

REVIEW

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# Epigenetic reprogramming of cell identity: lessons from development for regenerative medicine

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## Abstract

Epigenetic mechanisms are known to define cell-type identity and function. Hence, reprogramming of one cell type into another essentially requires a rewiring of the underlying epigenome. Cellular reprogramming can convert somatic cells to induced pluripotent stem cells (iPSCs) that can be directed to differentiate to specific cell types. Trans-differentiation or direct reprogramming, on the other hand, involves the direct conversion of one cell type into another. In this review, we highlight how gene regulatory mechanisms identified to be critical for developmental processes were successfully used for cellular reprogramming of various cell types. We also discuss how the therapeutic use of the reprogrammed cells is beginning to revolutionize the field of regenerative medicine particularly in the repair and regeneration of damaged tissue and organs arising from pathological conditions or accidents. Lastly, we highlight some key challenges hindering the application of cellular reprogramming for therapeutic purposes.

**Keywords:** Development, Epigenetic mechanisms, Transcription factors, Reprogramming, Regenerative medicine

## Background

Epigenetic mechanisms confer changes in the gene expression program without modulating the DNA sequence [1]. During mammalian development, the zygote undergoes a series of differentiation events to generate various cell types. The differentiation to various cell types requires the acquisition of cell-type-specific gene expression programs via epigenetic mechanisms [2–4]. These include DNA methylation, histone modifications, and noncoding RNAs such as micro-RNAs and long noncoding RNAs. The unique epigenetic landscape of each cell type determines its gene expression program that governs its identity and biological function [5, 6].

Over the years, numerous studies have attempted to convert differentiated cells into pluripotent cells or

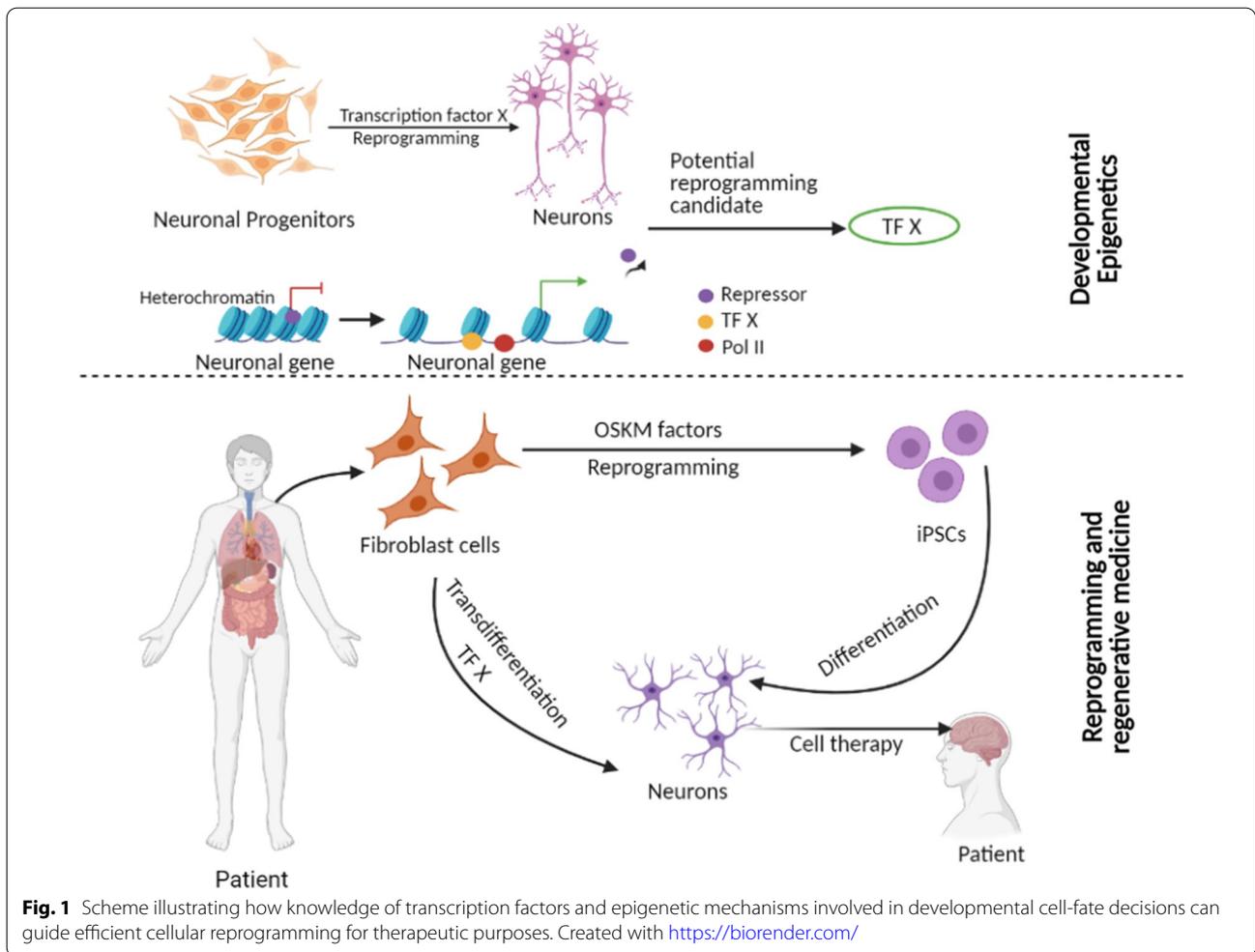
another cell type (direct reprogramming) using learnings from developmental biology (Fig. 1). The ultimate goal of generating the reprogrammed cell is to use them for regenerative medicine to restore structurally and functionally damaged tissues and organs. Currently, there are numerous clinical trials ongoing using reprogrammed cells and thus far have shown appreciable success. The reprogramming approaches include somatic cell nuclear transfer (SCNT), cell fusion, ectopic expression of specific transcription factors, micro-RNAs expression as well as by using small signaling molecules [7–10] (Table 1). It is becoming clear that such reprogramming involves remodeling of the epigenome eventually inducing a loss in molecular features of the original cell lineage and gain of new molecular features characteristic of the reprogrammed cell [11]:

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**I. Ectopic expression of transcription factors**

One of the most widely used methods for reprogramming cells is ectopic expression of transcription factors using adenovirus, lentivirus, retrovirus, etc., based transduction to deliver one or more transcription factors into primary cells. In stably reprogrammed cells, the epigenetic memory transmits across multiple cell divisions. The expression and activity of ectopically expressed transcription factors can alter the epigenetic state at the gene regulatory regions [12]. The presence of certain chromatin features has been shown to hinder the process of reprogramming of the cells, and hence, overcoming this barrier is an essential part of the reprogramming process [13]. We highlight below some examples where certain developmental transcription factors were used to reprogram cells and that function via epigenetic remodeling:

- a. The transcription factors Oct4, Sox2 and Klf4 are known to play a critical role in the pluripotency and differentiation potential of embryonic stem cells. A

landmark study in the field was the reprogramming of the mouse fibroblast cells into embryonic stem cell-like iPSCs (induced pluripotent stem cells) using a cocktail of transcription factors Oct4, Sox2, Klf4, and cMyc (OSKM) [14]. During reprogramming, these factors cooperate with Polycomb repressive complex (PRC2) proteins to repress lineage-specific genes in the differentiated cells used for reprogramming to iPSCs [15, 16]. Such reprogramming events also involve loss of the repressive histone mark H3K27me3 [17–19]. Interestingly, during reprogramming, the mesenchymal-to-epithelial transition (MET) pathway is induced involving loss of mesenchymal marks including transcription factors such as Zeb1 and Snail1 and activation of epithelial markers like Cdh1, Epcam, etc. [20]. The OSKM factors can carry out loss of repressive methylation at promoter regions of pluripotency genes and a corresponding

**Table 1** Summary of various successes in cellular reprogramming through ectopic expression of specific transcription factors or miRNAs, via CRISPR-Cas9 approach or via chemical inhibition of epigenetic machinery

Sl. no	Starting cell	Reprogrammed cell	Factors used	References
1	Fibroblast	Neurons	Ascl1, Brn2 and Myt11	Vierbuchen et al. 2010 [26]
2	Fibroblast	Cardiomyocytes	Gata4, Mef2c and Tbx5	Ieda et al. 2010 [40]
3	Fibroblast	Hepatocytes	HNF1 $\alpha$ , Foxa3 and Gata4	Huang et al. 2011 [44]
4	Fibroblast	iPSCs	Oct4, Klf4, Sox2 and cMyc	Yamanaka et al. 2006 [14]
5	Fibroblast	Myogenic cells	MyoD	Ito et al. 2017 [119]
6	Fibroblast	Neuron	miR-9/9* and miR-124	Yoo et al. 2011 [63]
7	Non-myocytes	Induced cardiomyocyte	miR-1, miR-133, miR-208 and miR-499	Jayawardena et al. 2012 [59]
8	B and T-cells	Macrophages	C/EBP $\alpha$	Xie et al. 2004 [45]
9	ESCs	Trophoectodermal cells	Cdx2	Strumpf et al. 2005 [120]
10	Acinar cells	Insulin producing B cells	MafA, Pdx1 and Ngn3	Xu et al. 2013 [48]
11	Astrocytes	Glutamatergic Neurons	NeuroD1	Guo et al. 2014 [121]
12	mESC	Neurons	NeuroD1	Pataskar et al. 2016 [29]
13	Neural precursor cell	Astrocyte	NFIA, ATF3 and RunX2	Tiwari et al. 2018 [33]
14	Fibroblast	Oligodendrocyte	SOX10, ZFP536, OLIG2	Yang et al. 2013 [36]
15	Brain Pericytes	Neurons	Ascl1 and Sox2	Karow et al. 2018 [122]
16	Pluripotent stem cell	Adipocyte	CEBP $\beta$ , PRDM16	Ahfeldt et al. 2012 [123]
17	Fibroblast	Osteoblast	OCT4, RUNX2, OSX, MYC	Yamamoto et al. 2015 [124]
18	Fibroblast	iPSCs	CRISPR-dCas9 activation-OSKM and Lin28	Weltner et al. 2018 [67]
19	Fibroblast	Myoblast	CRISPR-dCas9 activation of Myod enhancer	Liu et al. 2016 [68]
20	Neural progenitor cell	Neuron	CRISPR-dCas9 activation of Sox1 promoter	Baumann et al. 2019 [69]
21	Fibroblasts	Neurons	CRISPR-dCas9 activation of Brn2, Ascl1, and Myt11	Black et al. 2016 [70]

gain at the promoters of cell lineage-specific genes. The discovery of iPSCs has revolutionized the field of reprogramming, and several modifications to the original protocol have been made to generate better iPSCs and increase reprogramming efficiency [21–23]. There is numerous application of iPSC in regenerative medicine, some of which have been highlighted in the later section ‘Success stories of cellular reprogramming in regenerative medicine’.

- b. Ascl1 belongs to the basic helix-loop-helix (BHLH) family of transcription factors and was found to be essential for neuronal differentiation and functions via chromatin remodeling to generate neurons [24, 25]. The fibroblast cells can be converted directly to neurons with a cocktail of transcription factors Ascl1, Brn2 and Myt11 [26]. During reprogramming, Ascl1 triggers widespread chromatin accessibility in fibroblasts following Ascl1 overexpression and generates neurons [27]. The POU transcription factor Brn2 is known to be critical for neuronal differentiation during cortical development and is recruited via Ascl1 during reprogramming [28]. Myt11 is another established neuronal transcription factor essential for neurogenesis. Altogether, these three factors (BAM factors) induce rapid and efficient changes in the fibroblast transcriptome toward a neuronal one to

enable successful reprogramming. The induced neurons generated from fibroblast have similar characteristics as cortical neurons with integration potential to the existing neuronal network and thus suitable for therapeutic use.

- c. The bHLH transcription factor NeuroD1 is induced during cortical development and was shown to remodel the chromatin landscape at target neuronal genes toward an active state to induce neuronal differentiation [29]. In a study, NeuroD1 could successfully convert mouse microglial cells directly into neurons [30]. Another study demonstrated that NeuroD1 can convert astrocytes to neurons using NeuroD1 [31]. Importantly, the neurons generated after reprogramming were successfully used in recovering the mouse brain with ischemic injury, clearly highlighting how the knowledge from development can be used for making a visible impact in regenerative medicine.
- d. NFIA was established as a gliogenic switch in the previous study [32, 33]. NFIA can bring about chromatin remodeling and demethylation of astrocyte-specific glial fibrillary acidic protein (GFAP) promoter to trigger this differentiation [32]. Our study has recently shown that at the onset of astrogliogenesis, NFIA binds to the target distal regulatory ele-

- ments of critical astrocyte differentiation genes and converts primed to active chromatin to induce the required their expression [33]. Several studies have now shown that functional astrocytes can be generated via direct or indirect reprogramming using the transcription factor NFIA.
- e. Sox10 can regulate the expression of myelin protein and oligodendrocyte cell marker PDGFR $\alpha$  [34]. The bHLH transcription factor Olig2 is essential for oligodendrocyte development in collaboration with Nkx2.2. Zfp536 was shown to be induced late during oligodendrocyte differentiation [35]. Mouse fibroblasts can be converted to oligodendrocytes by expression of transcription factors Sox10, Olig2 and Zfp536 [36].
  - f. The zinc finger transcription factor Gata4 is an established regulator of cardiac differentiation and regulates different cardiac-specific genes [37]. Mef2c is a mad box transcriptional factor and found to be a cofactor of Gata4 that regulates the cardiac muscle differentiation [38, 39]. Tbx5 is a member of the T-box transcription factor family, which activates genes involved in cardiomyocyte maturation. Fibroblast cells were directly reprogrammed into cardiomyocytes by overexpression of these three transcription factors Gata4, Mef2c, and Tbx5 [40]. The transdifferentiated cardiomyocytes are suitable for the treatment of damages from myocardial infarction in heart patients.
  - g. Hepatocyte nuclear factor 1 $\alpha$  (HNF1 $\alpha$ ) is important for the maintenance of hepatocytes [41, 42]. It is an activator of transcription and can regulate several genes during hepatogenesis. Loss of HNF1 $\alpha$  function can cause fatty liver-related hepatocellular carcinoma. Foxa3 (hepatocyte nuclear factor 3 gamma) is a winged-helix transcription factor and helps maintain cellular glucose homeostasis [43]. A pioneering study showed how hepatocytes can be generated from fibroblasts by co-expression of HNF1 $\alpha$ , Foxa3, and Gata4 [44].
  - h. The differentiated B cells were successfully transdifferentiated to macrophages by the overexpression of C/EBP $\alpha$  and C/EBP $\beta$  [45]. These factors can inhibit the expression of Pax5 and consequently downregulate CD19.
  - i. Pancreatic and duodenal homeobox 1 (Pdx1) is involved in the differentiation and maturation of  $\beta$ -cells [46]. Musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) also plays an important role in preserving the function of the  $\beta$ -cells and an insulin activator in the cells. MafA can bind to the promoter region of the insulin gene and regulate its expression [47]. Neurogenin 3 (Ngn3) is required for islet-like cell production. In a well-recognized study, the pancreatic cells derived from the acinar cells were reprogrammed to insulin-producing cells by the expression of MafA, Pdx,1 and Ngn3 [48].
  - j. The Tet family dioxygenases mediate sequential oxidation of 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [49]. The Tet proteins include Tet1, Tet2 and Tet3, which are involved in the process of epigenetic reprogramming of the cells [50, 51]. During the process of reprogramming 5hmC modification is increased and knockout of Tet proteins prevent reprogramming [52]. Tets are believed to reactivate Oct4 gene by demethylation of its promoter and enhancer regions and Tet1 can replace Oct4 in the OSKM reprogramming cocktail [46]. The iPSCs generated with Tet1, Sox2, Klf4, and c-Myc (TSKM) cocktail were found to be fully pluripotent. An interesting study highlighted how the Tet proteins can induce reprogramming by triggering mesenchymal-to-epithelial transition (MET) [53]. Tet3 was shown to regulate DNA methylation in the neural precursor cells and maintain the neural stem cell identity [54]. Knockdown of Tet3 causes upregulation of pluripotency genes in neural precursor cells. Further observations suggested that Tet3 is required for efficient reprogramming of fibroblasts into neurons. It was shown that knockout of all three Tets in MEFs can halt their reprogramming by preventing activation of micro-RNAs that are essential for MET during reprogramming [53]. Vitamin C, which was known to enhance reprogramming [55], was found to regulate Tet1-dependent 5hmC formation at loci involved in MET [56].

## II. micro-RNAs

miRNAs have been shown to exhibit the capacity to reprogram cells alone or in combination with other transcription factors. Owing to their relatively small size, miRNAs can be easily delivered in the cells to initiate reprogramming. Micro-RNAs such as the miR-302 is known to facilitate the reprogramming of human skin cells to iPSC-like cells [57, 58]. Furthermore, reprogramming of fibroblasts to cardiomyocytes is enhanced by using miR-1, miR-133, miR-208 and miR-499 [59]. miR-1 and miR-133 are known to inhibit cardiomyocyte proliferation and G1/S phase transition [60]. Furthermore, miR-208 induces the expression of cardiac transcription factors [61]. In addition, miR-499 functions as a regulator of cell proliferation during the late stages of cardiac differentiation [62]. It was also shown that fibroblasts can be reprogrammed to neurons using miR-9\* and miR-124

which modulate the SWI/SNF-like BAF chromatin-remodeling complexes in neuronal progenitor cells [63]. Interestingly further, these miRNAs can work in synergy with the other transcription factor-like NeuroD2, Ascl1 and Myt1l [63].

**III. CRISPR-Cas9-based genomic editing for reprogramming**

Several recent studies have shown a successful application of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and catalytically inactive CRISPR-associated 9 (dCas9) nuclease for reprogramming of cells [64, 65]. This system is vastly robust and can be employed to correct disease-causing mutations or to repress or activate genes by targeting specific activators or repressors. A method to set up genome-wide reprogrammable transcriptional memory using CRISPR-based editing was recently reported, and it holds great potential for stable and specific editing of relevant genes for therapeutic purposes [66]. We present below some of the examples where CRISPR-dCas9 was successfully used for cellular reprogramming of cells using gene-specific targeting of selected epigenetic regulators.

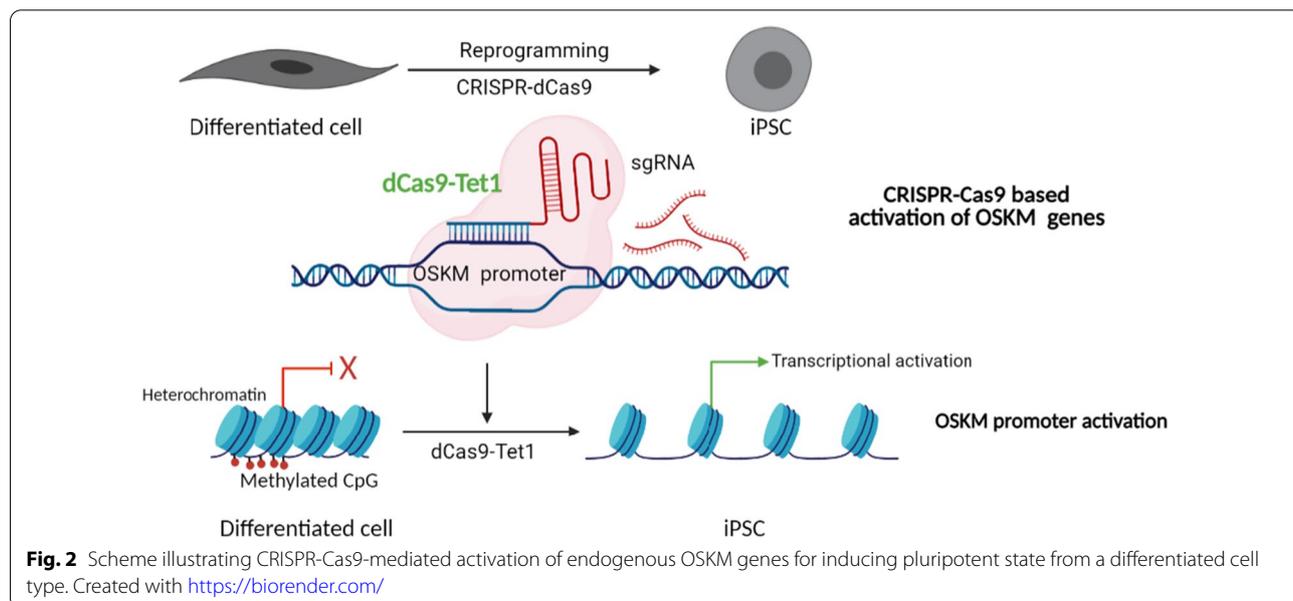
- a. CRISPR-dCas9 was used successfully to activate the promoters of Oct4, Sox2, Klf4, Myc, and Lin28 genes to convert human fibroblast cells into iPSC cells [67] (Fig. 2). The reprogramming efficiency was further enhanced by targeting the Alu-motif embryonic genome activation genes.
- b. The CRISPR-dCas9 can be used to bring about targeted alteration of DNA methylation state to con-

trol gene expression of cell-fate genes and drive cell reprogramming. The DNA methyltransferase Dnmt3a or the DNA demethylase Tet1 can be fused to Cas9 to specifically target the regulatory elements of genes which should be epigenetically reprogrammed [68]. For example, the Tet1 fused Cas9 was used to activate the Myod enhancer and convert fibroblast into myoblast cells [68].

- c. CRISPR-dCas9 has also been used to enhance reprogramming efficiency. For example, scientists targeted the promoter of the Sox1 gene in the neural progenitor cells (NPC) with dCa9-Tet1 protein, resulting in increased expression of Sox1. This resulted in an enhancement in the differentiation potential of the NPCs where Sox1 acts as a master regulator [69].
- d. CRISPR-dCas9-based simultaneous induction of multiple promoters of Brn2, Ascl1, and Myt1l genes (BAM factors) could successfully convert mouse embryonic fibroblasts into neurons [70]. Such endogenous gene induction was rapid and stable over time and involved triggered chromatin remodeling at the target sites. This method offered better reprogramming efficiency to induced neurons as compared to the other transient transfection-based reprogramming.

**IV. Using chemical inhibitors for reprogramming**

The field of reprogramming has greatly benefitted using small chemical molecules that have made a remarkable impact on increasing the efficiency as well as the scope



of direct differentiation of cells. We will highlight a few examples below that have involved inhibition of epigenetic regulators:

- a. DNA methyltransferase inhibitors: The DNA methyltransferase inhibitor 5'-azacytidine (5'-azaC) can improve the reprogramming efficiency induced by OSKM in a dose-dependent manner [71]. A partially reprogrammed cell can also be driven into a fully reprogrammed cell by 5'-azaC treatment [72]. Another DNA methyltransferase inhibitor RG108 was shown to increase the reprogramming efficiency of Oct4 and Klf4 in the presence of BIX (G9a histone methyltransferase inhibitor) [73].
- b. Histone deacetylase inhibitors: HDAC inhibitor valproic acid (VPA) can induce reprogramming in the absence of cMyc overexpression. Furthermore, VPA improves the reprogramming efficiency with OSKM [71]. During the generation of OSKM-induced pluripotent stem cells (piPSCs) from MEFs, VPA can significantly increase the reprogramming efficiency [74]. Moreover, Two other HDAC inhibitors suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) were found to promote the MEF reprogramming efficiency [71]. Sodium butyrate, an HDAC inhibitor, can enhance the reprogramming to human iPSC cells from adult or fetal fibroblast cells [75]. In addition, butyrate could induce the expression of certain pluripotency genes during reprogramming by catalyzing their promoter demethylation. Butyrate was suggested to be more efficient than VPA for Oct4 and Klf4-based reprogramming [76]. In another study, direct conversion of fibroblast cells into neurons was successfully carried out in the presence of VPA and some other inhibitors [77]. Moreover, the mouse fibroblasts can be directly reprogrammed into cardiomyocytes using a chemical cocktail including VPA [78]. Small molecules including VPA can also reprogram the astrocytes directly into neurons [79].
- c. Histone methyltransferase (HMT) inhibitors: BIX-01294, an HMT G9a inhibitor, can improve the reprogramming efficiency with Oct4/Klf4 in neural progenitor cells (NPCs) [73]. BIX is predicted to activate the Oct4 expression in the cells during reprogramming by inhibition of G9a-mediated H3K9me2 methylation.
- d. Histone demethylase inhibitor: Parnate is an LSD1 inhibitor, which in combination with CHIR99021(GSK-3 inhibitor) can reprogram the human primary keratinocytes into iPSCs upon overexpression of Oct4/Klf4 [80]. LSD1 inhibition with Parnate could partially convert the Epiblast stem cells (EpiSC) into pluripotent embryonic stem cell [81].

During this process, the expression of genes associated with the inner cell was found to be activated.

### Success stories of cellular reprogramming in regenerative medicine

The remarkable developments in the basic understanding and tools for reprogramming have begun to show the clinical impact of cellular reprogramming. The patient-derived cells have been successfully reprogrammed into different cell types and used for the treatment of underlying diseases. A few noticeable examples of such successful applications of reprogrammed cells for therapeutic use are highlighted below:

1. A Japanese woman was the first to receive cornea derived from iPSCs which significantly improved her vision [82]. The skin cells from a donor were reprogrammed into iPSCs, which were further differentiated into corneal cells. The use of such reprogrammed cornea can solve the problem associated with getting sufficient corneal tissue from the donor's eye for transplantation.
2. Reprogrammed neuronal precursors were successfully implanted into a Parkinson's disease patient in Japan [83]. The scientists used skin cells for reprogramming into iPSCs, which were differentiated into neuronal precursors that ultimately matured into dopamine-producing neurons. If successful, this treatment can be used to treat the tremors and walking issues in Parkinson's patients.
3. Cardiac tissues derived from reprogrammed iPSCs are currently under trials for use in patients with heart diseases [84]. The researchers plan to use the induced iPSCs to create sheets of heart muscle cells and grafted them into the heart. These sheets of heart cells can then produce growth factors that can help heal the damaged heart tissues in the adjoining regions.
4. Another potential application under testing involves the use of iPSCs generated precursor neurons to treat spinal cord injuries [85]. The precursor neuron cells could develop into neurons and glial cells when injected into the injured spinal cord in the monkey.
5. One of the earliest attempts in the treatment of a specific disease using iPSCs was for Duchenne Muscular Dystrophy (DMD), which results from mutations in the dystrophin gene that leads to muscular degeneration and ultimately loss of movement [41]. Here the approach involved converting the pluripotent stem cells into muscle cells by activation of MyoD. MyoD is a basic helix-loop-helix regulatory factor and responsible for the expression of muscle-

specific genes in the embryo. Specific manipulation of epigenetic circuitry with HDACi is suggested to play a vital role in this targeted differentiation [86]. Transplantation of these transformed myocytes into adults suffering from DMD is expected to improve their condition by muscle regeneration [87].

### Challenges in the field

Despite the revolutionary potential of reprogramming for therapeutics, several issues have created obstacles for a successful use of reprogrammed cells for therapeutic purposes. Some of these issues are highlighted below-

#### a) Incomplete resetting of epigenetic mark

During the process of reprogramming of cells, resetting of epigenetic marks such as DNA methylation is not complete [88]. This can lead to considerable differences between the individual reprogrammed cells and affect the differentiation potential and suitability of such cells for therapeutic purposes. In addition, such partially reprogrammed cells have higher tendency to become tumorigenic. The incomplete reprogramming can also lead to persistence of founder cell traits, which is not suitable for therapeutics.

#### b) Mutagenesis due to retroviruses

Many reprogramming protocols require retroviruses to deliver reprogramming factors into cells. These retroviruses can cause insertional mutagenesis in reprogrammed cells [89]. The integration of retrovirus can also lead to activation of retrotransposable elements in cells. To overcome these problems, there is a shift toward methods of reprogramming independent of retroviruses such as chemical-induced reprogramming and use of episomal vectors [90, 91].

#### c) Neoplastic development

The genes used to trigger the process of reprogramming such as OSKM can lead to neoplastic development in reprogrammed cells by getting activated during a later time point. This calls for the development of alternate approaches for reprogramming to minimize the carcinogenic potential of reprogrammed cells [92–94]. Tumors can also be initiated by the disruption of tumor suppression genes or the action of oncogenes during genomic integrations mediated by virus used for reprogramming.

#### d) Immunogenic incompatibility

In case the transplanted reprogrammed cell is derived from cells other than the patient itself, there is the possibility of immunogenic reaction in the receiver patient. The immune reaction elicited by such cells can decrease the survival of transplanted reprogrammed cells. In such cases, the patient is prescribed lifelong immunosuppressants which in turn can increase the susceptibility of the patient to certain opportunistic infections and other health complications [95, 96].

### Conclusions

The generation of iPSCs or specific transdifferentiated cells has created a new paradigm in the field of regenerative medicine with a wide range of applications including understanding the fundamental biology of cell specification, to drug screening to the treatment of patients [97–99]. The derived iPSCs can be used either for in vitro culturing for screening various drugs to treat the disease or for cell replacement therapy for the treatment of underlying diseases [100]. In patients suffering from diseases such as Parkinson's disease, patient-derived iPSCs are generated and underlying mutations corrected by gene therapy and subsequently differentiated into specific neurons [101]. These reprogrammed cells can be transplanted back to the patient for therapy. Similar approaches for diseases such as muscular dystrophy, Down syndrome, Fanconi anemia and Huntington's disease are under trial by various laboratories [102–105]. The use of patient-specific reprogrammed cells can circumvent various risks associated with the rejection of transplanted cells in the body as well as be a source of unlimited cells for therapy. In addition, the ability to study disease in a Petri plate using the iPSCs derived from the patient offers a unique opportunity to study the diseased phenotype for its better treatment. It would be vital to decipher the epigenetic mechanisms underlying these processes comprehensively and further optimize the protocol for the generation of iPSCs or transdifferentiated cells from patient cells. Exciting new approaches like CRISPR-cas9-based activation of transcription factors as well as computational modeling to screen large number of transcription factors for reprogramming ability offer an excellent opportunity to investigate the role of more than 2000 transcription factors for reprogramming [67, 106, 107]. Recently one of the focuses in regenerative therapeutics has been toward directed reprogramming of one cell type into another by transdifferentiation without the need to go through the intermediate pluripotent cell stage [108–110]. Transdifferentiated cells can

be generated at better efficiency and in a shorter interval of time compared to iPSC cells. Another huge advantage with transdifferentiation is that the cells can be reprogrammed directly in the affected tissue or organ without the need to derive pluripotent cells outside the body of the organism. Certain signaling molecules including growth factors present in the microenvironment of the transdifferentiated cells can enhance the transdifferentiation potential of the cell in vivo [111, 112].

Another important aspect for clinical application of these cells is regarding the safety including long-term behavior of these cells and tumorigenic potential once they are transplanted back into the patients [113, 114]. There have also been efforts to generate the iPSCs without viral genome integration or even without the use of viruses for delivery of the transcription factors in the cell as the integration of viral genome in recipient cell is associated with tumorigenic consequences [115]. The aim therefore should be to generate homogeneous reprogrammed cells that resemble the naturally occurring cell for therapeutics. A combinatorial approach using small chemical and transcription factors might pave the way for better-reprogrammed cells with increased reprogramming efficiency that might be a game-changer in the field of therapeutics [116]. The reprogrammed cells need to be mature as well as retain the ability to retain the reprogrammed memory across cell divisions [117]. The delivery of reprogrammed cells into the body can be critical depending on the target area of the body [118]. The successful application of reprogrammed cells in therapeutics is thus dependent on overcoming all these hurdles before we can apply this technique for the treatment of a wide range of diseases.

#### Abbreviations

iPSCs: Induced pluripotent stem cells; EpiSC: Epiblast stem cells; SCNT: Somatic cell nuclear transfer; PRC2: Polycomb repressive complex; CRISPR: Clustered regularly interspaced short palindromic repeats; Cas9: CRISPR-associated 9 ( $\beta$ ); NPC: Neural progenitor cells; 5'-azaC: 5'-Azacytidine; DMD: Duchenne muscular dystrophy.

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#### Authors' contributions

A.B. and V.T. conceptualized and wrote the review. Both the authors read and approved the final manuscript.

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#### Availability of data and materials

There are no data and material associated with this review.

## Declarations

#### Ethics approval and consent to participate

No such approval was needed for this review.

#### Consent for publication

All authors consent to publication.

#### Competing interests

The authors declare no competing financial interests.

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