex neuronal network which scientists are just beginning to understand. It is conceivable that many neurological and neuropsychiatric diseases preferentially affect one neuronal subtype over others and that e.g. identical genetic lesions lead to different pathophysiological effects depending on the neuronal subtype. This has led to the motivation to generate specific neuronal subtypes.

Cortical excitatory glutamatergic neurons

Cortical excitatory projection neurons are generated from the ventricular and subventricular zones in defined periods. Their precursors migrate and settle in distinct cortical layers in an inside-out manner [50]. Cajal-Retzius neurons occupying the marginal zone are born first, followed by corticothalamic neurons at layer VI and callosal and subcerebral projection neurons at layer V, and later layer IV and layer II/III pyramidal neurons [51]. Neurons in each cortical layer or areal (motor, somatosensory and visual) are endowed with specific marker expressions [51]. We and others have noticed Tbr1 expression in a fraction of the iN cells, indicating potential region-specific features of cortical (preplate and layer 6), olfactory bulb and eminentia thalami neurons [52]. Given the diversity of neocortical neurons, more cortical areal and layer specific markers have to be used to better define the iN cells generated. While mouse iN cells appeared of exclusive central nervous system identity, a fraction of the human fibroblast-derived iN cells expressed peripherin, a neuronal marker of

the peripheral nervous system [53]. How the same combination of transcription factors can give rise to iN cells with features of the central and peripheral nervous systems remains to be addressed.

Mesencephalic dopaminergic neurons

There are seventeen cell groups (A1–A17) of catecholamine neurons (dopamine, norepinephrine and epinephrine) within the mammalian brain ranging from medulla to hypothalamus. Only three of them A8 (retrorubral area), A9 (substantia nigra) and A10 (ventral tegmental area) are within mesencephalon. Given their involvement in Parkinson's disease, a common neurodegenerative disorder, much attention has been devoted to the developmental mechanisms of their generation. Several specific markers, signaling pathways and critical transcription factors have been described that can serve as a roadmap to recreate this particular lineage in reprogramming efforts. Unfortunately, the reported iDaN cells generated so far lack expression of authentic midbrain markers even with induction of six transcription factors characteristic of the dopaminergic lineage [32]. Furthermore, dopaminergic neurons in the midbrain can be further divided into dorsal and ventral tiers [54]. Dopaminergic neurons in the dorsal tier are calbindin positive and express low level of DAT while those in the ventral tier are calbindin negative, mostly GIRK2 positive and express high level of DAT [54]. Dorsal tier dopaminergic neurons are preferentially lost in aging while in Parkinson's disease degeneration of predominantly ventral tier dopaminergic neurons is observed [55]. Based on these markers, iDaN cells generated with Ascl1, Nurr1, and Lmx1a transcription factors are more similar to those in the dorsal tier as they are calbindin positive [31]. However, those cells also fail to induce endogenous midbrain-specific markers.

Spinal motor neurons

Spinal motor neurons are arranged in columns with their identity determined by their longitudinal position, axonal projections and Hox gene expression [56]. There are several distinct columns stretching along rostrocaudal direction and each of the distinct columns innervates different targets. Hypaxial, medial (Lim3+) and lateral (Foxp1+) motor column neurons each targets body wall, axial and limb muscles respectively. Lateral motor column neurons can be further divided into Isl1+ medial and Lim1+ lateral divisions which innervate ventral and dorsal muscles. Within each column, there are pools of motor neurons innervating a single specific muscle. Those pools usually contain different types of motor neurons (fast-fatigable, fast-fatigue resistant or slow-twitch alpha motor neurons and gamma motor neurons) each with different firing patterns and morphology [57]. Certain diseases appear to affect specific subsets of motor neurons, e.g. pathological examinations of skeletal muscles of ALS patients show that fast fatigable muscle units are preferentially affected [58], highlighting the importance of generating the right iN cells.

6. Conclusions and perspectives

In summary, the field of iN cell reprogramming has come a long way since it first discovery just three years ago [6]. In this review we have discussed the remarkable progress that has been made regarding functional maturation and subtype specification, but we have also

outlined the current limits and challenges to further improve the technology in order to be able to apply it for disease modeling. A common theme in the subtype specification of iN cells is that certain traits of neuronal subtypes that often include relevant functional properties can be induced but the cells do not adopt a complete “authentic” phenotype. While it is conceivable that further optimization of the reprogramming protocols will lead to generation of iN cells more similar to a specific authentic brain cell type, the question arises whether it will ever be possible to generate a perfectly matching cell in culture. This might be impossible even from ES or iPS cells given that the 3-dimensional context neurons usually are exposed to might provide essential clues for their final phenotype that might be very difficult to mimic in vitro. The question thus arises how close the neurons will have to resemble cells in the brain in order to acquire meaningful and relevant results that will inform about pathophysiology of the disease brain. A pessimistic view would be that the disease pathology of the brain is way too complex and pathophysiological traits need to be studied in iN cells perfectly matching affected cell types. An optimistic view would be that perhaps even suboptimally specified iN cells might be informative of pathophysiological processes going on in the brain. Along these lines, we would like to note that mouse iN cells carrying a mutation in Neuroligin 4 associated with the development of autism spectrum disorders exhibited the exact same specific synaptic phenotype that was previously observed in the mouse brain [59]. But even if iN cells show different phenotypes than observed in certain neurons in the brain, the phenomenon might be useful to find therapeutics. It is possible that even though phenotypes might be different, the underlying cell biological mechanism could be related among different neuronal subtypes and the molecular approach to rectify a phenotype (e.g. following a drug treatment) observed in iN cells might be identical in neurons of the brain.

Aside from this one concrete example of potential translation, iN cell reprogramming research paved the way for even more widespread applications. The discovery of iN cells triggered efforts to generate various kinds of cell types from fibroblasts and other non-related lineages. With respect to cell types of the brain alone, the field has made substantial progress. Various methods have been developed to generate neural stem/progenitor cells from murine fibroblasts [60–64]. More recently, induced oligodendroglial progenitor cells with the capacity to differentiate into myelinating oligodendrocytes were generated by two independent groups [65, 66]. Unlike postmitotic iN cells, these neural progenitor cell populations are particularly attractive for cell transplantation-based therapies.

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Table 1

Summary of induced neuronal cells conversion efficiency

Author	Starting Cells	Transcription factor combination	Efficiency/Purity
Heins 2002	Mouse postnatal astrocytes	Pax6	50–60% of clones comprising TuJ1 positive neuronal cells 7 d after transduction
Berninger 2007	Mouse postnatal astrocytes	Ngn2	71 ± 16% of clones comprising TuJ1-positive neuronal cells 12 d after transduction
	Mouse postnatal astrocytes	Ascl1	37 ± 11% of clones comprising TuJ1-positive neuronal cells 12 d after transduction
Heinrich 2010	Mouse postnatal astrocytes	Ascl1 and Mash1	93.0% of the Dlx2 expressing cells are B-III-tubulin positive 10 d after transduction
	Mouse postnatal astrocytes	Ngn2	91.4% % of the Ngn2 expressing cells areMAP2positive
Blum 2011	Rat postnatal astrocytes	Ngn2	89.7% of surviving cells are βIII-tubulin positive
Vierbuchen 2010	Mouse embryonic fibroblasts	Ascl1, Brn2, Myt1l	20% of the plated cells
Pang 2011	Human embryonic and postnatal fibroblasts	Brn2, Ascl1, Myt1l, NeuroD1	2–4% of the plated cells
Yoo 2011	Postnatal and adult dermal fibroblasts	NeuroD2, Ascl1 and Myt1l, miR-9/9* and miR-124	10% of the initially plated cells
Ambasudhan 2011	Postnatal and Adult dermal fibroblasts	Brn2, Myt1l miR-124	Postnatal: 4–8% relatively to the initially plated cells Adult: 1.5–2.9% (Line1) and 9.5–11.2% (Line 2) relatively to the initially plated cells
Pfisterer 2011	Embryonic fibroblasts	BAM, Lmx1a, FoxA2	10% of human induced neurons from embryonic and postnatal fibroblasts are TH positive
Caiazzo 2011	Mouse embryonic fibroblasts Human Fetal and adult and Parkinson's adult fibroblasts	Ascl1, Nurr1, Lmx1a	Mouse embryonic fibroblasts: 18% of TH positive cells Human fetal fibroblasts: 6% of TH positive cells over the infected cells Human fetal fibroblasts: 3% of TH positive cells over the infected cells
Kim 2011	Mouse tail tip fibroblasts	Ascl1, Pitx3, Lmx1a, Nurr1, Foxa2, En1	Mouse 9.1% of the remaining fibroblasts
Son 2011	Mouse embryonic and adult tail tip fibroblasts Human embryonic stem cells derived fibroblasts	Mouse: BAM, Lhx3, Hb9, Isl1, and Ngn2 Human: BAM, Lhx3, Hb9, Isl1, and Ngn2, NeuroD1	Mouse: 5–10% of Hb9:GFP of the MEF plated at 10 days Human 10–15 cells per 30000 plated at day 30
Ladewig 2012	Human postnatal and adult fibroblasts	Ascl1, Ngn2	Human postnatal fibroblasts: 200% (neuronal yield) relative to initially plated cells Human adult fibroblasts: 13.2% (neuronal yield)
Meng 2013	Mouse embryonic fibroblasts and adult ear fibroblasts	Ascl1, Ngn2, Brn3b	7% in MEF and <1% in ETF
Marro 2011	Mouse hepatocytes	Brn2, Myt1l, Ascl1	5% TauEGFP cells over the number of infected cells