European Review for Medical and Pharmacological Sciences 2016; 20: 4390-4400

Stem cells differentiation and probing their therapeutic applications in hematological disorders: a critical review

F. ALI[1,2], S. TARESH[3], M. AL-NUZAILY[3], P.L. MOK[4,5], A. ISMAIL[1], S. AHMAD[6]

[1]Department of Nutrition and Dietetics, Metabolism and Genomics Group, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[2]Hematology Department, University Hospital, Faculty of Medicine and Health Sciences, Sana'a University, Yemen
[3]Hematology Department, Faculty of Medicine and Health Sciences, Sana'a University, Yemen
[4]Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[5]Genetics and Regenerative Medicine Research Centre, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[6]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

Abstract. – Numerous lines of evidence support that bone marrow is a rich source of stem cells that can be used for research purposes and to treat some complex blood diseases and cancers. Stem cells are a potential source for regenerative medicine and tissue replacement after injury or disease, and mother cells that possess the capacity to become any type of cell in the body. They are cells without specific structure and characterized by their ability to self-renew or multiply while maintaining the potential to develop into other types of cells. Stem cells can normally become cells of the blood, heart, bones, skin, muscles or brain. Although, there are different sources of stem cells, all types of stem cells have the same capacity to develop into multiple types of cells. Stem cells are generally described as unspecialized cells with unlimited proliferation capacity that can divide (through mitosis) to produce more stem cells. Several types of adult stem cells have been characterized and can be cultured *in vitro*, including neural stem cells, hematopoietic stem cells, mesenchymal stem cells, cardiac stem cells and epithelial stem cells. They are valuable as research tools and might, in the future, be used to treat a wide range of diseases such as hematological hereditary diseases, Parkinson's disease, diabetes mellitus, heart disease and many other diseases. Currently, two types of stem cells have been identified based on their origins, namely embryonic stem cells and adult stem cells. Collectively, although many kinds of literature have been studying stem cell application in terms of clinical practice, stem cell-based therapy is still in its infancy stage.

Key Words:
Stem cell types, Stem cell biology, Hematological disorders therapy, Specific molecular and cellular markers, Therapeutic challenges.

Introduction

The main objectives of the studies presented in this review were, (1) to identify molecular and cellular markers of which expression and function are specifically associated with stem cell differentiation, (2) to present the main arguments in favor and against potential uses of human stem cell in clinical applications with special emphasis on hematological-related diseases.

Types of Stem Cells

The discovery of stem cells is largely referred to Professor Alexander A. Maximow (1922 to 1928), a Russian-American physician, embryologist, and histologist. He developed his theory that all cells come from the same precursor cell, which he later called as stem cells[1]. He proposed the existence of hematopoietic stem cells and described his unitarian theory of hematopoiesis during that event. He was the first person to use the term "stem cells" for scientific and medical purposes. Later, other physicians and medical researchers such as Joseph Altman, Gopal Das, Andre Gernez, McCulloch and Till discovered various characteristics of stem cells. They first

Corresponding Author: Faisal Ali, MD; e-mail: sshrmany@yahoo.co.uk

found methods to isolate stem cells from mouse and then human embryos. Later, they grew the cells in the laboratory for infertility purposes through *in vitro* fertilization procedures[2].

Usually, stem cells are defined as unspecialized cells with unlimited proliferation capacity that can differentiate into various cell types with specific functions[1,2]. Two types of stem cells have been identified based on their origin, namely a) embryonic stem cells (ES) or fetal stem cells and b) adult stem cells. ES cells are obtained from tissues of a developing human fetus and these cells have some characteristics of the tissues they are taken from. While adult stems cells are obtained from some tissues of the adult body, for example, bone marrow. It is also worth to mention that adult bone marrow is a rich source of stem cells that can be used to treat some blood diseases and cancers[2,3].

Fetal stem cells are initially formed during the early stages of embryonic development in the inner cell mass of the blastocyst[3]. By this property, the stem cell has the ability to divide for long periods and retain their capacity to make all cell types within the organism[3,4]. The fetal stem cells are thus called pluripotent stem cell. But, adult stem cells are called multipotent, indicating that they have a less wide-ranging potential than ES cells, and their differentiation capability is limited to particular cell types in the body. Adult stem cells are being resided in various tissues throughout the body where they are differentiated into somatic cells, and passing through stepwise stages of maturation[4]. Furthermore, stem cells receive multiple signals from their niche for proper development and tissue homeostasis[5]. These signals include cytokines and growth factors, as well as signals mediated by cell-cell interactions, which control the processes of stem cell inactivity and self-renewal and differentiation[5,6]. On the other hand, stem cells are not only demanded organogenesis and development but also for tissue maintenance and repair[7,8]. By these processes, the stem cell closet is essential to produce the full range of differentiated cells and also maintaining a group of undifferentiated cells (self-renew)[8]. This phenomenon can also occur by so-called asymmetrical cell division that reveals that upon cell dividing, one daughter cell becomes a committed progenitor cell that thereby goes for further differentiation, but the other daughter cell remains a stem cell with similar characteristics as the mother cell, consequently saving the size of the stem cell pool fit[6].

Stem Cell Biology

Studying stem cell biology and development will provide important information in such processes as early embryogenesis, tumor formation, and autoimmune diseases. Both adult stem cells and embryonic were shown to be able to differentiate into specialized cell types and participate to the renovation of damaged tissue after inoculation into animals[9]. Stem cell biology is thus of particular importance for its potential uses in regenerative medicine in which damaged cells are repaired in many cases such as Parkinson's disease, heart failure, diabetes mellitus. Several types of adult stem cells have been identified and can be cultivated *in vitro* such as hematopoietic stem cells, neural stem cells, epithelial stem cells, and mesenchymal stem cells[10]. The later cells have already been used in bone tissue formation, by growing them on three-dimensional scaffolds and thereby transplanted *in vivo* to generate new bone. In addition to their usage in bone tissue regeneration, the knowing of mesenchymal stem cell behavior can lead to understanding the pathogenesis of bone diseases such as osteoporosis[11]. To reveal the internal and external factors that block or stimulate stem cells to differentiate into a specialized cell lineage and by which mechanisms can such final product cells be characterized and control the self-renewal process?. A thorough understanding these matters is clinically important, since the development of stem cell culture techniques will probably provide options for managing stem cell behavior[12]. The major defiance might be to utilize their self-renewal capability to extend stem cells in culture to produce sufficient amounts of cells and also to promote the cells to differentiate into a specific cell type. Transplantation the differentiated cells into the body has promising potentials for replacing cells and tissue damaged by degenerative disorders[11-13].

Stem Cell Proliferation and Differentiation

Stem cell differentiation has become an important area of research, both in the context of the pluripotentiality of embryonic stem cells and the multipotentiality of adult stem cells.

The stem differentiations are the generation of specialized cells (functional cells) such as bone, nerve, muscle and blood cells. Scientists are being tried to differentiate stem cells into fully functioning specialized cells to use them to treat many diseases or for research purposes to study the development of diseases. The differentiation

reveals that complex stimulatory and regulatory pathways assign the irreversible commitment of such cells to generate a definite specialized function[14,15]. Moreover, the lineage commitment and differentiation of both embryonic and adult stem cells at the molecular level may be varied. Currently, scientists and physicians are excited about the potential of stem cells to be used as a tissue source for transplantation therapy. For example, insulin-secreting pancreas cells created from stem cells have the potential to treat diabetes. These stem cell-derived pancreas cells could replace dysfunctional cells in the pancreas, allowing a patient to regulate their blood sugar levels. Stem cells generate specialized cell types called progenitors with restricted differentiation capacity during asymmetric self-renewal. Therefore, progenitor cells can increase the number of mature cell types within a single lineage. In addition, progenitor cells still possess the capability to proliferate, but generally unlike stem cells they cannot self-renew. The differentiation of stem cell has been counted to be hierarchical in nature such that the generation of a limited number of cell types occurs gradually through determined intermediate stages[16,17].

Hematopoietic Stem Cell Differentiation

The best-characterized model of adult stem cell differentiation is that of the hematopoietic stem cell (HSC), since all cells of the blood and immune system are generated from HSC. The model presented in (Figure 1) is illustrative for lineage commitment and further differentiation of such cells. In particular, HSC can form two distinct progenitors, called a myeloid and a lymphoid progenitor cells. The lymphoid progenitor differentiates into mature immune cells such as B lymphocytes, T lymphocytes, and natural killer cells, while the myeloid progenitor generates erythrocytes, platelets, neutrophils, macrophages and monocytes. Stem cells produce restricted progenitor cells that become stepwise more specialized by losing their capacity to differentiate along an alternative pathway. Other hierarchical models have been described for differentiation of other adult stem cells such as mesenchymal stem cells, intestinal stem cells, epidermal stem cells

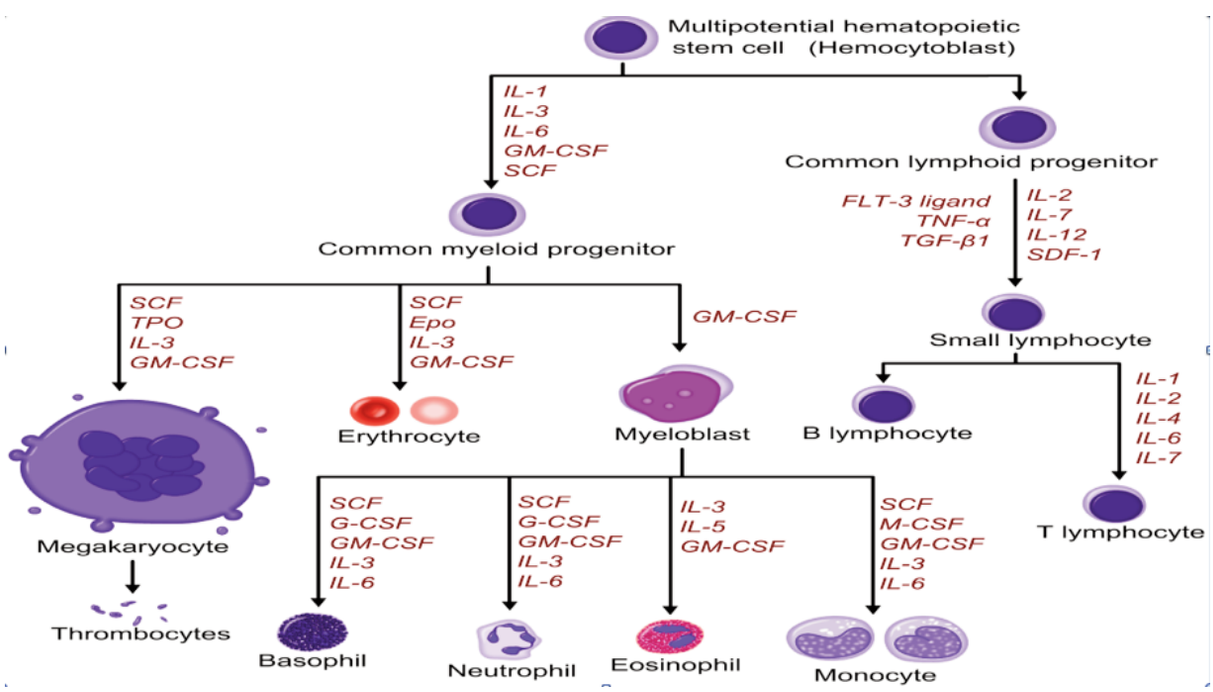

Figure 1. Diagram showing the development of different blood cells from hematopoietic stem cell to mature cells. Hematopoietic stem cells (HSC) differentiate into specialized mature cells via progenitor cells. It's including some of the important cytokines that determine which type of blood cells will be created. IL=Interleukins or cytokines (1-7); SCF=Stem cell Factor. Tpo=Thrombopoietin; GM-CSF=Granulocyte Macrophage-colony stimulating factor; EPo=Erythropoietin; M-CSF=Macrophage-colony stimulating factor; G-CSF=Granulocyte-colony stimulating factor; SDF-1=Stromal cell-derived factor-1, FLT-3 lligand=FMS-Like tyrosine kinases 3; TNFα=Tumor necrosis factor-alpha; TGFβ1=Transforming growth factor beta1. Adapted from (2).

and neural stem cells[15-17]. HCS fate decisions are modulated by differentiation factors, which activate transcription programmes that specify differentiation and lineage status. One of these factors, the transforming growth factor (TGF)-β family is substantial in both lineage selection and progression of differentiation of most cell and tissue types. Cytokines, growth factors (GF), and hormones are chemical messengers that mediate intercellular communication and stimulate cellular growth, proliferation and cellular differentiation of HSC as shown in (Figure 1). These factors and their receptors play an important role in the regulation of HSC differentiation into the cell of interest. By adding BMP, bone morphogenetic protein; EGF, epidermal growth factor; FGF, fibroblast growth factor; GM-CSF, granulocyte macrophage-colony stimulating factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor into specific media, HSC were directed toward becoming early and mature cells *in vitro*[16,17]. In the same context, rat bone marrow mesenchymal stem cells (BMSCs) could differentiate into hepatocytes in the differentiation media including hepatocyte growth factor (HGF) or beta-nerve growth factor (beta-NGF) and to find a new source for therapies of hepatic diseases[16-18]. Also, human BMSCs could differentiate into neural cells (NCs) in the presence of human epidermal growth factor (hEGF) and bovine fibroblast growth factor (bFGF) and neurobasal media plus B2. This differentiation was confirmed by culture BMSCs with hEGF and bFGF, whereby RNA expression of neuronal specific markers Nestin, MAP-2, and tyrosine hydroxylase (TH) were observed[18,19].

Mesenchymal Stem Cell Differentiation

Mesenchymal stem cells (MSC) are multipotent adult stem cells of mesodermal origin. These stem cells are able to differentiate into a number of different cell types including osteoblasts (bone cells), adipocytes (fat cells), chondrocytes (cartilage cells) and myoblasts (muscle cells)[20-23]. A schematic overview of potential differentiation pathways of MSC is given in (Figure 2).

Multipotent MSC has been isolated from various adult tissues, including bone marrow, skeletal muscle, adipose tissue, umbilical cord blood and fetal lung. MSC can most easily be harvested from bone marrow. When placed in culture, bone marrow –derived MSC retain their multipotential capacity and can differentiate into at least osteoblasts and adipocytes.

The origin and development of MSCs in the body is poorly understood, since it is not exactly known how adult stem cells arise from embryonic stem cells[19-22]. Pretreatment of embryonic stem cells with retinoic acid induces an MSC-like intermediate cell that can subsequently be triggered to differentiate into osteoblasts, chondrocytes or adipocytes, but the underlying mechanisms remain to be solved. Moreover, the exact nature of progenitor cells that are responsible for the lineage commitment of mesenchymal stem cells is less well understood than in the case of hematopoietic stem cells due to a lack of specific

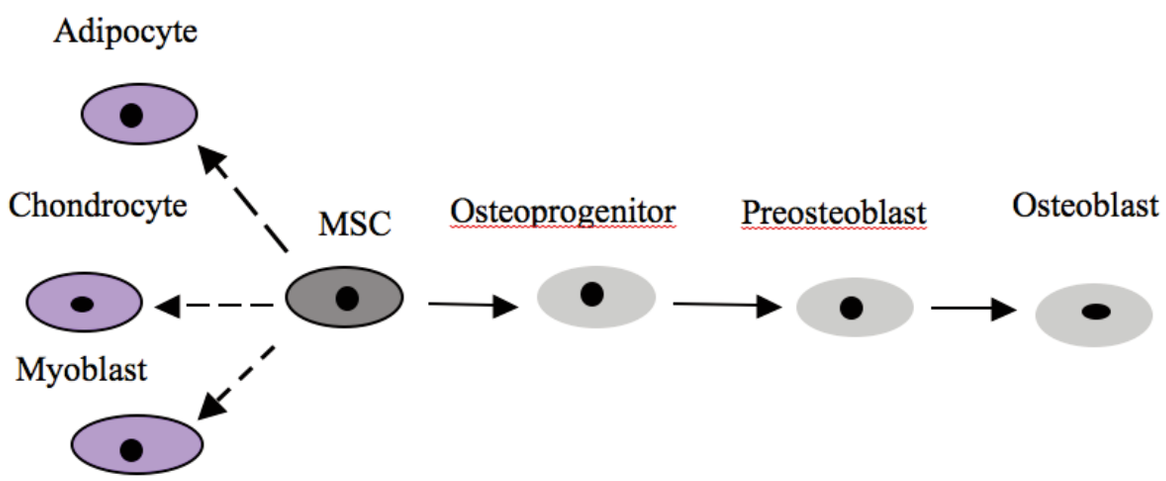

Figure 2. Bone marrow-derived mesenchymal stem cells capable of differentiation into osteoblasts, adipocytes, chondrocytes and myoblasts.

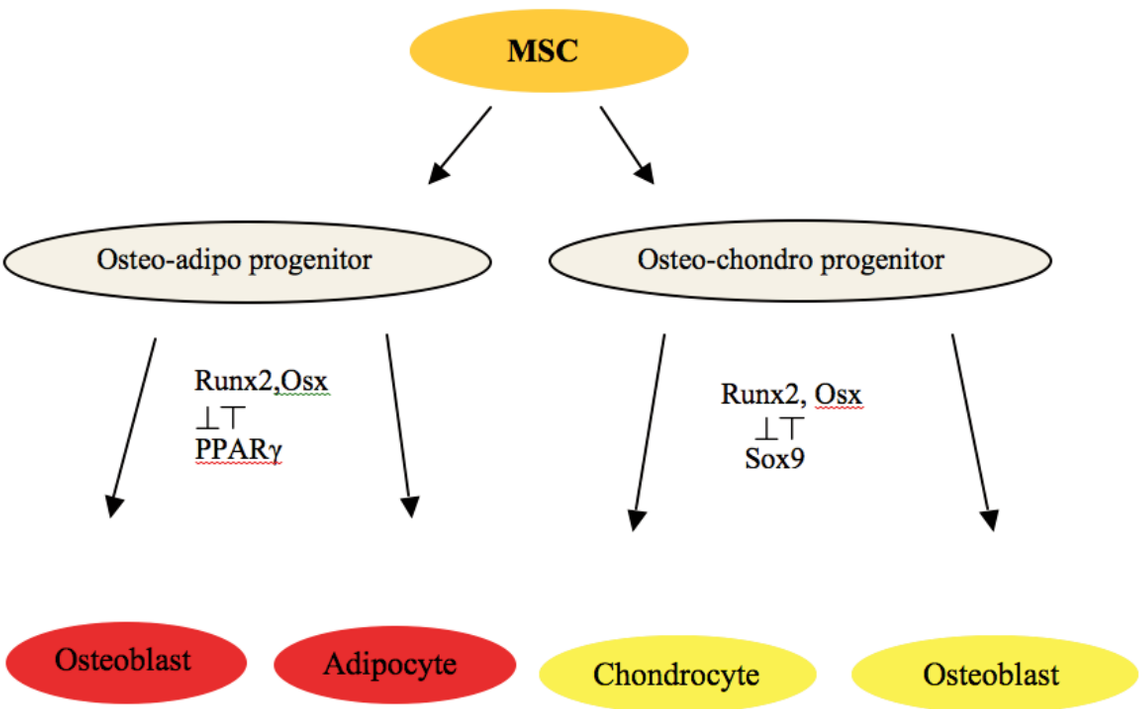

Figure 3. Model of mesenchymal stem cell commitment via bipotential progenitors. Osteoblasts, chondrocytes and adipocytes originate from bipotential precursor cells *in vivo*, in which the transcription factors Runx2, Pparg, Osx and Sox9 are key regulators of lineage specification. Osteo-chondroprogenitor cells express both Runx2 and Sox9 and have the capacity to differentiate into osteoblasts or chondrocytes. While, osteo-adipoprogenitor cells express both Runx2 and PPARγ and have the capacity to differentiate into osteoblasts or adipocytes. All these transcription factors inhibit (⊥) or activate (⊤) each other.

markers to characterize intermediate stages. In particular, the early commitment steps of mesenchymal stem cells and the identity of the progenitor cells involved need further elucidation. The commitment of MSC and succession along specific lineages needs the transcriptional control of specific members of genes[23,24]. Genes encoding transcription factors that have been assigned as driving powers in mesenchymal lineages, including PPARg2 (peroxisome proliferator-activated receptor gamma 2) and C/EBP (CCAAT-enhancer binding protein alpha) for adipocyte; SOX9 for chondrocyte; and RUNX2 (Runt-related transcription factor 2) and OSX (Osterix) for osteoblast differentiation. Transcription of these regulatory transcription factors in progenitor cells determines early differentiation and locates their lineage commitment[23-26]. The rigorous hierarchy and differentiation capacity of intermediate progenitors during MSC differentiation is not wholly known but it was proposed that it occurs either via tripotential or bipotential progenitors. Tripotential progenitors would have the capacity to differentiate into osteoblasts, chondrocytes, and adipocytes, while the bipotential progenitors can differentiate either into osteoblasts and chondrocytes (osteo-chondroprogenitor) or into osteoblasts and adipocytes (osteo-adipoprogenitor). Published study has been demonstrated the genetic evidence for the *in vivo* presence of notable osteo-chondro and osteo-adipoprogenitors, in which Runx2, Sox9, Osterix, and Pparg2 function in cell fate determination (Figure 3). Of particular importance, each of these genes induces a particular phenotype, by actively suppressing other lineage directions through inhibit or activate each other[25-28].

Previous studies[29,30] have widely studied the role of Wnt signaling pathway in the determination of MSC fat during proliferation in bone marrow. Wnt signaling was previously shown to regulate bone cell homeostasis and bone cell differentiation. The effect of Wnt is mainly appeared on the regulatory pathway of osteoblast differentiation and consequently bone mass. During the last years, it has been reported that high Wnt sig-

naling activity stimulates osteoblast differentiation and inhibits chondrocyte differentiation, but low Wnt activity suppresses osteoblast differentiation and promotes chondrocyte formation. Also, Wnt signaling activity has exerted effect on the cell fate of osteo-adipoprogenitor cells in their option to be either adipocytes or osteoblasts. These data confirmed that Wnt signaling pathway is partly one of the most important molecular mechanism involved in the regulation of MSC differentiation towards the osteoblast lineages by activation expression of Runx2 and decreasing expression of PPAR gamma or Sox9[31,32]. Furthermore, a group of researchers observed that disruption of β-catenin in bone marrow progenitor cells led to normal HSC self-renewal and differentiation, which demonstrates that Wnt indirectly regulates HSC through its effect on the stem cell niche[33,34].

Age-Related Changes of MSC in Bone Marrow

Age-related changes in the rate of bone formation are accompanied by a decrease in the number of bone-forming osteoblasts and an increase in the number of fat-storing adipocytes in the bone marrow of osteoporotic individuals, since the balance between osteogenesis and adipogenesis in bone marrow is gradually shifted in favor of adipogenesis[35,36]. Moerman et al[37] reported that aging causes a decrease in the commitment of marrow MSC to osteoblast lineage and an increase in the commitment to the adipocyte lineage *in vitro*. An increase in marrow adipocytes is associated with osteoporosis and age-related osteopenia, where in humans up to 90% of the marrow cavity is occupied by adipocytes resulting in the appearance of fatty marrow by the third decade of life. Thus, there is a clinical correlation between the appearance of bone marrow fat and reduced bone forming capacity[38,39]. The reciprocal relationship between osteogenesis and adipogenesis, in combination with the increased adipogenesis during aging and osteoporosis, opens opportunities to decrease adipocyte differentiation, accompanied by an increase of osteoblast formation providing a therapeutic target to prevent further increases in adipocytes formation[40-43].

Stem Cells and Age-Related Diseases

Characterization of the molecular mechanisms underlying MSC commitment can provide a new insight for the treatment of age-related diseases such as osteoporosis and aging. Osteoporosis is a bone disorder characterized by a decrease in the number of osteoblasts coupled with an increase in the number of adipocytes. Moreover, the balance between osteoblastogenesis and adipogenesis in bone marrow are being shifted in favor of adipogenesis. The differentiation of MSC into osteoblasts and adipocytes was shown to be reciprocal[44,45]. This correlation was supported by the result that PPARγ induced osteoblasts while adipogenesis was decreased in (+/-) mice. The shifting in MSC commitment during osteoporosis can consequently be attributed to a defect in differentiation potential of MSC. The mechanisms underlying these alterations in MSC fate still remain to be clarified. The mutual relationship between adipogenesis and osteogenesis in parallel with the increased adipogenesis during osteoporosis and aging will open a new approach to regulating bone mass through modulation the balance between adipocyte and osteoblast differentiation from MSC[46,47]. A new strategy can be utilized to increase osteoblast formation, coupled with a decrease in adipocyte differentiation. Most recently scientists have proposed that age-related diseases and some of the chronic diseases might be a stem cell disease. Although most drugs currently used are anti-catabolic, efficient drugs for medication of age-related diseases can act by increasing bone mineralization (anabolic treatment) or by suppression bone degradation (catabolic treatment). The anabolic drugs that can restore bone mass may be expected to decrease the risk of fractures more than catabolic drugs[47,48]. The close relation between stem cell development and bone loss-related diseases indicate that the importance of stem cell research for treating or preventing osteopenic disorder.

Stem Cell Transplantation and Hematological Diseases

Hematopoietic stem cell transplantation (HSCT) comprises the intravenous (IV) infusion of autologous or allogeneic stem cells to normalize hematopoietic function in patients whose bone marrow or immune system is damaged or defective. HSCT is able to medicate various types of malignant (e.g. leukemia, lymphoma) and non-malignant (e.g. thalassemias, a plastic anemia) hematological diseases. A transplant provides stem cells to replace the faulty white blood cells or make red blood cells. However, the process is still currently accompanied with significant morbidity and mortality[49,50]. Trans-

plantations are generally divided into two types of stem cell transplants, autologous from the patient or allogeneic from another person. In addition, autologous transplantations are considered to be less dangerous than allogeneic methods. A clear decision between autologous and allogeneic transplantation can be made after observation of patient's disease, stage, and donor availability. Stem cells in blood can be collected from an umbilical cord and placenta immediately after the birth. The blood is stored at a cord blood bank for future need. The umbilical cord blood is a voluble source of HSC and progenitor cells for treatment a variety of blood diseases. Although the availability of cord blood through blood bank and low level of graft versus host diseases compared to bone marrow, The number of nucleated cells and platelets are usually limited in a single unit of cord blood, which can less than optimal for grafting of many adults and children[51,52].

After giving a relatively high dose of chemotherapy or radiation therapy, stem cell transplantation (SCT) is provided to patients with a hematologic malignancy. The reasons behind this regime are to remove the underlying malignancy and to repress the immune system of patients in order to accept the donor stem cells. After replacing the stem cells in the bone marrow, SCT retrieves the capacity of the patient to activate an immune response against pathogens and to prevent cancer cell recurrence. However, Graft-versus-host disease (GVHD) is a potentially fatal complication of this therapy and occurs in 25%-60% of patients. Therefore, clinicians and researchers are perfectly working to reduce the rate of GVHD incidence and improve patient's health status. Currently, combinations of a steroid such as prednisone with other immunosuppressive drugs are a clinically given for GVHD treatment. Another method is called extracorporeal photopheresis to reduce the GVHD effect. By this approach, the blood of the patient is circulated using a device to remove the white blood cells and platelets, and then treat them with a chemical that is then activated by exposure to ultraviolet light, and returns them back to the circulation[53,54].

Challenges Facing Stem Cell Scientists

Scientists have been facing several challenges that should be managing before stem cell therapies can become a successful. The first clinical trials testing the safety of using specialized cells was from human embryonic stem cells (hESCs). However, scientists are still learning how to control the differentiation of embryonic stem cells into specialized cells. It is not yet possible to make pure and fully functional specialized cells of every type found in the body, starting from hESCs in the lab such as the nerve cells that are affected in Parkinson's disease. But more work is needed to understand and control hESCs, if they are to fulfill their potential for use in future treatments. The remarkable capacity of hESCs to mature into hundreds of kinds of cells that make up the tissues of the human body such as blood, skin, bone, nerve, heart, and so on. By this concept, the cells have big promise for treating various diseases like Alzheimer's, Parkinson's, cancer, and diabetes. Nevertheless, some consider human embryonic stem cell research controversial because, in some cases, the new stem cell lines are derived frozen human embryos that have been assigned for research purposes. New approaches have been currently evolved that overcome this issue by genetically reprogramming adult cells[53-57].

First Challenge Facing Stem cell Therapy: Ethical Status

Some groups have raised sensitive ethical and religious arguments, which are balanced against the possible great benefit of such research for the patients suffering from so far incurable diseases.

Against Arguments

1. Fertilized eggs are considered as human that should be protected from destruction and respected as we respect the adult human.
2. If one permits to destroy fertilized egg or pre-implantation stage embryos, then why not fetuses, newborns, and human beings.
3. Human embryos differ from human beings in the stage of development.
4. A human embryos are the human being in the embryonic stage.
5. Pre-implantation embryos deserve the same level of respect and protection as human being, just because they are humans.

Pro-arguments

1. Pre-implantation embryos do not have the psychological, physiological, emotional or intellectual properties that associate with personhood (criteria of personhood). Thus, they can be used to benefit those who are persons.
2. The embryo itself cannot develop into a child without being transferred into the uterus of a woman. Thus, the embryos at the early stage do not have the degree of value in the terms of moral status.

3. The probability that this fertilized egg via *in vitro* fertilization way will implant and grow until the birth stage is low.
4. The major tissues and organs are differentiated after 14 days. Therefore, the blastocysts before implantation cannot be harmed by being destroyed.

Second Challenge Facing Stem Cell Therapy: Immune Rejection

A major challenge limiting the use of ES cells in therapeutic applications is the potential for immune rejection of the engrafted stem cells by the recipient. In recent years, several studies have been done to generate large populations of patient-specific pluripotent stem cells. A potential solution for producing immunologically matched stem cells is to transfer the nucleus of the recipients owns cells into the enucleated ES cell. Currently, scientists across the world are trying to produce stem cells in the lab by inserting a small number of factors into ordinary adult skin cells to use them in transplantation medicine. The added factors reprogram the adult cells into stem cells that have all the important characteristics of human embryonic stem cells. As similar as ES cells, such reprogrammed adult cells, termed induced pluripotent stem cells (iPSCs), are able to produce all the cells of the body. Unlike ES cells, however, iPSCs are genetically identical to patients and are generated without destroying human embryos and without using human or animal eggs.

Isolation and Characterization of Stem Cells in Tissues

The most important difficulty that still also faces many scientists is the identification of stem cells in adult tissues. An attempt to locate the often scarce numbers of stem cells in tissues is difficult, due to that these tissues contain many different types of cells. The research needed is complicated and even after cells are obtained; the process and applications to successfully promote differentiation into the cell type of interests is another challenge for scientists. This issue needs an understanding of stem cell biology, control, and regulation that has yet to be fully clarified. Moreover, researchers of stem cells should utilize the correct laboratory facilities, e.g., medium or solution, to acquire the growth and this has proven also to be difficult.

General Critique

The above-mentioned studies do not show the origin and development of MSCs in the body probably since it is not exactly known how adult stem cells arise from embryonic stem cells?. Moreover, the possible mechanism behind the stem cell differentiation is poorly understood. In the same context, the exact nature of progenitor cells that are responsible for the lineage commitment of mesenchymal stem cells is less well understood than in the case of hematopoietic stem cells due to a lack of specific markers to characterize intermediate stages. However, the previous researches perfectly addressed the stem cell plasticity, which refer to the concept that adult stem cells may differentiate into specialized cell types of other lineages than where they are derived from. Although many studies on the effects of induction and reduction of Wnt signaling pathway in osteoblasts and bone, the definite mechanism of Wnt signaling during stem cell differentiation is not well known. The possible reason behind that, many proteins involved in Wnt signaling pathway that may communicate with other pathways need to be characterized at the cellular and molecular level. Generally, a better understanding the molecular mechanisms by which these signaling pathway control stem cell differentiation may open new insights to treat many chronic diseases[57-61].

Conclusions

It has become proven that blood could not continually renew, wounds would never heal, fertilized eggs would not grow into fetus, and fetus would not grow into adults without stem cells. A clinical application of stem cells for tissue engineering and regenerative medicine requires effective systems of differentiation. Stem cells are unspecialized precursor cells characterized by their abilities of self-renewal and pluripotency that means they can undergo numerous rounds of cell division maintaining the undifferentiated state and are able to develop into different types of cells such as skin, muscle or blood cells. Mammalian stem cells are generally comprised of two types: embryonic stem cells (ESCs) are considered pluripotent and capable of differentiating into any cell type of the adult body. Adult stem cells (also known as somatic stem cells) are undifferentiated cells found in the tissues of children and adults. Their main function is to replace dead or damaged specialized cells and to maintain the normal turnover of regenerative organs. Most adult

stem cells are lineage-restricted (multipotent) and characterized by a limited self-renewal and differentiation capacity usually restricted to cell types of the tissue in which they reside. In contrast, induced pluripotent stem cells (iPSCs) are not adult stem cells but specialized, somatic adult cells (e.g. epithelial cells) that have been genetically reprogrammed to pluripotent cells which are in many respects similar to natural pluripotent stem cells such as embryonic stem cells. Nevertheless, controlling cell proliferation and differentiation requires additional works on the molecular and genetic signals that regulate cell division and specialization. A complete understanding of the genetic and molecular controls of these processes may yield information about how such diseases arise and suggest new strategies for stem cell therapy.

Acknowledgements

We thank our group research members for their thorough review and helpful discussions during the preparation of this review and library for assistance with collection of references.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Rookmaaker MB, Schutgens F, Verhaar MC, Clevers H. Development and application of human adult stem or progenitor cell organoids. Nat Rev Nephrol 2015; 11:546-554.
2) Harvey L. Molecular cell biology, 5th edition: New York: W.H. Freeman and Co., 2003; p. 973. ISBN: 0-7167-4366-3.
3) Doss MX, Koehler CL, Gissel C, Hescheler J, Sachinidis A. Embryonic stem cells: a promising tool for cell replacement therapy. J Cell Mol Med 2004; 8: 465-473.
4) Lepper C, Conway SJ, Fan CM. Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. Nature 2009; 460: 627-631.
5) Fuchs E, Tumbar T, Guasch G. Socializing with the neighbors: stem cells and their niche. Cell 2004; 116:769-778.
6) Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science 2000; 287: 1427-1430.
7) Joseph NM, Morrison SJ. Toward an understanding of the physiological function of Mammalian stems cells. Dev Cell 2005; 9: 173-183.
8) Kruger GM, Morrison SJ. Brain repair by endogenous progenitors. Cell 2002; 110: 399-402.
9) Passier R, Mummery C. Origin and use of embryonic and adult stem cells in differentiation and tissue repair. Cardiovasc Res 2003; 58: 324-335.
10) Ramos CA, Venezia TA, Camargo A, Goodell MA. Techniques for the study of adult stem cells: be fruitful and multiply. Biotechniques 2003; 34: 572-591.
11) Bianco P, Robey PG. Stem cells in tissue engineering. Nature 2001; 414: 118-121.
12) Derubeis AR, Cancedda R. Bone marrow stromal cells (BMSCs) in bone engineering: limitations and recent advances. Ann Biomed Eng 2004; 32: 160-165.
13) Otto WR, Rao J. Tomorrow's skeleton staff: mesenchymal stem cells and the repair of bone and cartilage. Cell Proliferation 2004; 37: 97-110.
14) Radtke F, Clevers H. Self-renewal and cancer of the gut: two sides of a coin. Science 2005; 307: 1904-1909.
15) Lavker RM, Sun TT. Epidermal stem cells: properties, markers, and location. Proc Natl Acad Sci U S A 2000; 97: 13473-13475.
16) Wu S, Wu Y, Capecchi MR. Motoneurons and oligodendrocytes are sequentially generated from neural stem cells but do not appear to share common lineage-restricted progenitors in vivo. Development 2006; 133: 581-590.
17) Feng Z, Li C, Jiao S, Hu B, Zhao L. *In vitro* differentiation of rat bone marrow mesenchymal stem cells into hepatocytes. Hepatogastroenterology 2011; 58: 2081-2086.
18) Long X, Olszewski M, Huang W, Kletzel M. Neural cell differentiation in vitro from adult human bone marrow mesenchymal stem cells. Stem Cells Dev 2005; 14: 65-69.
19) Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. J Cell Physiol 1999; 181: 67-73.
20) Asakura A, Komaki M, Rudnicki M. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation 2001; 68: 245-253.
21) Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser, JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13: 4279-4295.
22) Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. Stem Cells 2003; 21: 105-110.
23) Hong KY, Bae H, Park I, Park DY, Kim KH, Kubota Y, Cho ES, Kim H, Adams RH, Yoo OJ, Koh GY. Perilipin + embryonic preadipocytes actively proliferate along growing vasculatures for adipose expansion. Development 2015; 15: 2623-2632.

24) Kawaguchi J, Mee PJ, Smith, AG. Osteogenic and chondrogenic differentiation of embryonic stem cells in response to specific growth factors. Bone 2005; 36: 758-769.

25) Pacios S, Xiao W, Mattos M, Lim J, Tarapore RS, Alsadun S, Yu B, Wang CY, Graves DT. Osteoblast lineage cells play an essential role in periodontal bone loss through activation of nuclear factor-Kappa B. Sci Rep 2015; 15: 16694-16706.

26) Nuttle MA, Gimble JM. Is there a therapeutic opportunity to either prevent or treat osteopenic disorders by inhibiting marrow adipogenesis?. Bone 2000; 27: 177-184.

27) Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M. Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. Biogerontology 2001; 2: 165-171.

28) Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stromal stem cells nature, biology, and potential applications. Stem Cells 2001; 19: 180-192.

29) Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418: 41-49.

30) Colaianni G, Brunetti G, Faienza MF, Colucci S, Grano M. Osteoporosis and obesity: Role of Wnt pathway in human and murine models. World J Orthop 2014; 5: 242-246.

31) Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 2005; 8: 739-750.

32) Inada M, Yasui T, Nomura S, Miyake S, Deguchi K, Himeno M, Sato M, Yamagiwa H, Kimura T, Yasui N, Ochi T, Endo N, Kitamura, Y, Kishimoto T, Komori T. Maturational disturbance of chondrocytes in Cbfa1-deficient mice. Dev Dyn 1999; 214: 279-290.

33) Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA. Novel mechanism of Wnt signaling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nature Cell Biol 2001; 3:683-686.

34) Dennis JE, Charbord P. Origin and differentiation of human and murine stroma. Stem Cells 2002; 20: 205-214.

35) Nuttall MA, Gimble JM. Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications. Curr Opin Cell Biol 2004; 4:290-294.

36) Verma S, Rajaratnam JH, Denton J, Hoyland JA, Byers RJ. Adipocyte proportion of bone marrow is inversely related to bone formation in osteoporosis. J Clin Pathol 2002; 55: 693-698.

37) Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways. Aging Cell 2004; 3: 379-389.

38) Longo KA, Wright WS, Kang S, Gerin I, Chiang SH, Lucas PC, Opp MR, MacDougald OA. Wnt10b inhibits development of white and brown adipose tissues. J Biol Chem 2004; 279: 35503-35509.

39) Nuttle MA, Gimble JM. Is there a therapeutic opportunity to either prevent or treat osteopenic disorders by inhibiting marrow adipogenesis?. Bone 2000; 27: 177-184.

40) Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M. Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. Biogerontology 2001; 2: 165-171.

41) Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stromal stem cells nature, biology, and potential applications. Stem Cells 2001; 19: 180-192.

42) Adegani FJ, Langroudi L, Arefian E, Shafiee A, Dinarvand P, Soleimani M. A comparison of pluripotency and differentiation status of four mesenchymal adult stem cells. Mol Biol Rep 2013; 40: 3693-3703.

43) Keller G. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. Genes Dev 2005; 19: 1129-1155.

44) Gimble JM, Robinson CE, Wu X, Kelly KA. The function of adipocytes in the bone marrow stroma: an update. Bone 1996; 19: 421-428.

45) Adler BJ, Kaushansky K, Rubin CT. Obesity-driven disruption of haematopoiesis and the bone marrow niche. Nat Rev Endocrinol 2014; 12: 737-748.

46) Nuttall ME, Gimble, JM. Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications. Curr Opin Pharmacol 2004; 4: 290-294.

47) Nuttall ME, Patton AJ, Olivera DL, Nadeau DP, Gowen M. Human trabecular bone cells are able to express both osteoblastic and adipocytic phenotype: implications for osteopenic disorders. J Bone Miner Res 1998; 13: 371-382.

48) Pan Z, Yang J, Guo C, Shi D, Shen D, Zheng Q, Chen R, Xu Y, Xi Y, Wang J. Effects of hind limb unloading on ex vivo growth and osteogenic/adipogenic potentials of bone marrow-derived mesenchymal stem cells in rats. Stem Cells Dev 2008; 17: 795-804.

49) Epperla N, Fenske TS, Lazarus HM, Hamadani M. Post-autologous transplant maintenance therapies in lymphoid malignancies: are we there yet?. Bone Marrow Transplant 2015; 50: 1393-1404.

50) Lee SJ, Fairclough D, Parsons SK, Soiffer RJ, Fisher DC, Schlossman RL, Antin JH, Weeks JC. Recovery after stem-cell transplantation for hematologic diseases. J Clin Oncol 2012; 19: 242-252.

51) Ballen KK, Spitzer TR, Yeap BY, McAfee S, Dey BR, Attar E, Haspel R, Kao G, Liney D, Alyea E, Lee S, Cutler C, Ho V, Soiffer R, Antin JH. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. Biol Blood Marrow Transplant 2007; 13: 82-89.

52) Silva AM, Diabangouaya B, Salaun PJd, Dieterlen-Lievre F. Mouse placenta is a major hematopoietic organ. Development 2003; 130: 5437-5444.

53) Alousi AM. Graft-versus-host disease: part I. Pathogenesis and clinical manifestations of graft-versus-host disease. J Am Acad Dermatol 2012; 66: 515.e1-515.e18.

54) Hymes SR, Alousi AM, Cowen EW. Graft-versus-host disease: part II. Management of cutaneous graft-versus-host disease. J Am Acad Dermatol 2012; 66: 535-551.

55) Date T, Doiguchi Y, Nobuta M, Shindo H. Bone morphogenetic protein-2 induces differentiation of multipotent C3H10T1/2 cells into osteoblasts, chondrocytes, and adipocytes in vivo and in vitro. J Orthop Sci 2004; 9: 503-508.

56) Brambrink T, Hochedlinger K, Bell G, Jaenisch R. ES cells derived from cloned and fertilized blastocysts are transcriptionally and functionally indistinguishable. Proc Natl Acad Sci U S A 2006; 103: 933-938.

57) Hug K. Therapeutic perspectives of human embryonic stem cell research versus the moral status of a human embryo – does one have to be compromised for the other?. Medicina (Kaunas, Lithuania) 2006; 42: 107-114.

58) Ali F, Ranneh Y, Ismail A, Vaes B. Impaired of a non-DNA dependent methylation status decides the fat decision of bone marrow-derived C3H10T1/2 stem cell. Springerplus 2013; 2: 2-10.

59) Herrmann M, Binder A, Ursula U, Zeiter S, Alini M, Verrier S. CD34/CD133 enriched bone marrow progenitor cells promote neovascularization of tissue engineered constructs in vivo. Stem Cell Res 2014; 13: 465-477.

60) Blaber EA, Dvorochkin N, Torres ML, Yousuf R, Burns BP, Globus RK, Almeida EA. Mechanical unloading of bone in microgravity reduces mesenchymal and hematopoietic stem cell-mediated tissue regeneration. Stem Cell Res 2014; 13: 181-201.

61) Ali F, Ranneh Y, Ismail A, Vaes B. Influence of demethylation pathway on the differentiation potential of human mesenchymal stem cells. J Stem Cell Res Ther 2013; 3: 1-6.