

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Advances in Stem Cell Therapies

---

Joel Sng and Thomas Lufkin

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55061>

---

## 1. Introduction

Four key milestones have to be realized for the ideal customized stem cell therapy to be successful. First, stem cells utilized in these therapies have to be genetically stable and epigenetically regulated to ensure the safety of stem cells employed in any future therapies. This is essential to ensure that patients undergoing stem cell therapy are not exposed to increased risks of tumorigenesis and other mutagenic diseases. Second, stem cells should be able to evade the innate immune response of patients, possibly via the secretion of immunosuppressive molecules that inhibit immune responses or by displaying host cellular recognition markers. The survival of transplanted stem cells is crucial for the design of an effective therapy. Additionally the ability of transplanted stem cells to evade immune detection and inflammatory responses will prevent undesired symptoms such as graft-versus-host-disease in patients. Third, stem cells employed in these therapies should be location specific. These stem cells should possess specific homing cell surface markers that will allow them to locate and migrate to specific localities. This will ensure that stem cells used in therapies will only accumulate in diseased tissues for targeted therapeutic effect, and not in other healthy regions where detrimental non-specific interactions might occur. Finally, the stem cells used in these therapies should be functionally specific and disease relevant. Transplanted stem cells should be designed to restore a healthy phenotype in patients. These cells should be able to restore organ and tissue function in regenerative therapies, either directly by replicating to replace damaged portions of these organs and tissues and/or indirectly by secreting therapeutic molecules to mediate their functional restoration. These stem cells should also be epigenetically primed for specific functions to ensure that they are able to reverse the effects of treated diseases while minimizing unwanted side effects.

Stem cells are commonly classified into three broad categories based on how they were derived. Embryonic stem cells (ESCs) are stem cells that are isolated from the inner cell mass of the early developing embryo. Adult stem cells assist in the natural regeneration and repair

in developed organisms and can be purified from their tissues. Induced pluripotent stem cells (iPSCs) are artificially derived stem cells that are formed via various genetic and epigenetic reprogramming procedures. Of the three broad categories of stem cells, adult stem cells are most widely utilized in clinical trials and experimental therapies worldwide. Most adult stem cells are multipotent and differentiate to form only a limited subset of cell types. Hence these stem cells are commonly classified according to their developmental commitment or tissue source. Examples of adult stem cells include mesenchymal stem cells (MSCs), neural stem cells (NSCs), hematopoietic stem cells, inner ear stem cells, mammary stem cells, endothelial stem cells, intestinal stem cells, and testicular stem cells.

Adult stem cells present the first success of human experimental stem cell therapy. There are several reasons why adult stem cells therapies are currently more successful than ESC and iPSC therapies. Firstly, stem cell therapies involving adult stem cells are often autotransplants with minimal potential for immune rejection. These adult stem cells can be harvested directly from individual patients before being utilized as transplants. Hence these adult stem cells will exhibit host cell recognition molecules unlike ESCs and iPSCs that may provoke an immune response when used in therapies. Secondly since most adult stem cells therapies involve minimally processed cells, there is a reduced possibility of genetic mutation or chromosome aberration occurring compared to ESCs and iPSCs that have to be cultured extensively in vitro before their use in therapies. Thirdly, adult stem cells do not readily form tumors when introduced into patients and are considered to be safer than ESCs and iPSCs that display greater carcinogenic potential. Finally, the use of adult stem cells in therapies is not considered to be controversial as they can be readily extracted from patient tissues and do not require the destruction of embryos to derive stable cell lines unlike ESCs. These key advantages of adult stem cells have led to their wider utilization in research and various clinical trials compared to ESCs and iPSCs.

## 2. Mesenchymal stem cell therapy

Mesenchymal stem cells (MSCs) are one of the first multipotent adult stem cells to be utilized in stem cell therapies. These stem cells have the ability to differentiate and form bone, cartilage, and adipose tissues. While the bone marrow is the most common source of MSCs for therapeutic purposes, they can also be found in adipose and synovial tissue, skeletal muscles, peripheral blood, breast milk, and the umbilical cord [1, 2].

While these stem cells are commonly referred to as MSCs, they actually form a heterogeneous population of cells as evidenced by differences in proliferative capacity, differentiation potential, cellular markers, and morphology. For example, MSCs derived from the bone marrow (M-MSC) have lower proliferative capacity, followed by adipose tissue MSCs (A-MSC) and umbilical cord blood MSCs (U-MSC) which have the highest proliferative capacity [3]. MSCs also have differing differentiation potentials. For example, bone marrow MSCs have a higher chondrogenic potential while adipose MSCs have a lower chondrogenic potential [4, 5]. In addition both bone marrow and adipose MSCs readily form cells with adipogenic

phenotypes unlike umbilical cord blood MSC that display a lower capacity to form adipocytes [3, 6]. MSCs also express different cellular markers. For example A-MSCs express CD34, CD49d, and CD54 at higher levels than M-MSCs while M-MSCs and U-MSCs express higher levels of CD 106 than A-MSCs [7]. M-MSCs and A-MSCs also have higher levels of CD90 and CD105 expression when compared to U-MSCs [3]. The morphology of MSCs can also differ significantly and even MSCs from the same source display heterogeneous morphologies. Various descriptions of MSCs in the literature include spindle shaped, round, fibroblastoid cells, flattened cells, and blanket cells [8, 9]. Further studies to understand these inherent differences in various subpopulations of MSCs could lead to an improved understanding of how epigenetic differences regulate stem cell differentiation fates, homing to specific recognition sites, proliferation rates, and senescence.

M-MSCs are currently the most widely used stem cells in clinical trials and therapies. Both autograft and allograft M-MSCs have been extensively tested for their therapeutic safety and effectiveness in alleviating the symptoms of several diseases. One of the key reasons for the success of M-MSCs therapy is because these cells possess intrinsic immunomodulatory properties that enable M-MSCs to inhibit and evade potential immune rejection when transplanted [10]. M-MSCs are able to inhibit the maturation and function of various immune cells including dendritic cells, natural killer cells, B cells, and T lymphocytes [11]. Additionally M-MSCs have reduced immunogenicity due to their minimal expression of surface MHC II proteins and the lack of T cell stimulatory proteins like CD80 and CD86 [12]. Another important reason for the early success of M-MSCs based therapies is that MSCs have low tumorigenic potential and are safer than therapies based on ESCs or iPSCs which display robust tumorigenicity [13].

Due to these intrinsic advantages of MSCs, clinical trials can be conducted to evaluate their safety and effectiveness in treating various diseases. For example, the safety and effectiveness of M-MSC transplantation for joint cartilage repair has been evaluated in several studies. In a clinical trial involving 41 patients studied over a period of between 5 to 137 months, M-MSC transplantation did not contribute to increased risk of tumors or infection [14]. Another study has reported the potential for M-MSC regenerative knee therapy to induce cartilage and meniscus growth and increase range of motion [15]. These results are supported in a larger scale M-MSC transplantation study involving 339 patients which reported no increased risk of tumor formation and a significant improvement of knee function in transplant patients [16]. While further clinical trials have to be conducted to verify these preliminary results, the successes of these initial clinical trials indicate that M-MSC therapy is likely to be safe and can catalyze cartilage repair.

The effectiveness of M-MSC and other MSC therapies for various autoimmune diseases has also been studied in several small clinical trials. These autoimmune diseases include multiple sclerosis, Crohn's disease, scleroderma, and systemic lupus erythematosus [17]. The causes of many of these autoimmune diseases are not well understood and it is likely that while the patients suffer from similar symptoms, contributing disease factors may vary significantly between patients. However the application of a generic MSC transplantation therapy was successful in alleviating the symptoms of these patients in several clinical trials.

Multiple sclerosis is a debilitating autoimmune disease caused by immune mediated damage of neural myelin sheath. Progressive neural damage results in many disabling symptoms including the loss of balance, vision and memory. M-MS C clinical trials for multiple sclerosis therapy have provided limited preliminary data indicating that M-MS C transplantation is safe, inhibits the progress of multiple sclerosis through immune regulated neuroprotection, and can repair limited damage to the CNS [18, 19]. For example, in a preliminary phase 2 clinical trial involving 10 patients diagnosed with progressive multiple sclerosis, autologous infusion of externally expanded M-MS Cs was shown to improve visual acuity and increase optic nerve area without any major side-effects [18].

Crohn's disease is a chronic autoimmune bowel disease characterized by inflammation of the gastrointestinal tract. In severe cases, this uncontrolled immune response may result in infection, hemorrhage, and intestinal fistulas. M-MS C clinical trials involving patients suffering from Crohn's disease have sought to harness the innate immunomodulatory capacity of MSCs to mitigate abnormal immune response in these patients and determine the safety of any potential therapies. In two phase one clinical trials a total of 22 adult Crohn's disease patients were enrolled to investigate the effects of M-MS C therapy. In the first trial it was determined that while autologous M-MS C infusion therapy did not result in adverse side effects, it only had a modest impact in alleviating the autoimmune response in these patients [20]. In the second trial in vitro expanded M-MS Cs were directly injected into the intestinal wall and lumen [21]. When M-MS Cs were directly injected, they were able to inhibit inflammation locally and mediate healing of intestinal tissue in these regions.

Scleroderma is an autoimmune connective tissue disorder characterized by accumulation of collagen in the skin, heart, kidneys or lungs. This buildup of collagen may lead to skin ulcers, pulmonary fibrosis, heart and kidney failure. Exploratory M-MS C clinical trials involving patients suffering from Scleroderma have sought to harness the regenerative and immunomodulatory capacity of MSCs to initiate ulcer healing and prevent organ failure while evaluating the safety of these therapies. In two separate phase one clinical trials a total of 7 adult scleroderma patients were enrolled to determine the effects of M-MS C therapy. In the first trial allogeneic transplantation of donor M-MS C was performed via intravenous infusion and was associated with possible pericardial calcification and increased risk of cardiac impairment. While patients in this trial displayed a slight improvement in MRSS score and healing of skin ulcers, the effects were on occasion only temporary and the disease regressed in some patients [22]. The second trial involved autologous transplantation of either M-MS C or peripheral stem cells in patients via intramuscular injection [23]. This local stem cell therapy was able to induce healing of skin ulcers in these patients and improved endothelial function of blood vessels.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that can affect the kidney, lung, brain, and other organs. Severe SLE may result in kidney failure, stroke, and inflammation of blood vessels. M-MS C clinical trials in SLE patients have attempted to treat progression of SLE symptoms by harnessing the immunomodulatory properties of MSCs. In two clinical trials a total of 19 patients suffering from SLE were treated with M-MS C transplants to determine if MSC therapy is safe and effective in reversing the symptoms of SLE patients.



In the first clinical trial SLE patients were treated with allogeneic M-MSC infusion [24]. Treatment with donor M-MSCs was shown to restore kidney function and reverse the progression of SLE. The second clinical trial involved a larger group of patients and provided additional evidence that M-MSC therapy could mitigate the symptoms of SLE in patients [25].

Other studies have also attempted to verify the effectiveness of M-MSC therapies for various diseases. M-MSC therapy has been shown to improve liver function in patients suffering from liver cirrhosis by encouraging hepatocyte proliferation [26]. The co-transplantation of M-MSCs and kidney transplants for patients with kidney failure can reduce the risk of acute transplant rejection and improve transplant function in treated patients [27]. M-MSC therapy can also catalyze functional recovery and improve survival rates in ischemic stroke patients [28, 29]. These clinical studies provide preliminary evidence that M-MSC therapy is safe and the regenerative properties of these stem cells can be harnessed to treat a wide variety of diseases.

To develop the ideal next generation stem cell therapy, it is necessary to evaluate currently available therapies to identify their current limitations and suggest areas for improvement. Next generation stem cell therapies will have to fulfill the four key milestones (safety, immune evasion, location specificity, and disease relevancy) of customized stem cell therapy. Human M-MSC therapy has been extensively studied in multiple experiments and clinical trials and is an ideal candidate for evaluation against these key milestones.

Firstly the safety of M-MSC therapies must be considered. Multiple clinical trials mentioned previously involving the infusion and injection of both autologous and allogeneic M-MSCs for therapeutic purposes stated that patients were generally not exposed to increased risks of cancer or other serious side-effects. However, a study stated that the infusion of M-MSC may lead to pericardial calcification and increased risk of cardiac impairment in some patients [22]. As currently completed clinical trials often only involve a relatively small patient population or are only conducted over a brief period of time, the risks of M-MSC therapies may not be fully understood and more studies have to be conducted to ensure that the benefits of these therapies outweigh their potential risks. Another source of concern is the use of in vitro cultured M-MSCs in therapies. M-MSCs exist naturally in low concentrations in the human bone marrow, and often have to be concentrated and expanded in vitro media to provide sufficient numbers of stem cells for therapeutic purposes. This process may expose M-MSCs to xenogeneic antigens such as in fetal calf serum in the media. Culture of M-MSCs in vitro also exposes cells to an atmospheric oxygen concentration of 21% that is radically different from physiological conditions of 1-7% [30]. These in vitro culture conditions may affect the genetic and epigenetic stability of these stem cells resulting in an increased chance of mutagenesis. In an effort to resolve these potential issues, several studies have attempted to identify the ideal M-MSC culture media. From these studies, it has been proposed that human platelet lysate can be used as a viable substitute to fetal calf serum to reduce unnecessary exposure to xenogeneic antigens [31]. M-MSCs should also be cultured in low oxygen concentrations of approximately 3% to reduce oxidative stress and telomere shortening and increase the proliferative lifespan and genetic stability of in vitro M-MSCs [32]. The implementation of these protocols will provide M-MSCs with culture conditions that are more similar to the M-MSC native environment and minimize the impact of in vitro expansion on the genetic and

epigenetic stability of M-MSCs. In conclusion, while some doubts about the safety of M-MSC based therapies remain, various clinical trials and experiments have indicated that the use of minimally expanded M-MSCs is relatively safe for patients, especially when coupled with the latest M-MSC expansion protocols.

Secondly the ability of M-MSCs to evade immune detection must be accessed. No incidents of acute immune rejection were reported in the various clinical trials involving autologous and allogeneic transplants of M-MSCs. This could possibly be attributed to the fact that autologous M-MSCs are extracted from the treated patients and present host cellular recognition markers. Additionally M-MSCs have reduced immunogenicity due to the naturally low expression levels of surface MHC II proteins and the lack of other T cell stimulatory proteins like CD80 and CD 86 in M-MSCs. The multifaceted immunomodulatory capacity of M-MSCs must also be considered. Various studies have indicated that M-MSCs are able to inhibit the proliferation of T lymphocytes possibly via the activation of regulatory T cells and secretion of immunosuppressive factors like transforming growth factor beta1 and hepatocyte growth factor [33-35]. The inhibition of T lymphocytes that are essential for the recognition and destruction of foreign transplants contributes to the ability of M-MSCs to evade immune detection. M-MSCs can also interfere with the development and function of antigen-presenting dendritic cells. Soluble factors secreted by M-MSCs can inhibit differentiation of monocytes to dendritic cells and suppress the production of cytokines [36]. M-MSCs can also affect the function of mature dendritic cells by suppressing the expression of various presentation and co-stimulatory molecules like CD1a, CD80, CD83, and CD86 [37]. This impedes dendritic cells from inducing T cells and B cells and prevents resistance of foreign transplants from developing. Finally M-MSCs can also inhibit the proliferation of B cells stimulated with anti-CD40 monoclonal antibody and IL-4 by halting the G<sub>0</sub>/G<sub>1</sub> cell cycle phase [38, 39]. The data from these studies indicate that M-MSCs are able to efficiently evade the innate immune response of patients via various mechanisms of cellular recognition and immunosuppression.

Thirdly the location specificity of M-MSCs employed in various therapies should be considered. While M-MSC clinical trials discussed previously indicate that M-MSC therapy is able to alleviate the conditions of various autoimmune diseases and induce cartilage repair, infusion of M-MSCs resulted in non-specific distribution of these cells within the patient. Non-specific infusion of M-MSCs resulted in a distribution of these cells in various organs including the heart muscle, liver, kidney, skin, and lung. This may result in undesirable side-effects such as pericardial calcification and increased risk of cardiac impairment in patients as described in a clinical study [22]. Hence further research is required to design a stem cell therapy that is more specific to the injury location. In the ideal therapy, stem cells could be engineered with receptors for mobilization to the location of injury. Alternatively, stem cells could be integrated within a scaffold that would then be implanted into patients to improve the specificity of these therapies. The direct injection of M-MSCs near sites of injury may also provide increased specificity to these therapies.

The fourth consideration is stem cells utilized in these therapies should be disease relevant. Disease relevant stem cells should be epigenetically primed to treat specific underlying causes of disease in each patient. M-MSCs utilized in these clinical trials are not disease relevant and

cure or alleviate various disease symptoms through their general immunomodulatory and regenerative properties. While non-specific M-MSCs may still be a viable therapy for a wide range of diseases, the lack of specificity in these therapies may result in potentially lethal consequences. For example, the general immunosuppressive properties of M-MSCs can increase the severity of breast cancer by increasing the concentration of regulatory T cells and inhibiting the innate immune response against cancer cells [35, 40]. M-MSCs could also secrete soluble factors that accelerate tumor growth, such as through the activation of the phosphatidylinositol-3-kinase/Akt signaling pathway which can prevent apoptosis and induce proliferation of cancer cells [41]. Hence additional studies have to be performed to understand how stem cells can be epigenetically reprogrammed to enhance their specificity for disease treatment and reduce undesirable side effects.

In conclusion, it can be seen that while current clinical data demonstrates that M-MSC based therapies are relatively safe and M-MSC transplants can evade immune detection and survive in patients, these therapies rely on the general immunosuppressive and regenerative properties of M-MSCs and are neither specific nor disease relevant. Hence although the utilization of M-MSC based therapies may potentially result in cures for various diseases, more research is necessary for developing the ideal stem cell therapy.

### 3. Other MSC and adult stem cell therapies

While the bone marrow is the most commonly mentioned source of MSCs, MSCs can also be extracted from other sources including adipose and synovial tissue, skeletal muscles, peripheral blood, breast milk, and the umbilical cord [1, 2]. In particular, adipose MSCs (A-MSCs) have been increasingly studied because these cells can be readily purified from adipose tissue via liposuction and is a relatively non-invasive procedure compared to bone marrow extraction of M-MSCs [42, 43]. A-MSCs have similar immunomodulatory effects compared to M-MSCs and can be utilized for treatment of similar diseases such as scleroderma [44, 45]. A-MSCs also possess a similar capacity to regenerate cartilage and bone tissues and mediate some symptoms in patients with osteonecrosis and osteoarthritis [46]. Hence the discovery of A-MSCs provides patients with an alternative source of MSCs in the event that they are unable to undergo M-MSC extraction.

MSCs are also present in human and animal synovial fluid. These synovial MSCs (S-MSCs) have a greater ability to proliferate and differentiate compared to other MSCs and can form osteoblasts, adipocytes, chondrocytes, and neurons [47, 48]. S-MSCs also possess greater cartilage regenerative potential than other MSCs with 60% of S-MSCs placed on cartilage defects attaching to the defect within 10 minutes [49]. S-MSCs possess similar regenerative potential as M-MSCs and can also initiate regeneration of the nucleus pulposus in the damaged rabbit intervertebral disc by suppressing inflammation and inducing the synthesis of type II collagen which acts as a supportive framework for nucleus pulposus repair [50]. In addition S-MSCs can be readily harvested via punch biopsy [9]. The greater innate proliferative ability of S-MSCs and the relative ease of obtaining S-MSCs indicate that it may be an excellent source of MSCs for future regenerative therapies



Neural Stem Cells (NSCs) have also been studied in an attempt to harness their regenerative potential for therapeutic purposes. NSCs can be found in various tissues including the bone marrow and striatum [51, 52] and their regenerative properties have been assessed by both NSC transplantation and endogenous NSC functional studies. NSCs can initiate axon remyelination, neuroprotection, proliferation of oligodendrocyte progenitors, and functional recovery when transplanted into mice experimental autoimmune encephalomyelitis (EAE) models of multiple sclerosis [53]. NSCs also possess similar immunomodulatory properties as MSCs. For example, NSCs can inhibit dendritic cell and antigen-specific T cell maturation through the release of morphogens such as bone morphogenetic protein 4 [54]. Additionally, NSCs can suppress T-cell proliferation through the release of prostaglandin E2 and nitric oxide [55]. This innate immunomodulatory property of NSCs has been harnessed to induce stable pancreatic islet graft function in mice, without the need for long-term immunosuppression [56]. The immunosuppressive potential of NSC can also be enhanced by engineering NSCs to produce anti-inflammatory cytokines such as IL-10 [57]. Engineered NSC transplants have greater therapeutic potential than ordinary NSCs and give rise to enhanced functional recovery of EAE mice.

Functional studies of endogenous NSCs have revealed the complex regulatory pathways governing *in vivo* neuronal regeneration. While neural stem cell niches exist in the subventricular zone and the subgranular zone of the hippocampal dentate gyrus [58], NSCs in these niches are unable to initiate spontaneous neural regeneration in many diseases. Hence recent research has been concerned with elucidating the regulators of neurogenesis and repair. For example neurogenesis can be initiated by suppressing *Olig2* resulting in increased neurogenesis for brain injury repair [59]. Other molecular regulators of neurogenesis include morphogens like *Shh* and *Wnt*, transcription factors like *Sox2*, growth factors like Fibroblast Growth Factor family, and cell surface molecules like *Notch1* [60]. An improved understanding of the molecular pathways that regulate the differentiation, mobilization, and proliferation of endogenous NSCs and the development of molecular tools to manipulate these pathways may lead to the development of novel minimally invasive regenerative therapies.

Other adult stem cells that have been evaluated for therapeutic use include hematopoietic stem cells, inner ear stem cells, mammary stem cells, intestinal stem cells, and adult germline stem cells. Hematopoietic stem cells are multipotent and can form various blood cells such as those from the lymphoid and myeloid lineages. Allogeneic hematopoietic stem cell transplantation (HSCT) therapy in leukemia patients can lead to remission by inducing an immune antitumor response [61]. HSCT has also been utilized to cure other diseases including sickle cell anemia, acquired aplastic anemia and thalassemia [62, 63]. HSCT can also halt neurological deterioration in X-linked adrenoleukodystrophy patients [64]. HSCT is also useful for alleviating symptoms of Hurler Syndrome and other lysosomal storage diseases and these grafts can replace metabolic enzymes that are lacking in host cells.

Inner ear stem cells are important progenitors of auditory hair cells and exist endogenously in both the utricular sensory epithelium and the dorsal epithelium of the cochlear canal [65, 66]. An improved understanding of molecular regulatory pathways in these stem cells could lead to the development of regenerative therapies for treating hearing impairment. Ongoing

studies have revealed that the over-expression of *SKP2* can induce proliferation of non-sensory cells that can differentiate to form hair cells through the co-expression of *Atoh1* [67]. Developmental studies have also provided insight into the *Notch* signaling pathway, and its influence on the lateral-inhibition mediated differentiation of hair cells [68, 69]. Further studies could lead to the development of a viable stem cell therapy for regenerating auditory hair cells and a cure for hearing impairment.

Mammary stem cells are indispensable in the formation of mammary glands and can possess the capacity to form myoepithelial cells, alveolar epithelial cells, and ductal epithelial cells [70, 71]. The deregulation of various signaling pathways including the Notch, Wnt, and Hedgehog pathways in mammary stem cells has been implicated in breast cancer development [71, 72]. These studies could lead to the development of anti-cancer drugs that target specific signaling pathways.

Intestinal stem cells are multipotent progenitors of the intestinal epithelial cell lineages. Studies of intestinal stem cells have revealed the role of the Notch and Wnt signaling pathways in intestinal stem cell maintenance, differentiation, and proliferation and how deregulation of these pathways can promote intestinal carcinogenesis [73, 74]. An impaired differential capacity of intestinal stem cells has also been linked to inflammatory bowel diseases like Crohn's disease and ulcerative colitis [75]. Future studies based on these discoveries could lead to more effective cures for these diseases.

Adult germline stem cells are essential for gamete generation and can be derived from testis spermatogonial cells. These stem cells are pluripotent and share characteristics similar to ESCs [76]. The pluripotent nature of these stem cells may allow the development of regenerative therapies not possible with other multipotent adult stem cells. Adult germline stem cell transplantation can also be utilized for fertility restoration in animals [77-79]. This regenerative ability could be utilized for maintaining the fertility of patients undergoing radiotherapy, chemotherapy and other therapies that may cause infertility.

A comparison to determine how epigenetic differences inherent to these different classes of adult stem cells lead to a wide variation in differentiation, homing, proliferation, and immunomodulation capacities will enable the design of novel stem cell therapies for specific diseases. The differentiation potentials of adult stem cells can vary widely, for instance hematopoietic stem cells tend to form cells from the lymphoid and myeloid lineages, while neural stem cells tend to form neural cells like neurons, oligodendrocytes and astrocytes. Differences in differential predisposition also exist within a similar class of stem cells. A-MSCs tend to form adipocytes and cardiomyocytes, while M-MSCs form chondrocytes more readily [4, 5]. Further studies to map epigenetic differences between these stem cell populations will reveal how differentiation is regulated. This will lead to an improved ability to prime and select optimal stem cell transplants for disease therapy. For example, a better understanding of underlying pro-chondrogenic factors will enable the engineering of stem cells specialized in cartilage regeneration. Detailed studies of other intra-population epigenetic variations will also lead to better understanding of how these differences lead to differences in other properties of stem cells and augment the safety, effectiveness, and specificity of stem cell therapies. In conclusion, it can be seen that while the safety of adult stem cell therapies remains a key

concern especially in the less studied stem cells, the innate immunomodulatory and regenerative capacity of adult stem cells can be exploited for curing a wide range of diseases.

#### 4. Induced pluripotent stem cells

The discovery of iPSCs has led to a revolution in stem cell research. The ability to reprogram adult somatic cells to iPSCs using an increasing array of novel vectors and strategies has opened up a myriad of possibilities for therapeutic stem cell development. iPSC based therapies possess several advantages over adult stem cell and ESC based therapies. First, since iPSCs can be derived from patients like adult stem cells, they will exhibit host cellular recognition markers and can evade immune rejection more readily than ESCs. Additionally since iPSC lines can be derived from patients, they do not face the ethical concerns associated with ESC derivation. The use of iPSCs is also advantageous because iPSCs can be modified to produce desired cell phenotypes that may not be naturally available in adult stem cell and ESC populations. Hence iPSCs can be customized for treating specific diseases unlike other stem cells whose curative properties tend to be more general.

However currently available iPSCs face several limitations that prevent their use in patient therapies. First the iPSC derivation process commonly involves the use of viral vectors such as lentiviral and retroviral vectors which results in the integration of viral DNA in iPSCs [80, 81]. Second, many iPSC derivation processes involve the over-expression or integration of proto-oncogenes such as *Oct4*, *c-Myc*, and *Sox2* [82-86]. Third, iPSC cultures are genetically unstable and contain numerous genetic abnormalities including protein coding mutations, copy number variations, and chromosomal aberrations [87-89]. Fourth, the iPSC reprogramming process may be incomplete and iPSCs can retain epigenetic memory from parental somatic cells [90, 91]. Finally, the transformation efficiency of adult somatic cells to iPSCs is inefficient (0.001% - 4.4%) and only a small fraction of adult somatic cells can be transformed to iPSCs via existing methods.

These current shortcomings hinder the development of iPSCs suitable for patient therapy. The use of viral reprogramming vectors, over-expression of proto-oncogenes, and sub-optimal culture conditions contribute to widespread genetic mutation and increased tumorigenic potential in iPSCs. Consequently, iPSCs can readily form tumors in immune deficient mice and mice derived from iPSCs have a high chance of developing tumors [82, 92]. To overcome these limitations, new methods for iPSC reprogramming were developed to enhance the genetic integrity of iPSCs. Advances in reprogramming enabled the generation of iPSCs without *c-Myc* and mice without tumors could be derived from these iPSCs [93]. Additionally, non-integrating viral vectors like adenoviruses were used to prevent the introduction of foreign viral DNA into iPSCs [94]. More recently iPSCs have been generated via transfection of modified mRNA, this DNA free method results in a higher efficiency of iPSC generation compared to previous methods and does not introduce any exogenous DNA into reprogrammed cells [95]. Optimized iPSC culture and reprogramming conditions will also be essential for maintaining genetic stability and increasing transformation

efficiency. High atmospheric oxygen concentration (~21%) exposes stem cells to increased oxidative stress and DNA damage [32]. Lowering the oxygen concentration to 5% can improve iPSC generation efficiency and genetic stability of stem cells [96]. The addition of vitamin C and other antioxidants can also improve the efficiency of iPSC generation by preventing the accumulation of reactive oxygen species and promoting epigenetic modifications required for reprogramming to occur [97]. Reprogramming and iPSC culture maintenance also requires precise manipulation of other medium conditions. iPSC progenitors have to be cultivated in conditions that facilitate their survival but these original conditions may have to be modified to enhance reprogramming efficiency and maintain iPSC populations [98, 99]. The search for improving the efficiency of iPSC generation has also led to the use of miRNA sequences in reprogramming. Viral aided *miR302/367* cell reprogramming can reprogram fibroblasts to iPSCs with up to 10% efficiency [100].

The reprogramming of adult somatic cells to ideal iPSCs will involve a complex epigenomic transformation of the cellular epigenome to resemble ESC epigenetics. However iPSCs derived with current procedures retain unique epigenetic signatures that differ from the ESC epigenome. Some common epigenetic differences include variations in DNA methylation at CpG islands and histone modifications [101, 102]. The epigenetic memory of iPSCs is an artifact from the reprogramming process and parental cell epigenetics and can affect the differentiation predisposition of iPSCs [90]. This iPSC epigenetic signature can also be transmitted to successive generations of iPSCs and their differentiated progeny [102]. A failure to reset the epigenetic memory of iPSCs to more closely resemble the epigenetic ground state of ESCs could affect the function and safety of differentiated cells derived from these iPSC lines.

The issue of residual iPSC epigenetic memory can be partially addressed. For example, somatic cell nuclear transfer has been proposed as a viable method for resetting epigenetic memory [91]. Sodium butyrate, a short-chain fatty acid, could also assist in programming iPSCs closer to the epigenetic ground state by inhibiting histone deacetylase, directing the acetylation of specific genes, and encouraging stem cell renewal [103, 104]. DNA methyltransferase inhibitors can also be used to direct DNA methylation at specific sites for more complete epigenetic reprogramming [105]. The iPSC culture environment can also be manipulated to achieve a desired epigenetic state. For example, reducing culture oxygen concentration to 2% can induce epigenetic modifications that increase the expression of the retinal genes *Six3* and *Lhx2* in iPSCs while an oxygen concentration of 5% can increase the efficiency of iPSC regeneration [96, 106]. However despite the increased availability of tools for epigenetic modification, more studies are required to determine the ideal epigenomic ground state for therapeutic stem cells. Comprehensive epigenetic mapping of ESCs and adult stem cells could provide important clues and enable the development of improved experimental procedures for reprogramming adult somatic cells to mirror this ideal epigenomic ground state.

When evaluated against the four key milestones, iPSCs are clearly inferior to M-MSCs and other adult stem cells currently being evaluated in experimental therapies. First, iPSCs are neither genetically nor epigenetically stable, this inherent property of iPSCs, along with the integration of proto-oncogenes as a byproduct of some iPSC reprogramming procedures results in increased propensity for tumorigenesis in vivo. Hence the safety of iPSC based



therapies remains a key concern and must be resolved before they can be tested in clinical therapies. Second, while iPSCs can be derived from patients and should be able to evade patient immune response, abnormal expression of genes in iPSCs and their differentiated progeny has been shown to induce immune responses in recipients [107]. A possible consequence of the genetic and epigenetic instability, the inability of iPSCs to evade the innate immune response of patients could lead to the rejection of iPSC grafts. Similarly, genomic and epigenetic instability of iPSCs will frustrate efforts for developing iPSCs with specific function and homing abilities. Hence more research is required before iPSCs suitable for use in patient therapies will be available.

While current limitations of iPSC technology forestalls their direct use in patient therapy, the versatility of iPSCs and their ease of derivation from patients has enabled their use in disease modeling and in vitro drug screening. iPSCs can be derived from patients affected by various diseases including LEOPARD syndrome, Schizophrenia, and X-linked adrenoleukodystrophy and used in drug and functional tests [108, 109]. This has enabled the molecular pathways and genetic mutations that cause these diseases to be studied in greater detail and led to the development of new therapies for patients. Hence the discovery of iPSCs continues to contribute to an improved knowledge of the underlying molecular mechanisms of various diseases and catalyze the development of novel drugs for their treatment.

## 5. Embryonic stem cells

The first breakthrough technique for isolating and growing human ESCs *in vitro* was developed at the University of Wisconsin-Madison in 1998 [110]. Since then, interest in developing more efficient methods for deriving ESCs and research into potential therapies involving ESCs has increased exponentially. ESC based therapies possess several natural advantages over other stem cell therapies. Since ESCs are directly derived from the developing embryo, they possess greater innate pluripotent capacity compared to most adult stem cells and could potentially be used in a wider range of therapies. Additionally, while the pluripotent potential and number of autogenic adult stem cells available may decline as patients age, ESC based therapies do not share the same limitation and a potentially limitless source of stem cells can be derived and cultured from blastocysts. Finally ESCs occur naturally in the inner cell mass of blastocysts and can be easily derived with minimal genetic or epigenetic manipulation unlike iPSCs.

The effectiveness and safety of ESCs therapies for treating various medical conditions including spinal cord injury, Stargardt's disease, and macular degeneration have been tested in animal and human clinical trials [111-115]. These studies demonstrate that it is relatively easy to obtain high quality and pathogen free human ESC cells, stimulate hESCs to form pure populations of differentiated cells for transplantation, and obtain sufficient quantities of cells for transplantation. Some studies of human ESC based transplants have also demonstrated that ESC is potentially safe and can be conducted with minimal risk of teratoma formation and graft rejection. The results of these animal and human ESC based clinical trials also indicate



that human ESC transplantation can rescue animal models of retinal degeneration, Stargardt, and spinal cord injury, and catalyze limited visual improvement in human macular degeneration patients.

Despite these apparent advantages of human ESCs, its use in research and medical therapy has been fairly controversial historically as the derivation of human ESC lines requires the destruction of human embryos. Pro-life advocates have strongly opposed the destruction of embryos for research on the basis that human life begins when a human egg cell is fertilized, and the belief that human life is inviolable. More recently, these objections have been partially overcome through the development of human ESC derivation procedures that do not require embryo destruction [116] and the use of surplus frozen embryos from in vitro fertilization clinics.

When evaluated against the four key milestones, it can be determined that while ESCs are potentially safer than iPSCs, several key concerns continue to forestall their wider use in human clinical trials. First while some studies of human ESC based transplants in animals and humans have suggested that there is minimal risk of teratoma formation or uncontrolled proliferation of transplanted cells, other studies contend that *in vitro* culture conditions can result in potentially hazardous epigenetic modifications [117]. Second since ESC transplants are allogeneic, there is a higher likelihood of immune rejection compared to autographs of adult stem cells. Third ESC transplants potentially share similar location specificity limitations as adult stem cell transplants. This limitation has to be addressed for the development of a viable next generation stem cell therapy. Fourth since ESCs have a higher innate pluripotent capacity than adult stem cells and iPSCs, it may be easier to obtain pure populations of functionally specific and disease relevant transplant cells from ESC lines. In conclusion, while more clinical trials will be required to assess the viability of ESC therapy, studies have indicated that ESC therapy seems to offer a promising alternative for treating currently incurable diseases.

## 6. The promise of transdifferentiation therapy

Transdifferentiation is the direct conversion of one cell type to another without the involvement of an intermediate pluripotent state. Transdifferentiation could be a viable alternative therapy to stem cell therapies and relatively abundant adult somatic cells like fibroblasts and adipocytes could be harvested from patients and directly converted by transdifferentiation to neurons or cardiomyocytes before being used as autologous grafts in regenerative therapies. Transdifferentiation is advantageous compared to adult stem cell therapy because cell grafts could be designed specifically for each disease therapy resulting in improved functional and positional specificity. Transdifferentiation is also advantageous compared to iPSC based therapy because conversion to a desired cell type is a one step process requiring lesser epigenetic modification, and is a more rapid and direct process than dedifferentiation to form iPSCs followed by controlled differentiation into the desired cell type.

Transdifferentiation can be a relatively spontaneous process such as the *in vitro* transdifferentiation of chick retinal cells to lens cells [118]. It can also be induced via the guided expression of various molecular factors and genes. For example overexpression of *Atoh1* can induce the transdifferentiation of non-sensory supporting cells in the cochlea to auditory hair cells [67]. The expression of the microRNAs *miR-9/9\** and *miR-124* can also induce the transdifferentiation of human fibroblasts to functional neurons [119]. However despite initial successes, currently available methods for inducing transdifferentiation remain too inefficient *in vivo* therapeutic purposes and further research is required to improve the process before it can be considered as a viable therapeutic alternative.

## 7. Summary

The use of stem cells for therapeutic purposes will be increasingly widespread as improved knowledge leads to the development of safer and more effective therapies. Stem cells derived from patients have also been successfully used in disease modeling and therapy evaluation. Further studies will enable the innate regenerative and immunomodulatory properties of stem cells to be harnessed more effectively for treating a larger variety of diseases and injury. The study and use of adult stem cells will continue to play a pivotal role in the ongoing search for novel therapies due to their availability and safety, while further developments in iPSC and ESC derivation and cultivation processes will be required before they can be used in therapies. An improved understanding of genetic and epigenetic control continues to be a prerequisite for the development of an ideal stem cell therapy. Finally further improvements in inducing direct transdifferentiation of adult non-stem cells to the desired cell types may be an alternative regenerative therapy that may circumvent the use of stem cells entirely.

## Author details

Joel Sng and Thomas Lufkin\*

\*Address all correspondence to: [lufkin@gis.a-star.edu.sg](mailto:lufkin@gis.a-star.edu.sg)

Stem Cell and Developmental Biology Genome Institute of Singapore, Singapore

## References

- [1] Zhang, Y, et al. Mechanisms underlying the osteo- and adipo-differentiation of human mesenchymal stem cells. *ScientificWorldJournal*, (2012). , 793823.
- [2] Patki, S, et al. Human breast milk is a rich source of multipotent mesenchymal stem cells. *Hum Cell*, (2010). , 35-40.

- [3] Kern, S, et al. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells*, (2006). , 1294-1301.
- [4] Yang, J, et al. Differentiation potential of human mesenchymal stem cells derived from adipose tissue and bone marrow to sinus node-like cells. *Mol Med Report*, (2012). , 108-113.
- [5] Huang, J. I, et al. Chondrogenic potential of progenitor cells derived from human bone marrow and adipose tissue: a patient-matched comparison. *J Orthop Res*, (2005). , 1383-1389.
- [6] Chang, Y. J, et al. Disparate mesenchyme-lineage tendencies in mesenchymal stem cells from human bone marrow and umbilical cord blood. *Stem Cells*, (2006). , 679-685.
- [7] De Ugarte, D. A, et al. Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. *Immunol Lett*, (2003). , 267-270.
- [8] Pevsner-fischer, M, Levin, S, & Zipori, D. The origins of mesenchymal stromal cell heterogeneity. *Stem Cell Rev*, (2011). , 560-568.
- [9] Sakaguchi, Y, et al. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum*, (2005). , 2521-2529.
- [10] Nauta, A. J, & Fibbe, W. E. Immunomodulatory properties of mesenchymal stromal cells. *Blood*, (2007). , 3499-3506.
- [11] Zhao, S, et al. Immunomodulatory properties of mesenchymal stromal cells and their therapeutic consequences for immune-mediated disorders. *Stem Cells Dev*, (2010). , 607-614.
- [12] Barry, F. P, et al. Immunogenicity of adult mesenchymal stem cells: lessons from the fetal allograft. *Stem Cells Dev*, (2005). , 252-265.
- [13] Knoepfler, P. S. Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. *Stem Cells*, (2009). , 1050-1056.
- [14] Wakitani, S, et al. Safety of autologous bone marrow-derived mesenchymal stem cell transplantation for cartilage repair in 41 patients with 45 joints followed for up to 11 years and 5 months. *J Tissue Eng Regen Med*, (2011). , 146-150.
- [15] Centeno, C. J, et al. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. *Pain Physician*, (2008). , 343-353.
- [16] Centeno, C. J, et al. Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. *Curr Stem Cell Res Ther*, (2011). , 368-378.

- [17] Tyndall, A. Successes and failures of stem cell transplantation in autoimmune diseases. *Hematology Am Soc Hematol Educ Program*, (2011). , 280-284.
- [18] Connick, P, et al. Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of-concept study. *Lancet Neurol*, (2012). , 150-156.
- [19] Freedman, M. S, et al. The therapeutic potential of mesenchymal stem cell transplantation as a treatment for multiple sclerosis: consensus report of the International MSCT Study Group. *Mult Scler*, (2010). , 503-510.
- [20] Duijvestein, M, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. *Gut*, (2010). , 1662-1669.
- [21] Ciccocioppo, R, et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut*, (2011). , 788-798.
- [22] Keyszer, G, et al. Treatment of severe progressive systemic sclerosis with transplantation of mesenchymal stromal cells from allogeneic related donors: report of five cases. *Arthritis Rheum*, (2011). , 2540-2542.
- [23] Nevskaya, T, et al. Autologous progenitor cell implantation as a novel therapeutic intervention for ischaemic digits in systemic sclerosis. *Rheumatology (Oxford)*, (2009). , 61-64.
- [24] Sun, L, et al. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. *Stem Cells*, (2009). , 1421-1432.
- [25] Liang, J, et al. Allogenic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: a pilot clinical study. *Ann Rheum Dis*, (2010). , 1423-1429.
- [26] Terai, S, et al. Timeline for development of autologous bone marrow infusion (ABMi) therapy and perspective for future stem cell therapy. *J Gastroenterol*, (2012).
- [27] Tan, J, et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. *JAMA*, (2012). , 1169-1177.
- [28] Lee, J. S, et al. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. *Stem Cells*, (2010). , 1099-1106.
- [29] Bang, O. Y, et al. Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol*, (2005). , 874-882.
- [30] Fehrer, C, et al. Reduced oxygen tension attenuates differentiation capacity of human mesenchymal stem cells and prolongs their lifespan. *Aging Cell*, (2007). , 745-757.

- [31] Perez-ilzarbe, M, et al. Comparison of ex vivo expansion culture conditions of mesenchymal stem cells for human cell therapy. *Transfusion*, (2009). , 1901-1910.
- [32] Estrada, J. C, et al. Culture of human mesenchymal stem cells at low oxygen tension improves growth and genetic stability by activating glycolysis. *Cell Death Differ*, (2012). , 743-755.
- [33] Rasmusson, I, et al. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. *Transplantation*, (2003). , 1208-1213.
- [34] Di Nicola M., et al., Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood*, (2002). , 3838-3843.
- [35] Djouad, F, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. *Blood*, (2003). , 3837-3844.
- [36] Nauta, A. J, et al. Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. *J Immunol*, (2006). , 2080-2087.
- [37] Jiang, X. X, et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. *Blood*, (2005). , 4120-4126.
- [38] Corcione, A, et al. Human mesenchymal stem cells modulate B-cell functions. *Blood*, (2006). , 367-372.
- [39] Glennie, S, et al. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood*, (2005). , 2821-2827.
- [40] Patel, S. A, et al. Mesenchymal stem cells protect breast cancer cells through regulatory T cells: role of mesenchymal stem cell-derived TGF-beta. *J Immunol*, (2010). , 5885-5894.
- [41] Torsvik, A, & Bjerkvig, R. Mesenchymal stem cell signaling in cancer progression. *Cancer Treat Rev*, (2012).
- [42] Schreml, S, et al. Harvesting human adipose tissue-derived adult stem cells: resection versus liposuction. *Cytotherapy*, (2009). , 947-957.
- [43] Dubois, S. G, et al. Isolation of human adipose-derived stem cells from biopsies and liposuction specimens. *Methods Mol Biol*, (2008). , 69-79.
- [44] Lee, J. M, et al. Comparison of immunomodulatory effects of placenta mesenchymal stem cells with bone marrow and adipose mesenchymal stem cells. *Int Immunopharmacol*, (2012). , 219-224.
- [45] Scuderi, N, et al. Human Adipose-Derived Stem Cells for Cell-Based Therapies in the Treatment of Systemic Sclerosis. *Cell Transplant*, (2012).



- [46] Pak, J. Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: a case series. *J Med Case Reports*, (2011). , 296.
- [47] Koyama, N, et al. Pluripotency of mesenchymal cells derived from synovial fluid in patients with temporomandibular joint disorder. *Life Sci*, (2011). , 741-747.
- [48] Ju, Y. J, et al. Synovial mesenchymal stem cells accelerate early remodeling of tendon-bone healing. *Cell Tissue Res*, (2008). , 469-478.
- [49] Sekiya, I, et al. Articular cartilage regeneration with synovial mesenchymal stem cells]. *Clin Calcium*, (2011). , 879-889.
- [50] Miyamoto, T, et al. Intradiscal transplantation of synovial mesenchymal stem cells prevents intervertebral disc degeneration through suppression of matrix metalloproteinase-related genes in nucleus pulposus cells in rabbits. *Arthritis Res Ther*, (2010). , R206.
- [51] Yang, J, et al. Evaluation of bone marrow- and brain-derived neural stem cells in therapy of central nervous system autoimmunity. *Am J Pathol*, (2010). , 1989-2001.
- [52] Reynolds, B. A, & Weiss, S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*, (1992). , 1707-1710.
- [53] Pluchino, S, et al. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature*, (2003). , 688-694.
- [54] Pluchino, S, et al. Immune regulatory neural stem/precursor cells protect from central nervous system autoimmunity by restraining dendritic cell function. *PLoS One*, (2009). , e5959.
- [55] Wang, L, et al. Neural stem/progenitor cells modulate immune responses by suppressing T lymphocytes with nitric oxide and prostaglandin E2. *Exp Neurol*, (2009). , 177-183.
- [56] Melzi, R, et al. Co-graft of allogeneic immune regulatory neural stem cells (NPC) and pancreatic islets mediates tolerance, while inducing NPC-derived tumors in mice. *PLoS One*, (2010). , e10357.
- [57] Yang, J, et al. Adult neural stem cells expressing IL-10 confer potent immunomodulation and remyelination in experimental autoimmune encephalitis. *J Clin Invest*, (2009). , 3678-3691.
- [58] Conover, J. C, & Notti, R. Q. The neural stem cell niche. *Cell Tissue Res*, (2008). , 211-224.
- [59] Buffo, A, et al. Expression pattern of the transcription factor Olig2 in response to brain injuries: implications for neuronal repair. *Proc Natl Acad Sci U S A*, (2005). , 18183-18188.

- [60] Saha, B, Jaber, M, & Gaillard, A. Potentials of endogenous neural stem cells in cortical repair. *Front Cell Neurosci*, (2012). , 14.
- [61] Nishida, T, et al. Development of tumor-reactive T cells after nonmyeloablative allogeneic hematopoietic stem cell transplant for chronic lymphocytic leukemia. *Clin Cancer Res*, (2009). , 4759-4768.
- [62] Lucarelli, G, et al. Hematopoietic stem cell transplantation in thalassemia and sickle cell anemia. *Cold Spring Harb Perspect Med*, (2012). , a011825.
- [63] Kim, H, et al. Allogeneic Hematopoietic Stem Cell Transplant for Adults over 40 Years Old with Acquired Aplastic Anemia. *Biol Blood Marrow Transplant*, (2012).
- [64] Shapiro, E, et al. Long-term effect of bone-marrow transplantation for childhood-onset cerebral X-linked adrenoleukodystrophy. *Lancet*, (2000). , 713-718.
- [65] Breuskin, I, et al. Strategies to regenerate hair cells: identification of progenitors and critical genes. *Hear Res*, (2008). , 1-10.
- [66] Li, H, Liu, H, & Heller, S. Pluripotent stem cells from the adult mouse inner ear. *Nat Med*, (2003). , 1293-1299.
- [67] Minoda, R, et al. Manipulating cell cycle regulation in the mature cochlea. *Hear Res*, (2007). , 44-51.
- [68] Dabdoub, A, et al. Sox2 signaling in prosensory domain specification and subsequent hair cell differentiation in the developing cochlea. *Proc Natl Acad Sci U S A*, (2008). , 18396-18401.
- [69] Kiernan, A. E, et al. The Notch ligands DLL1 and JAG2 act synergistically to regulate hair cell development in the mammalian inner ear. *Development*, (2005). , 4353-4362.
- [70] Shackleton, M, et al. Generation of a functional mammary gland from a single stem cell. *Nature*, (2006). , 84-88.
- [71] Liu, S, Dontu, G, & Wicha, M. S. Mammary stem cells, self-renewal pathways, and carcinogenesis. *Breast Cancer Res*, (2005). , 86-95.
- [72] Ercan, C, Van Diest, P. J, & Vooijs, M. Mammary development and breast cancer: the role of stem cells. *Curr Mol Med*, (2011). , 270-285.
- [73] Katoh, M. Notch signaling in gastrointestinal tract (review). *Int J Oncol*, (2007). , 247-251.
- [74] Crosnier, C, Stamatakis, D, & Lewis, J. Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat Rev Genet*, (2006). , 349-359.
- [75] Gersemann, M, Stange, E. F, & Wehkamp, J. From intestinal stem cells to inflammatory bowel diseases. *World J Gastroenterol*, (2011). , 3198-3203.

- [76] Conrad, S, et al. Generation of pluripotent stem cells from adult human testis. *Nature*, (2008). , 344-349.
- [77] Dobrinski, I. Germ cell transplantation and testis tissue xenografting in domestic animals. *Anim Reprod Sci*, (2005). , 137-145.
- [78] Honaramooz, A, et al. Fertility and germline transmission of donor haplotype following germ cell transplantation in immunocompetent goats. *Biol Reprod*, (2003). , 1260-1264.
- [79] Brinster, R. L, & Avarbock, M. R. Germline transmission of donor haplotype following spermatogonial transplantation. *Proc Natl Acad Sci U S A*, (1994). , 11303-11307.
- [80] Stadtfeld, M, Brennand, K, & Hochedlinger, K. Reprogramming of pancreatic beta cells into induced pluripotent stem cells. *Curr Biol*, (2008). , 890-894.
- [81] Takahashi, K, & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, (2006). , 663-676.
- [82] Takahashi, K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, (2007). , 861-872.
- [83] Yu, J, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*, (2007). , 1917-1920.
- [84] Neumann, J, et al. SOX2 expression correlates with lymph-node metastases and distant spread in right-sided colon cancer. *BMC Cancer*, (2011). , 518.
- [85] De Nigris, F, et al. c-Myc oncoprotein: cell cycle-related events and new therapeutic challenges in cancer and cardiovascular diseases. *Cell Cycle*, (2003). , 325-328.
- [86] Wang, P, et al. The POU homeodomain protein OCT3 as a potential transcriptional activator for fibroblast growth factor-4 (FGF-4) in human breast cancer cells. *Biochem J*, (2003). Pt 1): , 199-205.
- [87] Gore, A, et al. Somatic coding mutations in human induced pluripotent stem cells. *Nature*, (2011). , 63-67.
- [88] Hussein, S. M, et al. Copy number variation and selection during reprogramming to pluripotency. *Nature*, (2011). , 58-62.
- [89] Mayshar, Y, et al. Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. *Cell Stem Cell*, (2010). , 521-531.
- [90] Bar-nur, O, et al. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. *Cell Stem Cell*, (2011). , 17-23.
- [91] Kim, K, et al. Epigenetic memory in induced pluripotent stem cells. *Nature*, (2010). , 285-290.

- [92] Okita, K, Ichisaka, T, & Yamanaka, S. Generation of germline-competent induced pluripotent stem cells. *Nature*, (2007). , 313-317.
- [93] Nakagawa, M, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol*, (2008). , 101-106.
- [94] Stadtfeld, M, et al. Induced pluripotent stem cells generated without viral integration. *Science*, (2008). , 945-949.
- [95] Warren, L, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell*, (2010). , 618-630.
- [96] Yoshida, Y, et al. Hypoxia enhances the generation of induced pluripotent stem cells. *Cell Stem Cell*, (2009). , 237-241.
- [97] Esteban, M. A, et al. Vitamin C enhances the generation of mouse and human induced pluripotent stem cells. *Cell Stem Cell*, (2010). , 71-79.
- [98] Maherali, N, & Hochedlinger, K. Guidelines and techniques for the generation of induced pluripotent stem cells. *Cell Stem Cell*, (2008). , 595-605.
- [99] Wernig, M, et al. A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. *Nat Biotechnol*, (2008). , 916-924.
- [100] Anokye-danso, F, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. *Cell Stem Cell*, (2011). , 376-388.
- [101] Doi, A, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet*, (2009). , 1350-1353.
- [102] Lister, R, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*, (2011). , 68-73.
- [103] Govindarajan, N, et al. Sodium butyrate improves memory function in an Alzheimer's disease mouse model when administered at an advanced stage of disease progression. *J Alzheimers Dis*, (2011). , 187-197.
- [104] Ware, C. B, et al. Histone deacetylase inhibition elicits an evolutionarily conserved self-renewal program in embryonic stem cells. *Cell Stem Cell*, (2009). , 359-369.
- [105] Hogley, G, et al. Development of rationally designed DNA N6 adenine methyltransferase inhibitors. *Bioorg Med Chem Lett*, (2012). , 3079-3082.
- [106] Bae, D, et al. Hypoxia enhances the generation of retinal progenitor cells from human induced pluripotent and embryonic stem cells. *Stem Cells Dev*, (2012). , 1344-1355.
- [107] Zhao, T, et al. Immunogenicity of induced pluripotent stem cells. *Nature*, (2011). , 212-215.

- [108] Robinton, D. A, & Daley, G. Q. The promise of induced pluripotent stem cells in research and therapy. *Nature*, (2012). , 295-305.
- [109] Carvajal-vergara, X, et al. Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. *Nature*, (2010). , 808-812.
- [110] Thomson, J. A, et al. Embryonic stem cell lines derived from human blastocysts. *Science*, (1998). , 1145-1147.
- [111] Schwartz, S. D, et al. Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet*, (2012). , 713-720.
- [112] Lu, B, et al. Long-term safety and function of RPE from human embryonic stem cells in preclinical models of macular degeneration. *Stem Cells*, (2009). , 2126-2135.
- [113] Lukovic, D, et al. Concise review: human pluripotent stem cells in the treatment of spinal cord injury. *Stem Cells*, (2012). , 1787-1792.
- [114] Wichterle, H, et al. Directed differentiation of embryonic stem cells into motor neurons. *Cell*, (2002). , 385-397.
- [115] All, A. H, et al. Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitors Aid in Functional Recovery of Sensory Pathways following Contusive Spinal Cord Injury. *PLoS One*, (2012). , e47645.
- [116] Klimanskaya, I, et al. Human embryonic stem cell lines derived from single blastomeres. *Nature*, (2006). , 481-485.
- [117] Tompkins, J. D, et al. Epigenetic stability, adaptability, and reversibility in human embryonic stem cells. *Proc Natl Acad Sci U S A*, (2012). , 12544-12549.
- [118] Eguchi, G, & Okada, T. S. Differentiation of lens tissue from the progeny of chick retinal pigment cells cultured in vitro: a demonstration of a switch of cell types in clonal cell culture. *Proc Natl Acad Sci U S A*, (1973). , 1495-1499.
- [119] Yoo, A. S, et al. MicroRNA-mediated conversion of human fibroblasts to neurons. *Nature*, (2011). , 228-231.