A review of induced pluripotent stem cell, direct conversion by trans-differentiation, direct reprogramming and oligodendrocyte differentiation

Rapid progress in the field of stem cell therapy and cellular reprogramming provides convincing evidence of its feasibility in treating a wide range of pathologies through autologous cell replacement therapy. This review article describes in detail on three widely used approaches of somatic cell reprogramming: induced pluripotent stem cells, direct conversion and direct reprogramming, in the context of demyelination in the CNS. The potential limitations of each reprogramming technique are reviewed along with their distinct molecular approach to reprogramming. This is followed by an analysis on the scopes and challenges of its translational applications in deriving oligodendrocyte progenitor cells and oligodendrocytes for cell replacement treatment of demyelinating conditions in the CNS.

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It was long thought that cell diversity is unidirectional: primitive cells could differentiate into specialized cells, but the reverse was not plausible. Also, stem cells from either embryos, fetus or postnatal were recognized to be the only source of pluripotent cells specifying organogenesis. However, several breakthrough studies in the field of cellular reprogramming collectively indicated toward 'plasticity' of adult cells, and that it was possible to return a differentiated cell to an embryonic state. In 1962, John Gurdon inserted the nucleus of a specialized cell from an adult frog into an enucleated oocyte, resulting in the formation of a tadpole [1]. This cloning of frogs from adult cells provided the first evidence of the possibility to reverse the natural differentiation process. However, owing to low efficiency of the process and the lack of reproducibility in other species, it failed to dispel the doubts of cellular plasticity. In 1983, Blau *et al.* conducted a cell fusion experiment where diploid human

amniocytes were fused with differentiated mouse muscle cells to derive heterokaryons expressing human muscle genes [2]. This provided convincing evidence of reactivation of silenced genes in differentiated cells, thereby enabling cell fate conversions. In 1990, Wilmut and colleagues performed a somatic cell nuclear transfer experiment by transplanting the nuclei of epithelial cells into enucleated oocytes and created Dolly the sheep, making a significant shift from *Xenopus* to mammal cloning [3]. Similarly, Wakamaya *et al.* derived mice by introducing nuclei from somatic cells (Sertoli, neuronal and cumulus cells) taken from adult mice into enucleated mouse oocytes [4]. A decade later, transcription factor transduction was recognized as a promising technique to reprogram adult cells owing to the breakthrough research by Yamanaka *et al.* who demonstrated retrieval of pluripotency in somatic cells by a disruption in the stoichiometry of their transcriptional regulators [5]. The aforementioned

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models of reprogramming suggested that cellular memory is dynamically controlled and it is possible to achieve cell fate conversions in differentiated cells. Utilizing this plasticity of somatic cells to produce cells of the desired lineage, possessing properties and capacities of their native counterparts, forms the basis of an emerging field of patient-specific cell-based therapies for clinical applications. A summary of the various approaches of deriving cells of the target lineage is presented in Figure 1.

Induced pluripotent stem cells: reprogramming somatic cells to pluripotency

Before the breakthrough research of inducing pluripotent stages in the somatic cells by Yamanaka's four transcription factors [5], embryonic stem cells (ESCs) derived from the inner cell mass of a blastocyst were seen as a viable source of pluripotent cells [6]. The successful derivation of ESCs capable of being developed into all three germ layers and being expanded without causing genomic abnormalities paved the way for research focusing on its applications in regenerative medicine [7]. Although ESCs provide a practical model to study basic cellular differentiation and regeneration, its application to clinical studies is limited by ethical issues of using human embryos, immune response, population impurity during differentiation and proliferation as well as potential for tumorigenicity [8].

In 2006, a hallmark publication by Yamanaka redefined the field of stem cell biology and regenerative medicine. For the first time, adult fully differentiated somatic cells (mouse fibroblasts) were dedifferentiated

to the pluripotent stages through the expression of specific proteins using four transcription factors (*Sox2*, *Sox2*, *Klf4* and *c-Myc*) [5]. These induced pluripotent stem cells (iPSCs) bear similar morphology with ESCs, which are capable of generating teratomas and differentiating into all three germ layers. They could successfully generate a fertile adult mice and be validated by the tetraploid blastocyst complementation assay. In 2007, two groups reported the generation of iPSCs from human fibroblasts with a combination of different factors [9,10]. Yamanaka *et al.* used the above-mentioned four transcription factors while Thompson *et al.* used *Oct4*, *Sox2*, *Nanog* and *Lin28*.

There are mainly three limitations of using iPSCs in clinical applications:

- Viral transfection – safety versus efficacy: most iPSC derivation techniques use integrative delivery systems employing retroviral or lentiviral delivery which are sufficiently efficient and easy to implement in various cell types [11,12]. However, integration of viral transgenes into the cell genome raises concerns about their safety [13]. Advances in the development of nonintegrative methods like adenovirus [14], sendai virus [15], plasmids [16], *Cre-LoxP* [17] and inducible lentivirus [18] have relieved some of the concerns but they still fail to meet the safety standards for clinical applications. Various other reprogramming methods using recombinant proteins [19], miRNA [20] or small-molecule compounds [21], are being investigated to derive safer iPSCs for translational application. Nevertheless, most of these techniques are labor intensive with

Figure 1. Various techniques of acquiring cells of target lineage through pluripotent, multipotent and unipotent pathways. (A) SCNT through the nuclear transfer generates a blastocyst and the derived embryonic stem cells can be directed toward target lineage. Cell fusion involves altering the nuclear ratio of the two distinct cell types in fusion followed by programming the heterokaryon to produce target cell type [78]. Stem cells can be acquired from embryo, fetus and postnatal and their differentiation can be directed toward the target lineage. iPSC reprograms a somatic cell to a pluripotent stage and then directs its differentiation toward the desired cell type. All these approaches give rise to pluripotent cells which can differentiate to various cell lineages. (B) Direct reprogramming differentiates somatic cells to cells of target lineage through multipotent pathways without achieving pluripotency. Adult stem cells are self-renewing cells which can differentiate to some specialized cell types. (C) Direct conversion is based on the dominant action of transcription factors involved in the transdifferentiation process where the cells undergo a lineage switch to precursor cells of one specific target lineage.

hESC: Human embryonic stem cell; iPSC: Induced pluripotent stem cell; SCNT: Somatic cell nuclear transfer.

low efficiency and poor reproducibility. Newer studies focus on nonintegrative approaches in order to develop safe and efficient induction protocols;

- Epigenetic memory retention in reprogrammed cells: although there exists convincing evidence of the functional equivalence of ESC and iPSC, epigenetic analysis of the derived cells documents a significant difference in their gene expression profiles [22]. Genome-wide profiling of ESCs and iPSCs has identified over a thousand differentially methylated regions due to inefficient silencing of the somatic cell genes (epigenetic memory) or acquisition of new methylation pattern (epigenetic mutation) [23]. Retention of epigenetic memory in iPSCs was confirmed by the results of gene expression profiles which showed persistent donor cell gene expression in iPSC [24]. In this context, since not all the genomic variances have significant implications, it is important to reveal if any functional abnormalities occur and persist in or during differentiation post-pluripotency induction;
- Tumorigenicity: single nucleotide polymorphism analysis of iPSC showed that the pluripotent cells have increased frequency of 'sub chromosomal copy number variations'. Also, multiple passages of the iPSCs could lead to oncogenic gene duplication and silencing of tumor suppressor genes [25]. It has also been demonstrated that iPSC-derived chimera develop tumors either due to failure to demethylate pluripotency genes [26], or reactivation of oncogenes *c-Myc* and *Klf4* [27]. Also Gore *et al.* characterized genetic mutations in human induced pluripotent cells (hiPSCs) to be largely nonsynonymous or splice variants with oncogenic implications [28].

Direct conversion: a lineage switch through transdifferentiation

Direct conversion is an alternative approach to generate lineage-specific terminal cells. The process converts and commits adult, matured, fully differentiated cells to one specific terminal cell type without going through pluripotency. As opposed to the iPSC reprogramming technique, where the epigenetic information is erased to attain a pluripotent 'ground state', direct conversion primarily aims at inducing the epigenetic features of the desired cell type to achieve conversion between two distinctly unrelated cell types [29].

Initial breakthrough in the field of direct conversion occurred following reports suggesting that activation of some 'master control genes' regulates the commitment of cells toward a particular cell lineage [30]. Overexpression of these 'master control genes' in somatic cells was found to lead to a cascade of cell fate chang-

ing processes [31]. These master control genes were also, possibly specifying the body plan, organogenesis and cellular identity. The earliest evidence of the possibility of a single gene inducing a cell fate changes was provided by Weintraub *et al.* who confirmed a phenotypic conversion of fibroblasts to myogenic lineage by transfection of a master regulatory gene (*MyoD*) for myogenesis [32]. In 2009, the master control genes for cardiogenesis were identified to be *Gata* and *Baf60c* as they could directly convert mouse mesoderm cells into cardiomyocytes [33]. Further evidence was provided by Ieda *et al.*, who through progressive elimination defined a minimal combination of three transcription factors – *Gata4*, *Mef2c* and *Tbx5* needed for reprogramming fibroblasts into cardiomyocyte-like cells [34]. Later *Pax6* was also recognized as a master gene responsible for neuronal differentiation. *Pax6* plays a critical role in generation of neurons from astroglial stem cells [35]. Vierbuchen *et al.* identified *Ascl1* as a master gene regulating neuronal versus glial specification. This in combination with *Brn2* and *Myt11* could efficiently convert mouse embryonic fibroblasts into mature neurons [36]. Human fibroblasts, similarly, could be reprogrammed by adding neural differentiation transcription factor NeuroD1 along with *Ascl1*, *Brn2* and *Myt11* [37]. On the other hand, Ring *et al.* have achieved conversion of human fibroblasts to multipotent neural stem cells with just one transcription factor – *Sox2* [38]. There have also been reports of derivation of neuronal subtypes such as dopaminergic neurons using different combination of transcription factors (*Ascl1*, *Nurr1* and *Lmx1α* or combining *Ascl1*, *Brn2* and *Myt11* with *Lmx1α* and *FoxA2*) [39,40].

Direct reprogramming: reprogramming bypassing the pluripotency stage

Direct reprogramming is a cell lineage conversion procedure which closely follows the conventional iPSC reprogramming method but bypasses the pluripotency stages. While iPSC induction requires 1 or 2 weeks of transcription factor expression, direct reprogramming uses transient expression of the Yamanaka factors (less than a week) to propel the cells toward an epigenetically unstable, 'plastic' state (no pluripotency), before directing it toward the target cell lineage. Direct reprogramming also combines transient expression of iPSC factors with different signaling environments such as growth factors or signaling pathway modulators which promote cell lineage conversion under appropriate culture conditions [41]. Although the direct reprogramming avoids pluripotency, the differentiation process may still require an intermediate multipotency stage.

Efe *et al.* converted mouse fibroblasts into functional (spontaneously contracting) cardiomyocytes

by transiently (4–6 days) inducing the iPSC factors (*Oct4*, *Sox2*, *Klf4* and *c-Myc*) along with a JAK inhibitor (to prevent establishment of pluripotency) followed by an addition of cardio-inductive growth factor *BMP4* [42]. Similarly, Kim *et al.* derived neural stem cells from both mouse and human fibroblasts by culturing the epigenetically unstable intermediates in neural reprogramming medium containing FGF2, EGF and FGF4 [43]. On the other hand, Wang *et al.* restricted *Oct4* activity to the initial phase of reprogramming and constitutively induced *Sox2*, *Klf4* and *c-Myc* to generate neural stem cells (reported as neurospheres), which differentiated into neurons, astrocytes and oligodendrocytes [44].

Direct reprogramming versus direct conversion

At this point, it is critical to elucidate the inherent difference between direct conversion through transdifferentiation and direct reprogramming. Both approaches derive specific cells by a lineage conversion without attaining pluripotency. However, direct conversion is defined as transdifferentiation achieved by overexpression of lineage factors, without going through pluripotency or multipotency. On the other hand, direct reprogramming involves the transient opening of multipotent pathways to direct differentiated cells toward epigenetically unstable, ‘plastic’ intermediates that could be converted into the desired cell type before they attain pluripotency. Also, direct reprogramming generates multipotent precursor cells whereas direct conversion ends with terminal cells.

Molecular dynamics of somatic cell reprogramming: an integrative reprogramming model

Understanding the molecular mechanisms defining the reprogramming process, and studying its intermediates provide an insight on the sequence of events underlying acquisition of pluripotency or a specific lineage identity. Studies exploring the molecular basis of cell fate conversions focus on the transient intermediates of the reprogramming process, revealing the crucial time points to be targeted when aiming for efficient reprogramming [45–47].

Polo *et al.* described the iPSC reprogramming process in terms of two transcriptional waves: one primarily driven by *C-myc* and the other by *Oct4* and *Sox2* while *Klf4* represses somatic targets in early stages and activates pluripotent targets in the later phase [47]. They also studied the epigenetic changes during reprogramming and detected that it too followed a two wave pattern; with histone modification occurring in the early phase followed by DNA methylation events in the later phase. This is consistent with the pattern observed

during natural cell differentiation where transcription and chromatin changes precede the DNA methylation which ‘locks’ the cell in its committed lineage. Hansson *et al.* applied dynamic proteomic analysis to characterize the differentially expressed proteins during the course of reprogramming of fibroblasts to iPSCs and corroborated that epigenetic and proteomics changes occur in a highly coordinated manner which can be reflected in the two waves model described by Polo *et al.* [46]. The key attribute of this two wave model is the demarcation of an intermediate ‘resting phase’, where cells can remain resistant to reprogramming or commit to traverse the second wave. Also, while reprogramming could follow an early and late deterministic phase, the intermediate phase is largely stochastic or ‘probabilistic’ implying that the Yamanaka factors could repress or activate targets or induce transient reprogramming (dedifferentiation and redifferentiation). This model could potentially explain direct reprogramming, where manipulating this intermediate stage could achieve direct lineage conversions from a mature cell to another mature cell type [48]. Combining all the above-mentioned models of reprogramming and modifying the wave model proposed here [49], a more integrative model can be derived that encompasses the more recent reprogramming approaches like direct reprogramming and transdifferentiation as represented in Figure 2.

Various differentiation mechanisms targeting oligodendrocyte phenotype

The scope of this section is to highlight challenges of deriving oligodendrocytes (OLs) with remyelinating potential that can be used in clinical settings to treat patients with demyelination pathologies (spinal cord injury, multiple sclerosis, amyotrophic lateral sclerosis, transverse myelitis, trauma) through two main mechanisms, induced pluripotency and direct conversion.

CNS trauma leads to neuronal degradation due to demyelination, a pathological process where axons lose the insulating myelin sheath. This is mainly caused by destruction of oligodendrocytes post-trauma and consequently depletion of oligodendrocyte population [50]. Since OLs play a critical role in myelination, protecting and maintaining neuronal conductivity, various cell replacement therapies recognize transplantation of stem cell derived oligodendrocyte progenitor cells (OPCs) and OLs as a potential treatment of demyelinating CNS disorders [51].

It is important to recapitulate the natural process of oligodendrogenesis to identify the key stages that defines its differentiation process. As we reported, iTRAQ-based quantitative temporal proteomic analy-

Figure 2. Illustration of the wave models of reprogramming. The reprogramming of a somatic cell can be represented by a biphasic process. An early phase comprising of chromatin changes leading to erasure of somatic cell identity and a late phase where pluripotency is established and DNA methylation locks the cell in its reprogrammed state. These two phases are separated by an intermediate phase consisting of unstable, highly plastic group of partially reprogrammed cells. iPSC approach (blue arrows) runs the entire sequence of events where these unstable intermediates are pushed toward pluripotency and then the differentiation of iPSCs is directed toward the target cell lineage. Direct reprogramming follows the first crest of the wave till the intermediate stage, but instead of inducing pluripotency it directs the differentiation of the unstable intermediates toward the target cell lineage (red arrows) via multipotent pathways. Transdifferentiation involves induction of a target cell type through ectopic expression of lineage-specific transcription factors and hence may not travel the route of the other two approaches (green arrow). iPSC: Induced pluripotent stem cell.

sis of OPC differentiation has identified key stages of gene-specific expression as follows: OPCs progress toward oligodendroglial lineage as evident by the downregulation of *PDGFRα* and *NG2* markers and enhanced expression of oligodendrocyte marker *O4*, which is characteristic of an early, premyelinating state. The mid-stage OPCs express *GalC* and *O1* whereas the late OPCs are identified by the expression of *PLP* and *CNPase*. Finally, the expression of *MBP* indicates the onset of OL maturation [52,53].

iPSCs reprogramming to generate oligodendrocyte progenitor cells

Before the advent of iPSC reprogramming, several groups reported a successful derivation of OPCs and OLs from human ESCs (hESCs) [54–59]. Wernig *et al.* were among the first to explore the potential of iPSCs for neural cell replacement strategies as they successfully derived neural precursor cells through retroviral,

ectopic expression of the Yamanaka factors in mouse fibroblast cells. Upon transplantation of these neural precursor cells into the mouse brain, the cells could migrate and differentiate into neurons and glial cells. Generation of OLs using this approach was deemed unsuccessful as the appearance of *O4*⁺ oligodendrocytes was ‘rare’ [60]. Subsequently, Onorati *et al.* used a standard protocol of *in vitro* OL differentiation [61] on mouse fibroblast-iPSC and derived neural stem cells [62]. However, only 25% of total cells expressed the oligodendrocyte markers (*NG2*, *O4*), and the myelinating potential of these cells was not evaluated as the study did not report staining for mature OL markers (*MOBP*, *MBP*). Another study by Czepiel *et al.* reported the generation of functional OLs by sequential induction of oligodendrocytic cell lineage through appropriate growth factors such as *FGF2* and *PDGF* [63]. The derived OPCs showed decreased expression of pluripotency factors *Nanog* and *Oct4*

and increased expression of *Olig1* and *Olig2* during the final transition into OL lineage, thereby validating the reprogramming procedure. The myelin production ability of the derived OLs *in vitro* was tested by co-culturing with dorsal root ganglion neurons and was also transplanted in demyelinated corpus callosum of cuprizone-fed mice *in vivo*. The OLs were observed to form myelin around the unsheathed axons in 2 weeks *in vitro*. The transplanted OPC did develop into *MBP* positive mature OLs *in vivo* and remyelinated the corpus callosum axons. However, 80% of implanted cells did not survive the injection procedure or the hostile microenvironment and were 'eliminated by microglia'.

The first report of induction of OLs from human iPSCs was in 2011, when Ogawa *et al.* described the production of OLs from adult human fibroblasts by using PDGF and EGF [64]. However, the differentiation efficiency of *O4*⁺ OLs was extremely low (0.01%). In 2013, Wang *et al.* demonstrated the possibility of generating enriched population of autologous glial cells (astrocytes and OLs) from hiPSC which could be purified using fluorescence-activated cell sorting [65]. Significantly, they reported no evidence of *in vivo* tumorigenesis for as long as 9 months post-transplantation; perhaps because the robust differentiation protocol eliminated the risk of teratoma formation either due to residual partially differentiated or undifferentiated cells. However, since this extensive *in vitro* differentiation took about 125 days, it might limit clinical applications for treating time sensitive diseases. There are some other promising reports of OL differentiation from autologous iPSC from patients suffering from multiple sclerosis. While Song *et al.* used an integrating retroviral vector to induce differentiation of OLs from iPSCs derived from patients with multiple sclerosis [66], a recently published study by Douvaras and colleagues developed a protocol for integration-free method to generate patient-specific, functional OPC and OLs [67]. Their approach consisting of adherent cultures, dual inhibition of SMAD proteins and retinoid additions could generate *O4*⁺ cells in 75 days, much faster than the protocols which needed 125 days to acquire this stage. In 2015, Ali *et al.* demonstrated the derivation of high-purity OPCs from hiPSCs using both a nonintegrating plasmid and a retroviral approach. Based on their evaluation of cell survival and differentiation capacity of the transplanted cells, they advocated for an early transplantation (within 24 h) for highest therapeutic benefit post-spinal cord injury [68].

Direct reprogramming to generate oligodendrocyte progenitor cells

As it was defined above by direct reprogramming, mature cells are dedifferentiated and redifferentiated without going through the pluripotency but their repro-

gramming requires these cells to be genetically unstable and pass through an intermediate stage of multipotency. Surprisingly, the literature search revealed little information about direct reprogramming of OPCs or OLs.

Direct conversion to generate oligodendrocyte progenitor cells: a viable alternative to induced pluripotent stem cells

In 2013, direct conversion of fibroblasts into OPCs and OLs was reported by two groups who achieved successful conversion and proliferation of OPCs from rat fibroblasts using a set of three transcription factors [69,70]. Najm *et al.* used *Sox10*, *Olig2* and *Nkx6.2* to produce OPCs exhibiting morphological and molecular consistency with 'bonafide' OPCs in the CNS. They started with a cluster of eight transcription factors which could induce morphological changes in cells, converting flat spindle shaped fibroblasts to small bipolar cells, which after characterization were identified as OPCs that could differentiate into OLs upon growth factor removal and thyroid hormone (T3) addition. Furthermore, they also demonstrated the formation of myelin in *ex vivo* organotypic slice cultures and *in vivo* by transplantation in the dorsal region of shiverer mouse which lack compact myelin. An interesting observation reported by them was that while in slice cultures, the derived OPC showed clear property of oligodendrocytes being able to myelinating multiple axons. However, the same cells transplanted *in vivo* exhibited Schwann cell-like characteristics by myelinating single axons, though it is generally held that Schwann cell myelination occurs in the PNS and in the absence of astrocytes [71]. In addition, Zawadzka *et al.* reported that resident glial progenitors cells could differentiate to both oligodendrocytes and Schwann cells following demyelination in CNS [72]. Nevertheless, the mechanism by which newly generated (by direct conversion and transdifferentiation) OLs inter-differentiate to Schwann cells and remyelinate nude axons in CNS is less understood. On the other hand, Yang *et al.* used ectopic expression of *Sox10*, *Olig2* and *Zfp536* to generate OPCs which stained positive for appropriate OPC markers (*NG2*, *A2B5*, *S100Beta*) and proliferate and differentiate into OLs *in vitro* (expressing *MBP* and *CNPase* markers) [70]. However, they acknowledged the low efficiency of differentiation of OPC into mature OLs. They also reported dissimilarity in gene expression profiles of reprogrammed OPCs and the 'primary' (endogenous) OPCs in spite of steady downregulation of fibroblast-specific genes. They also observed heterogeneous population of reprogrammed cluster of cells expressing *GFAP*⁺ and *O4*⁺ characteristic of astrocytic cells and OPCs respectively. A simple schematic of OL derivation history is reported in Figure 3.

Discussion & conclusion

hESCs are an excellent source of pluripotent, self-renewing cells which can be easily differentiated into cells of target lineage with no retention of epigenetic memory. However, their tendency to become carcinogenic and the possibility of eliciting host immune response limit their clinical application. In the current scenario, the most important limitation in using hESC would be the ethical implications of using cells derived from the embryo. These limitations necessitate development of alternative techniques to derive pluripotent cells.

iPSCs overcome the ethical hurdles of hESCs since they are obtained from dedifferentiation of adult cells and not from embryo, to a pluripotent stage, following which they can be easily differentiated into any cell type. In addition, iPSCs could be patient-specific cells, which reduces chances of immunogenicity and would make them desirable for translational applications. However, the time required to generate sufficient number of iPSCs and desired target cells is much higher than any other cell generation process. Most importantly, recent studies raised concerns on iPSCs potential for tumorigenicity and epigenetic memory retention significantly restricts their potential use in clinical settings.

Although further investigation regarding the integration of the directly converted cells within the host tissue is needed and the challenge of procuring sufficient number of cells has to be considered, it appears that presently, direct conversion could and should be considered a clinically relevant form of autologous stem cell replacement therapy. Through the direct conversion process, mature somatic cells can be converted to cells of the target lineage in a reasonable period of time, which is critical for time sensitive pathologies. Most importantly, direct conversion through transdifferentiation bypasses the intermediate pluripotent stages and hence could avoid tumorigenicity arising from the acquisition of pluripotency. Also, the technique involves less genomic reprogramming, thus potentially exerts less mutational load on the cells. Moreover, there have been no reported contraindications of development, generation and application of directly converted stem cells with regard to their potential immunogenicity, retention of epigenetic memory and ethical concerns.

Future challenges & perspective

As with the other stem cell approaches, the lineage commitment of offspring cells derived by direct reprogramming or direct conversion through transdifferentiation has to be verified by quantitative and genomic comparison studies. A recently developed network biology computational platform (CellNet) provides the tools to determine the extent to which the engineered cells have acquired the gene regulatory network that govern the target cell

type [73]. A successful derivation of reprogrammed cells is defined by the possibility to maintain the functionality of the converted cells even after withdrawal of exogenous factors. In this regard, it is useful to understand the endogenous niche of the target cell and coculture the reprogrammed cells with supporting cells from its natural environment, to mimic the microenvironment [74]. In other studies, they have reported that within the CNS the astrocytes could be converted to neurons [75,76], and expression of *Sox10* could prompt satellite glia to differentiate into OLs *in vivo* [77]. Nevertheless, it remains to be seen if this could be extended to acquire OPCs and OLs subsequent to a spinal cord injury.

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Figure 3. Timeline of the development of stem cell approaches in derivation of oligodendrocyte progenitor cells and oligodendrocytes. Major advances in generation of oligodendrocytes are highlighted. Orange, blue and green indicate cells derived from hESC, iPSC and direct conversion, respectively. Texts to the right of the timeline indicate studies with human cell source and texts to the left indicate studies with rodent cell source. hESC: Human embryonic stem cell; hiPSC: Human induced pluripotent stem cell; iPSC: Induced pluripotent stem cell.

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Executive summary

Utilizing plasticity of adult cells for clinical applications

- The feasibility of reprogramming somatic cells toward desired cell lineage provides a viable cell source for autologous cell-based therapeutic treatment of demyelination in the CNS by transplantation of oligodendrocyte progenitor (OPCs) and oligodendrocytes (OLs).

Induced pluripotent stem cells: reprogramming somatic cells to pluripotency

- This is a promising reprogramming technique which converts adult fully differentiated somatic cells to pluripotent cells through the expression of specific proteins using four transcription factors (Oct4, Sox2, Klf4 and c-Myc).
- The clinical relevance of this approach is limited by the need of viral transfection for high efficiency, length to time required to generate sufficient number of cells and concerns on induced pluripotent stem cells (iPSCs) potential for tumorigenicity and epigenetic memory retention.

Direct conversion: a lineage switch through transdifferentiation

- Direct conversion converts and commits adult, matured, fully differentiated cells to one specific terminal cell type without going through pluripotency, by induction of lineage-specific transcription factors.
- Its application in clinical setting is promising as the process converts cells in a shorter period of time without going through pluripotency (avoiding tumorigenicity concerns) and exerts less mutational load on the cells.

Direct reprogramming: reprogramming without pluripotency

- Direct reprogramming is an alternative approach which avoids pluripotency by using transient induction of iPSC transcription factors to create an epigenetically unstable, 'plastic', multipotent cell state, which can then be directed toward target cell lineage.

Various differentiation mechanisms in oligodendrocytes for remyelination in CNS

- OLs play a critical role in myelination, protecting and maintaining neuronal conductivity, thus various cell replacement therapies recognize transplantation of stem cell derived OPCs and OLs as a potential cell-based treatment of demyelinating CNS disorders.
- Currently, iPSC reprogramming established the feasibility of generating OPCs and OLs from autologous cell source. But the time period required to get reprogrammed cells (about 2–3 months) makes it difficult to treat time-sensitive CNS pathologies.
- Recent studies have successfully converted mouse fibroblasts into OPCs using the direct conversion approach but there have not been reports of generation of OPCs from human cell sources in this context.

Future challenges & perspective

- Direct conversion appears to be a safe and viable reprogramming approach, but the need to derive oligodendrocytes from human origin cells is essential for its clinical relevance.
- The most critical validation required for successful reprogramming of cells is establishing the fidelity of the converted cells toward the target lineage by advanced quantitative and genomic comparison studies.
- Understanding the endogenous niche of the target cell and coculturing the reprogrammed cells with supporting cells from its natural environment to mimic the microenvironment may be a good approach to maintain the functionality of converted cells in an *in vivo* setting.

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