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REVIEW ARTICLE

Induced pluripotent stem cells for spinal cord injury therapy: current status and perspective

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Abstract Spinal cord injury (SCI) is induced by a variety of damages such as trauma, ischemia, and iatrogenic injury, resulting in sense and motion dysfunction. Despite the improvements in medical and surgical care, current treatment methods for SCI demonstrate poor and delayed efficiency, leading to different degree of permanent loss of neural function and disability in the patients. Rapid advances in stem-cells research suggest that stem cells may be applied in SCI therapy. Indeed, SCI is a major field in which stem-cell therapy has been proposed and practised, and most recently the clinical trials of stem-cell therapy were initiated, which aroused a number of clinical concerns. In this review, we summarize current status of SCI repair, then introduce the sources and biological characteristics of induced pluripotent stem cells (iPSCs), and discuss the differentiation potential of iPSCs and the perspective of the application of iPSCs in SCI therapy.

Keywords Spinal cord injury · Stem cells · Induced pluripotent stem cells · Transplantation therapy

Introduction

Spinal cord injury (SCI) is a common disease in clinical practice. SCI can be induced by a variety of damages such as trauma, ischemia, and iatrogenic injury, resulting in the dysfunction of sense and motion under damaged plane or bowel and bladder. Despite the improvements in medical and surgical care, current treatment methods for SCI demonstrate poor and delayed efficiency, leading to different degree of permanent loss of neural function and high disability rate in the patients. Therefore, there is an urgent need to develop novel therapy strategies that would improve the outcome after SCI. The pathogenesis of SCI is a complex process that involves an initial mechanical insult followed by a cascade of cellular and molecular events that constitute the secondary injury such as glutamatergic excitotoxicity, free radical generation, lipid peroxidation, inflammation, and ischemia that ultimately result in necrosis or apoptosis [1]. Based on these pathological events of SCI, several approaches have been proposed for SCI repair: (1) to minimize progressive cell death, (2) to replace lost cells by transplantation or to stimulate the injured cord to produce new cells, a process called neurogenesis, (3) to reconnect injured nerve fibers with their original targets or with substitute targets (axon regeneration and sprouting), (4) to maximize the function of spared pathways by altering connectivity (synaptic plasticity), (5) to maximize the function of spared nerve fibers by repairing their myelin sheath (re-myelination), (6) to rehabilitate muscle and function, and (7) to use prostheses, robotics or other technologies to restore function [2].

Rapid advances in stem-cells research in recent years suggest that stem cells may be applied in many or even all of the above proposed approaches for SCI therapy. Indeed, SCI is a major field in which stem-cell therapy has been proposed and practised, and most recently the clinical trials of stem-cell therapy were initiated, which aroused a number of clinical concerns [3]. In this review, we summarized current status of SCI repair, then introduced the sources and biological characteristics of induced pluripotent stem cells (iPSCs), and discussed the differentiation potential of iPSCs and the perspective of the application of iPSCs in SCI repair.

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Current status of SCI repair

SCI includes primary injury and secondary injury, the former refers to the irreversible damage caused by direct mechanical insult, while the latter refers to the damage caused by secondary injuries of trauma such as traumatic inflammation/immune response, secondary cell necrosis and/or apoptosis, excitotoxin, intracellular and extracellular ionic imbalance, oxygen free radical, lipid peroxide and axon reaction [4]. In the earlier stage of injury, spinal cord swells and bleeds, followed by spinal cord ischemia which leads to necrosis of diseased region. The early acute phase (2–48 h) is characterized by inflammation, free radicals production, ion imbalance, immune-related neuron toxicity and other secondary responses, thus neuroprotective intervention is of great significance during this period. The subacute stage (48 h–2 weeks) is characterized by significant phagocytosis, in which a huge number of necrotic cells and tissue fragments are engulfed to create a favorable microenvironment for axonal regeneration and other reactions and this period is the window stage of transplantation [5]. The distinct features of intermediate phase are glial scar formation and axonal regeneration [6]. Finally, during the chronic phase syringomyelia and/or fistula gradually form, which indicates that the SCI enters into stable stage [5].

The difficulty for SCI repair is that nerve and neuroglia cells are destroyed due to necrosis and/or apoptosis, and nearby undamaged neurons could not effectively supplement due to limited regeneration potency. On the other hand, changes in local microenvironment lead to imbalance of the proliferation of glial cells, such as insufficient proliferation of oligodendrocytes which produce and form axonal myelin sheath, and hyperplasia of astrocytes and microglia, which prevents axon regeneration and the reconstruction of synaptic connections as well as function recovery [7].

SCI repair methods include surgery, medicine therapy and biological therapy. Surgical decompression in early stage of SCI (<24 h) and the reconstruction of spinal stability have been suggested to be favorable for SCI repair [8, 9]. Most of the medicines commonly used to treat SCI are still under clinical trials such as corticosteroids, minocycline, erythropoietin and gangliosides, which are effective in acute stage of SCI by reducing SCI secondary injury and protecting the remaining nerve tissue [10–12]. However, surgery and medicine treatment methods are not satisfactory for SCI repair because the damaged/lost neurons and axons whose remyelination is impaired cannot be replenished and synaptic connections cannot be rebuilt.

With the development of biotechnology, biological therapy represented by cell transplantation has shown promise for SCI repair. It has been reported that in mouse models SCI could be treated by transplantation of Schwann cells, olfactory ensheathing cells, neural stem cells, mesenchymal stem cells, embryonic stem cells (ESCs) and iPSCs [13–16]. These transplantation methods indeed have certain therapeutic effects, but exhibit some shortages. Schwann cell transplantation can promote nerve regeneration by generating matrix, secreting neurotrophic factors, and improving local microenvironment, but the lost nerve cells cannot be replenished due to the limited distance of Schwann cell migration. Furthermore, Schwann cells, which are difficult to amplify, can only be sampled by invasive methods which may cause neurological deficits in donor sites, thus limiting their application [17]. Olfactory ensheathing cell transplantation also faces the similar problems [18]. The application of neural stem cells is also restricted due to limited source from specific sites, the difficulty in being sampled, and host immune rejection [19]. Human umbilical cord blood (hUCB) cells are immune naïve and they show the therapeutic potential as a safe, feasible and effective cellular source for transplantation in SCI [20]. However, clinical studies with hUCB cells are still rare due to the concerns about their safety and efficiency. Recent utilization of adipose-derived stem cells and bone marrow mesenchymal stem cells help overcome the problems of limited source and ethical issues, but these stem cells are not homogenous, and further technical improvement is required to purify and amplify them [21]. In contrast, iPSCs have many advantages that cannot be matched by other stem cells, such as multi-differentiation potential, abundant source, easily being sampled, no host immune rejection, and no ethical or legal issues.

The sources and biological characteristics of iPSCs

iPSCs are developed from differentiated cells by the expression of specific transcription factors which are regulated by direct reprograming techniques. The first iPSCs were created by retrovirus-mediated transfer of Oct3/4, Sox2, Klf4, and c-Myc genes into mouse fibroblast cells [22]. Currently, a variety of methods have been developed to derive iPSCs (summarized in Table 1): (a) viral transduction: IPSCs can be successfully induced by the introduction of different amounts/types of transcription factors using several types of virus [23–26], but exogenous genes of these viruses are prone to integrate with host genes and increase the risk of tumorigenesis; (b) plasmid transfection: although exogenous genes might integrate with host genes during plasmid transfection, the probability is much smaller than using retrovirus, thus plasmid transfection is relatively safe [27, 28]; (c) episomal vector: transcription factors are transfected into adult cells using episomal vector and then the episomal vector is removed, thus

Table 1 Summary of the methods by which IPSCs are derived

Methods	Factors	Exogenous gene integration	Cell type	Efficiency	Year	Reference
Virus						
Retrovirus	4 factors (Oct3/4, Sox2, Klf4, c-Myc)	Yes	Mouse embryonic fibroblasts	~0.1 %	2006	[22]
	2 factors (Oct4, Klf4 or c-Myc)	Yes	Mouse neural stem cells	~0.14 %	2008	[24]
	1 factor (Oct4)	Yes	Mouse neural stem cells	~0.1 %	2009	[26]
Lentivirus	4 factors (Oct4, Sox2, Nanog, Lin28)	Yes	Human fibroblasts	~0.01 %	2007	[23]
Adenovirus	4 factors (Oct4, Sox2, Klf4, c-Myc)	No	Mouse hepatocyte	~0.0006 %	2008	[25]
Plasmid	4 factors (Oct4, Sox2, Klf4, c-Myc)	No	Mouse embryonic fibroblasts	~0.0015 %	2008	[27]
	6 factors (Oct4, Sox2, Klf4, c-Myc, Nanog, and Lin28)	No	Human foreskin fibroblasts	~0.1 %	2008	[28]
Episomal plasmid	6 factors (Oct4, Sox2, Klf4, c-Myc, Nanog, and Lin28)	No	Human foreskin fibroblasts	~1 %	2009	[29]
Transposon	4 factors (Oct4, Sox2, Klf4, c-Myc)	No	Mouse embryonic fibroblasts	~0.1 %	2009	[30]
		No	Mouse and human fibroblasts	~2.5 %	2009	[31]
		No	Equine fibroblasts	~0.028 %	2011	[32]
Cell penetrating peptide	Protein of 4 factors (Oct4, Sox2, Klf4, c-Myc)	No	Human fibroblasts	~0.001 %	2009	[33]
		No	Mouse embryonic fibroblasts	–	2009	[34]
Chemical cocktail	BIX01294 and 2 factors (Oct3/4, Klf4)	Yes	Mouse fetal neural progenitor cell	~0.02 %	2008	[35]
	Metabolism modulating compounds (PS48, NaB, VPA) and 1 factor (Oct4)	Yes	Human epidermal keratinocyte	~0.015 %	2010	[36]
	Kenpaullone and 3 factors (Oct4, Sox2, c-Myc)	Yes	Mouse fibroblasts	–	2009	[37]
RNA retrovirus/ electroporation	Mir-302	Yes/no	Human skin cancer cells	–	2008	[38]
Lentivirus	Mir-302/367 and VPA	Yes	Mouse and human fibroblasts	100-folds	2011	[39]
Direct transfection	Mir-200c, Mir-302s and Mir-369 s	No	Mouse and human ASCs	~0.01 %	2011	[40]

avoiding the integration of exogenous genes [29]; (d) translocation system: transcription factors are transferred into cell using piggyback translocation system, the transposons (carrying transcription factor genes) are removed by translocase after the induction of iPSCs through re-programing, then iPSCs without the integration of exogenous genes are successfully prepared [30–32]; (e) recombinant protein: adult cells can be reprogramed by transcription factor genes encoded proteins (reprograming protein) to develop into iPSCs [33, 34]. This approach is much safer than virus or plasmid mediated gene transfer, but the efficiency is greatly reduced; (f) chemical method: several groups have successfully got iPSCs using "cocktail" which involves chemical substances and transcription factors [35–37]; (g) RNA: iPSCs can also be induced using miRNA [38–40]. This approach avoids the transfer of the genes encoding the above-mentioned transcription factors and is an ideal method of preparing iPSCs because it is more efficient and secure. However, the productivity is still low. We expect that with the continued revolution of iPS technology, efficient and safe methods of preparing iPSCs will be available in the near future [41].

Pluripotency is one of the main characteristics of iPSCs, indicating the potency to develop into three germ layers and differentiate into all types of cells. Initial studies have shown that iPSCs are similar to ESCs in many aspects such as morphology, proliferation, expression of ESCs marker genes and teratoma formation [22]. Another feature of iPSCs is that they come from the recipient, thus avoid the ethical and legal issues raised from ESCs in clinical application. In addition,

iPSCs have several advantages such as wide source, large quantity, and being easily obtained.

Neuron differentiation potential of iPSCs

iPSCs can be induced to differentiate into neurons, and these neurons indeed have the corresponding physiological functions. iPSCs have been shown to differentiate into dopaminergic neurons which could improve the Parkinson's symptoms after being transplanted into rat models of Parkinson's disease [42]. Dimos et al. [43] reprogramed the fibroblasts of amyotrophic lateral sclerosis patients into iPSCs, which then differentiated into neural cells after being treated with SHH agonist and RA. Ebert et al. [44] reprogramed the fibroblasts of a child with spinal muscular atrophy into iPSCs and found that neural cells differentiated from these iPSCs exhibited the genotypes and phenotypes of spinal muscular atrophy, suggesting that IPSCs have broad application in disease modeling and drug screening. Karumbayaram et al. [45] induced iPSCs to differentiate into motor neurons and demonstrated their electrophysiological properties for the first time.

IPSCs and SCI repair

Mouse embryonic fibroblasts-induced pluripotent stem cells-secondary neurospheres (MEF-IPSCs-SNSs) were shown to produce functional neurons, astrocytes, and oligodendrocytes in vitro. Furthermore, when these neurospheres were transplanted into the spinal cord 9 day after contusive injury, they differentiated into all three neural lineages without forming teratomas and participated in remyelination and induced the axonal regrowth of host 5HT+ serotonergic fibers, resulting in locomotor function recovery (Fig. 1) [16]. It has been proposed that the therapeutic effects of iPSCs transplantation for SCI are related to the following mechanisms: reconstructing neural synaptic connections by neural cells derived from the transplanted cells, the remyelination of demyelinated axons by oligodendrocytes, and neurotrophic factors secreted by neural cells derived from the transplanted cells [16]. Furthermore, when human-induced pluripotent stem-cell-derived neurospheres (hiPSC-NSs) were transplanted into nonobese diabetic (NOD)-severe combined immunodeficient (SCID) mice, the grafted hiPSC-NSs survived, migrated, and differentiated into the three major neural lineages within the injured spinal cord. In addition, both cell-autonomous and noncell-autonomous effects were observed, including synapse formation between hiPSC-NS-derived neurons and host mouse neurons, the expression of neurotrophic factors, angiogenesis, axonal regrowth, and increased amounts of myelin in the injured area. Consequently, significantly better functional recovery was observed which persisted through the end of the observation period 112 day post-SCI [46]. Taken together, these results provide strong evidence that treating SCI with iPSCs transplantation could improve or even restore the impaired function in mice.

However, to promote the application of iPSCs in the clinic for SCI therapy, several issues should be addressed. First is the efficiency of obtaining iPSCs. iPSCs can be derived using the above-mentioned methods, although the quantity and quality of the derived iPSCs are different. Up to now the efficiency of obtaining iPSCs is low, ranging from 0.001 to 10 % [27, 39]. Given the huge need of iPSCs in clinical practice, the clinical application of iPSCs will undoubtedly be restricted by low efficiency of obtaining them. Second is the selection of source cells for iPSCs. The efficiency and safety of iPSCs derived from different tissues are not exactly the same. Therefore, it is important to select appropriate source cells. Third is the immunological response induced by iPSCs. In early period, it was believed that iPSCs could avoid immune rejection of cell transplantation, but further experiments have shown that iPSCs have immunogenicity [47]. If immune response is induced by transplanted iPSCs, the host's immune system will attack transplanted cells, which may lead to the failure of transplantation. Fourth is the safety of iPSCs. It is known that transplantation of undifferentiated iPSCs may cause the formation of teratoma [48]. Although flow cytometry allows the selection of differentiated iPSCs and the exclusion of undifferentiated iPSCs, thus avoiding the formation of teratomas after transplantation, this method is not feasible in the clinic due to the huge need of iPSCs. In addition, DNA methylation and phenotypic analysis have shown that iPSCs are not exactly the same as ESCs and traditional virus-mediated gene transfer has the risk of exogenous gene integration [49]. All of these account for the safety issues of iPSCs. Fifth is the clinical application of iPSCs in SCI repair. No study has investigated the appropriate quantity of implanted iPSCs to achieve the best therapeutic effects because too little or too much implanted iPSCs cells will cause the failure of cell transplantation therapy. In addition, hyperalgesia symptoms may occur which might be related to functional disorder caused by abnormal conduction pathway formed after stem-cell transplantation [5]. Further studies are necessary to optimize iPSCs transplantation strategy to avoid similar complications.

Perspectives for iPSCs in SCI therapy

ES cells transplantation has been approved by FDA for phase I clinical trials of SCI therapy [50]. Given that

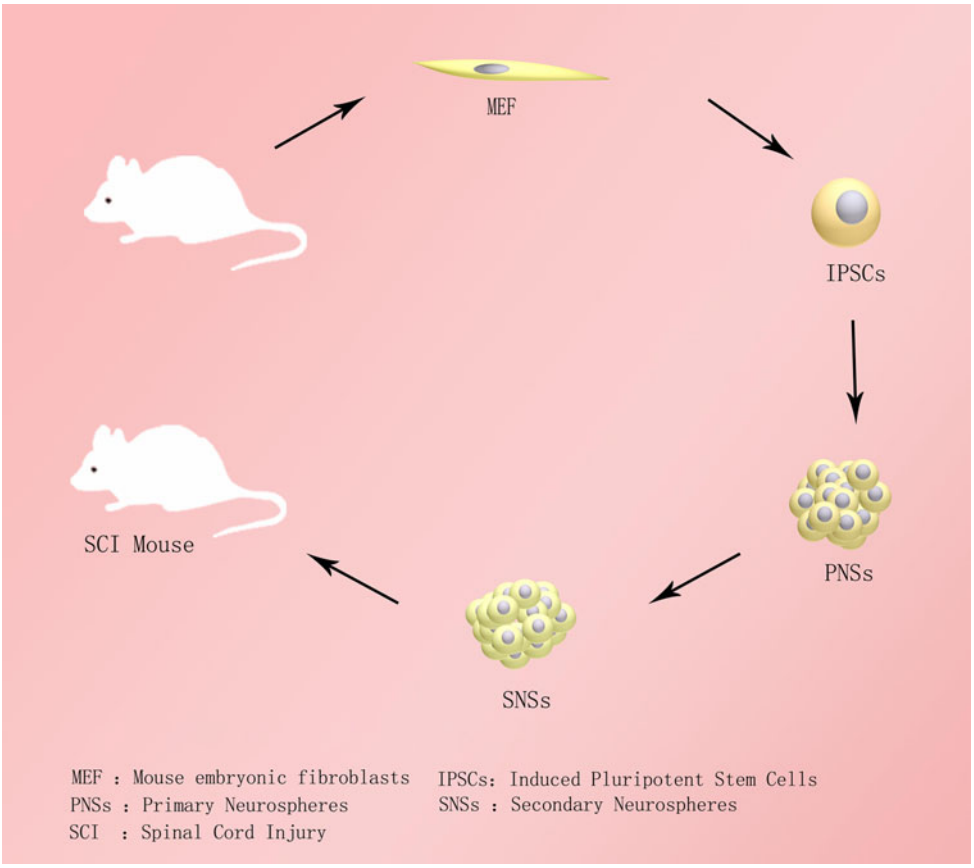

Fig. 1 Schematic presentation of the application of MEF-IPSCs-SNSs in SCI repair in mouse model

iPSCs have many advantages compared to ES cells, we can expect the clinical application of iPSCs in SCI therapy in the near future provided that the pitfalls of iPSCs are addressed. Interestingly, it was reported very recently that the fibroblasts could be directly reprogramed into neural cells and liver cells [51–53], suggesting that the intermediate state of iPSCs could be bypassed (Fig. 2). Does this mean that future IPSCs treatment of SCI will be changed? Somatic cells can be induced directly to trans-differentiate into target cells such as neural cells and glial cells without reversing to IPSCs, thus the oncogenic risk of iPSCs can be avoided in clinical application. Although this method has the problem of low conversion rate, it undoubtedly brings the breakthrough in the application of stem-cell transplantation for SCI therapy. Recently, Son et al. [54] directly converted mouse and human fibroblasts into induced motor neurons by defined factors and these neurons exhibited engraftment capacity and could be differentiated into functional neural subtypes in vivo. This study lays the experimental foundation for further investigations on the transplantation of these converted neurons for SCI treatment. However, it is important to note that stem-cell transplantation has been shown to work relatively less by direct cell replacement, and the induction

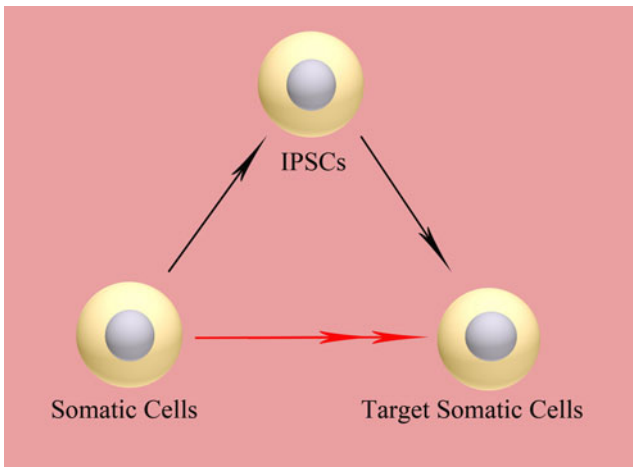

Fig. 2 Schematic presentation of the reprograming of somatic cells directly into target somatic cells

of tissue repair by local progenitors appears more efficient. Although the direct reprograming technology undoubtedly brings a new approach to SCI therapy, further optimization of the strategy for the large-scale preparation of clinical-grade and patient-specific transplantable iPSCs should pave the way for the application of iPSCs to SCI treatment in the clinic.

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Conflict of interest The authors declare no potential conflicts of interest.

References

1. Salewski RP, Eftekharpour E, Fehlings MG (2010) Are induced pluripotent stem cells the future of cell-based regenerative therapies for spinal cord injury? J Cell Physiol 222:515–521
2. Thomas KE, Moon LD (2011) Will stem cell therapies be safe and effective for treating spinal cord injuries? Br Med Bull 98:127–142
3. Illes J, Reimer JC, Kwon BK (2011) Stem cell clinical trials for spinal cord injury: readiness, reluctance, redefinition. Stem Cell Rev 7:997–1005
4. Ronaghi M, Erceg S, Moreno-Manzano V et al (2010) Challenges of stem cell therapy for spinal cord injury: human embryonic stem cells, endogenous neural stem cells, or induced pluripotent stem cells? Stem Cells 28:93–99
5. Rowland JW, Hawryluk GW, Kwon B et al (2008) Current status of acute spinal cord injury pathophysiology and emerging therapies: promise on the horizon. Neurosurg Focus 25:E2
6. Hill CE, Beattie MS, Bresnahan JC (2001) Degeneration and sprouting of identified descending supraspinal axons after contusive spinal cord injury in the rat. Exp Neurol 171:153–169
7. Sahni V, Kessler JA (2010) Stem cell therapies for spinal cord injury. Nat Rev Neurol 6:363–372
8. Fehlings MG, Perrin RG (2006) The timing of surgical intervention in the treatment of spinal cord injury: a systematic review of recent clinical evidence. Spine (Phila Pa 1976) 31:S28–S35 (discussion S36)
9. Furlan JC, Noonan V, Cadotte DW et al (2011) Timing of decompressive surgery of spinal cord after traumatic spinal cord injury: an evidence-based examination of pre-clinical and clinical studies. J Neurotrauma 28:1371–1399
10. Rabchevsky AG, Patel SP, Springer JE (2011) Pharmacological interventions for spinal cord injury: where do we stand? How might we step forward? Pharmacol Ther 132:15–29
11. Kwon BK, Tetzlaff W, Grauer JN et al (2004) Pathophysiology and pharmacologic treatment of acute spinal cord injury. Spine J 4:451–464
12. Robert AA, Zamzami M, Sam AE, Al Jadid M, Al Mubarak S (2011) The efficacy of antioxidants in functional recovery of spinal cord injured rats: an experimental study. Neurol Sci (Epub ahead of print)
13. Li BC, Li Y, Chen LF et al (2011) Olfactory ensheathing cells can reduce the tissue loss but not the cavity formation in contused spinal cord of rats. J Neurol Sci 303:67–74
14. Salazar DL, Uchida N, Hamers FP et al (2010) Human neural stem cells differentiate and promote locomotor recovery in an early chronic spinal cord injury NOD-scid mouse model. PLoS One 5:e12272
15. Erceg S, Ronaghi M, Oria M et al (2010) Transplanted oligodendrocytes and motoneuron progenitors generated from human embryonic stem cells promote locomotor recovery after spinal cord transection. Stem Cells 28:1541–1549
16. Tsuji O, Miura K, Okada Y et al (2010) Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. Proc Natl Acad Sci USA 107:12704–12709
17. Park HW, Lim MJ, Jung H et al (2010) Human mesenchymal stem cell-derived Schwann cell-like cells exhibit neurotrophic effects, via distinct growth factor production, in a model of spinal cord injury. Glia 58:1118–1132
18. Ramon-Cueto A, Munoz-Quiles C (2011) Clinical application of adult olfactory bulb ensheathing glia for nervous system repair. Exp Neurol 229:181–194
19. Xu L, Xu CJ, Lu HZ et al (2010) Long-term fate of allogeneic neural stem cells following transplantation into injured spinal cord. Stem Cell Rev 6:121–136
20. Lee JH, Chung WH, Kang EH et al (2011) Schwann cell-like remyelination following transplantation of human umbilical cord blood (hUCB)-derived mesenchymal stem cells in dogs with acute spinal cord injury. J Neurol Sci 300:86–96
21. Dominici M, Le Blanc K, Mueller I et al (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315–317
22. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676
23. Yu J, Vodyanik MA, Smuga-Otto K et al (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318:1917–1920
24. Kim JB, Zaehres H, Wu GM et al (2008) Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. Nature 454:646–654
25. Stadtfeld M, Nagaya M, Utikal J et al (2008) Induced pluripotent stem cells generated without viral integration. Science 322:945–949
26. Kim JB, Sebastiano V, Wu G et al (2009) Oct4-induced pluripotency in adult neural stem cells. Cell 136:411–419
27. Okita K, Nakagawa M, Hong HJ et al (2008) Generation of mouse induced pluripotent stem cells without viral vectors. Science 322:949–953
28. Liao J, Wu Z, Wang Y et al (2008) Enhanced efficiency of generating induced pluripotent stem (iPS) cells from human somatic cells by a combination of six transcription factors. Cell Res 18:600–603
29. Yu J, Hu K, Smuga-Otto K et al (2009) Human induced pluripotent stem cells free of vector and transgene sequences. Science 324:797–801
30. Woltjen K, Michael IP, Mohseni P et al (2009) piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458:766–770
31. Kaji K, Norrby K, Paca A et al (2009) Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature 458:771–775
32. Nagy K, Sung HK, Zhang P et al (2011) Induced pluripotent stem cell lines derived from equine fibroblasts. Stem Cell Rev 7:693–702
33. Kim D, Kim CH, Moon JI et al (2009) Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 4:472–476
34. Zhou H, Wu S, Joo JY et al (2009) Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 4:381–384
35. Shi Y, Do JT, Desponts C et al (2008) A combined chemical and genetic approach for the generation of induced pluripotent stem cells. Cell Stem Cell 2:525–528
36. Zhu S, Li W, Zhou H et al (2010) Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell Stem Cell 7:651–655
37. Lyssiotis CA, Foreman RK, Staerk J et al (2009) Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4. Proc Natl Acad Sci USA 106:8912–8917

38. Lin SL, Chang DC, Chang-Lin S et al (2008) Mir-302 reprograms human skin cancer cells into a pluripotent ES-cell-like state. RNA 14:2115–2124
39. Anokye-Danso F, Trivedi CM, Juhr D et al (2011) Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 8:376–388
40. Miyoshi N, Ishii H, Nagano H et al (2011) Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem Cell 8:633–638
41. Leeb C, Jurga M, McGuckin C et al (2010) Promising new sources for pluripotent stem cells. Stem Cell Rev 6:15–26
42. Wernig M, Zhao JP, Pruszak J et al (2008) Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci USA 105:5856–5861
43. Dimos JT, Rodolfa KT, Niakan KK et al (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 321:1218–1221
44. Ebert AD, Yu J, Rose FF Jr et al (2009) Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 457:277–280
45. Karumbayaram S, Novitch BG, Patterson M et al (2009) Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. Stem Cells 27:806–811
46. Nori S, Okada Y, Yasuda A et al (2011) Grafted human-induced pluripotent stem-cell-derived neurospheres promote motor functional recovery after spinal cord injury in mice. Proc Natl Acad Sci USA 108:16825–16830
47. Zhao T, Zhang ZN, Rong Z et al (2011) Immunogenicity of induced pluripotent stem cells. Nature 474:212–215
48. Keirstead HS, Nistor G, Bernal G et al (2005) Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J Neurosci 25:4694–4705
49. Dolgin E (2011) Flaw in induced-stem-cell model. Nature 470:13
50. Alper J (2009) Geron gets green light for human trial of ES cell-derived product. Nat Biotechnol 27:213–214
51. Vierbuchen T, Ostermeier A, Pang ZP et al (2010) Direct conversion of fibroblasts to functional neurons by defined factors. Nature 463:1035–1041
52. Pang ZP, Yang N, Vierbuchen T et al (2011) Induction of human neuronal cells by defined transcription factors. Nature 476: 220–223
53. Huang P, He Z, Ji S et al (2011) Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. Nature 475:386–389
54. Son EY, Ichida JK, Wainger BJ et al (2011) Conversion of mouse and human fibroblasts into functional spinal motor neurons. Cell Stem Cell 9:205–218