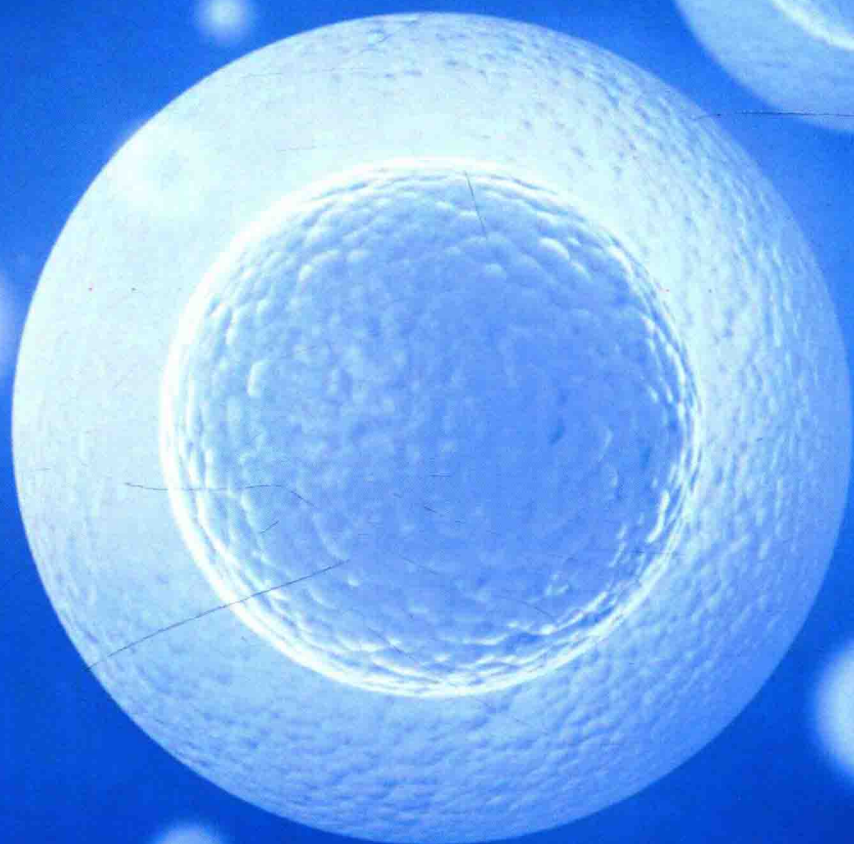


Pluripotent Stem Cells

Current Researches



Jack Collins

Pluripotent Stem Cells: Current Researches

Edited by **Jack Collins**



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Pluripotent Stem Cells: Current Researches

Preface

I am honored to present to you this unique book which encompasses the most up-to-date data in the field. I was extremely pleased to get this opportunity of editing the work of experts from across the globe. I have also written papers in this field and researched the various aspects revolving around the progress of the discipline. I have tried to unify my knowledge along with that of stalwarts from every corner of the world, to produce a text which not only benefits the readers but also facilitates the growth of the field.

This book is an up-to-date guide on pluripotent stem cells. Several types of stem cells are being examined for their regenerative ability. Stem cells have developed a great amount of excitement among the clinicians, researchers and the public alike. Slight advantage because of transplanting autologous stem cells in several distinct clinical conditions has been suggested to be a progress factor effect rather than actual regeneration. In comparison, several pre-clinical analyses have been carried out, with the help of differentiated cells from induced pluripotent stem cells or embryonic stem cells have displayed functional advancement, promise and zero signs of teratoma formation. This book is a compilation of studies, starting with an introduction to the pluripotent stem cells and encompassing insightful information regarding the latest standing on the pluripotent stem cells biology and elucidation of the potential therapeutic implications and ethical concerns of these in vitro developed cells in different diseases, along with the related pros and cons are covered in this book.

Finally, I would like to thank all the contributing authors for their valuable time and contributions. This book would not have been possible without their efforts. I would also like to thank my friends and family for their constant support.

Editor

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Therapeutic Implications and Ethical Concerns

Advances in Stem Cell Therapies

Joel Sng and Thomas Lufkin

Additional information is available at the end of the chapter

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_0/G_1 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