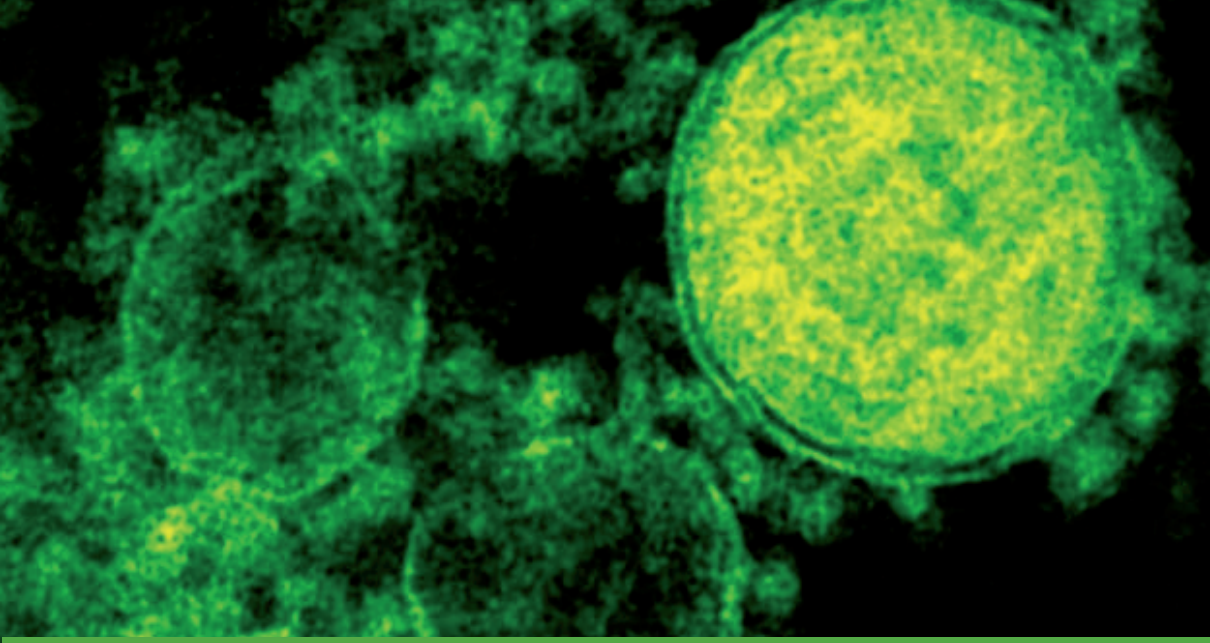
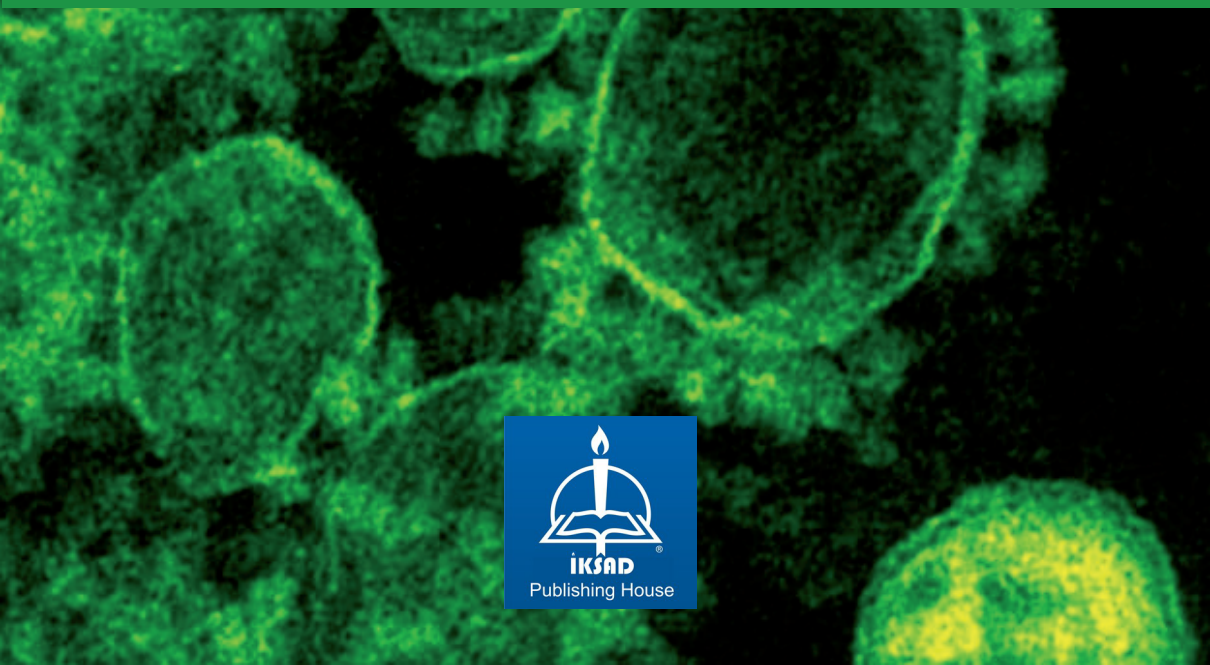


Mesenchymal Stem Cells (Especially Adipose-Derived Stem Cells): Innovative Therapeutic Approachs

İlhan ÖZDEMİR

Şamil ÖZTÜRK



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İlhan ÖZDEMİR[1], Şamil ÖZTÜRK[2]

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PREFACE

Mesenchymal Stem Cells (MSC) have become a valuable resource because of their abundance and isolation. It is prominent that MSCs may provide a therapeutic modality for the treatment of any disease. After, bone marrow, umbilical cord blood and the third molar, scientists have look for stem cells in human fat tissue, and they have discovered that there are much more stem cells in human fat tissue than in any other resource. In vitro studies done on these cells show that direct stem cell soybean optimization can be done from these cells depending on many variables. Part of the most important population of adult stem cells, mesenchymal stem cells are full-featured cells that reside usually in blood vessel walls and they participate in all rehabilitative functions. They form both such different tissues as bones and cartilage, and they take charge of increasing blood build up in the wounded area and of speeding the healing process. Recent studies have contended that there are 300-500 times more stem cells in 1 ml fat tissue than in bone marrow. Although there are stem cells in every tissue in the body and although stem cells have been obtained from such tissues as heart muscle, brain, and bone marrow, the fat tissue has proven to be the most prolific on this issue. In this book, we aimed to contribute to clinical practice. While writing our book, we benefited from many local and foreign sources. We sincerely thank the authors and those who contributed. We wish it to contribute to science and be useful. We look forward to your warnings, suggestions and support.

Regards.

İÇİNDEKİLER

FIGURE LISTS

INTRODUCTION

Stem cells are self-regenerating and undifferentiated cells that can transform into many specialized cell types when they receive appropriate signals in body and laboratory settings. Stem cell is defined as " functionally undifferentiated cell with heterogeneous reproductive potential ". According to another definition, the stem cell is a primitive cell that renews itself by dividing, keeps its numbers constant, forms specialized organs such as blood, liver and muscle, and is capable of differentiation (Akashi et al., 2000). Stem cell studies started with hematopoietic stem cell discovery in the 1960s. This was followed by the presence of stromal stem cells (mesenchymal cells). In the 1990s, scientists detected nerve stem cells in the mammalian brain. In later years, the presence of stem cells in the epidermis, liver and many other organs has been scientifically proven. Adult stem cells, stem cells derived from cord blood and embryonic stem cells are the three main sources of stem cells known today. These cells have the potential to transform into very different specialized tissue cells when they are stimulated with special biological signals as well as they can differentiate into specialized cells of the tissues they originate from (Karaşahin, 2012).

General Properties of Stem Cells

Differentiation (Plasticity)

Differentiation is used to describe a series of changes that cells that make up multicellular organisms undergo in the process of maturation and specialization (Figure 1). Differentiation is a complex set of

complex events achieved by the combined effect of cytokines, growth and difference factors, extracellular matrix proteins and intercellular communications. The cell, which is noticed, is prepared to respond to the signals coming from its environment, while also stopping the division. To do this, it usually reveals enzyme-dependent surface receptors, intracellular receptors and activation pathways, triggering the onset of certain events in the cell. For example, Eiraku et al. showed that a neuronal stem cell was noticed in glial precursors in the presence of Notch signaling (Eiraku et al., 2005). If the cell expressing the Notch signaling receptor interacts with its ligand, DNER (Delta-notch-like epidermal growth factor-related receptor), the glial cell formation is induced (Figure 2). In contrast, some oncogene products may reverse discrimination; In this way, an adult cell can acquire pluripotent property and turn into a malignant tumor cell. Cutaneous Kaposi's Sarcoma is a tumor tissue caused by human herpesvirus 8 and is one of the diseases that indicate AIDS (Acquired Immune Deficiency Syndrome). The forward recognition process for a cell usually starts from the point at which the proliferation process of that cell ends. Therefore, both processes generally do not occur at the same time. The cell in question reaches a sufficient number in the proliferation process, then the cell surface and intracellular pathways related to proliferation (ie self-renewal process) are usually closed and mechanisms for recognition are activated. During this process, the cell leaves the division cycle permanently or temporarily and enters the G0 phase. Ensuring that the stem cells are noticed in a certain line or directed differentiation in the laboratory; It is accomplished either by fulfilling

certain chemical and physical conditions or by directly modifying the genetic program of the cell. For example; Natural hormones and artificial chemicals such as dexamethasone, indomethacin, isobutyl methylxanthin and insulin are added to the culture medium to differentiate an adult stem cell into the fat cell. Although it is not known whether these substances stimulate the transformation of stem cells into fat cells in vivo, the fat cells obtained in this way usually mature in a few weeks compared to their in vivo counterparts. Similarly, when dexamethasone, ascorbic acid and β-glycerophosphate are added to the culture medium, osteogenic differentiation is provided (Matur and Solmaz, 2011).

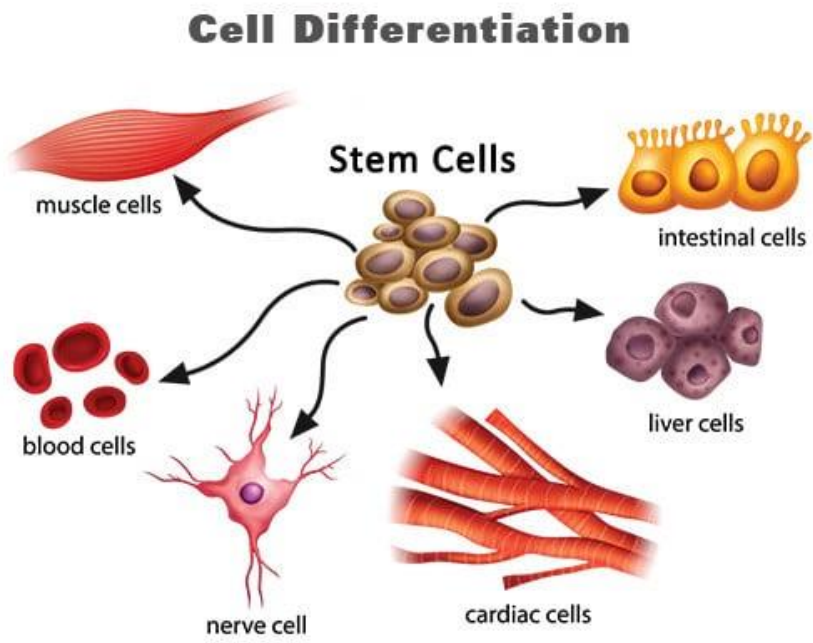

Figure 1. Cells differentiation (https://www.allevi3d.com/reprogramming-the-fate-of-cells/, Erişim 30.09.2020).

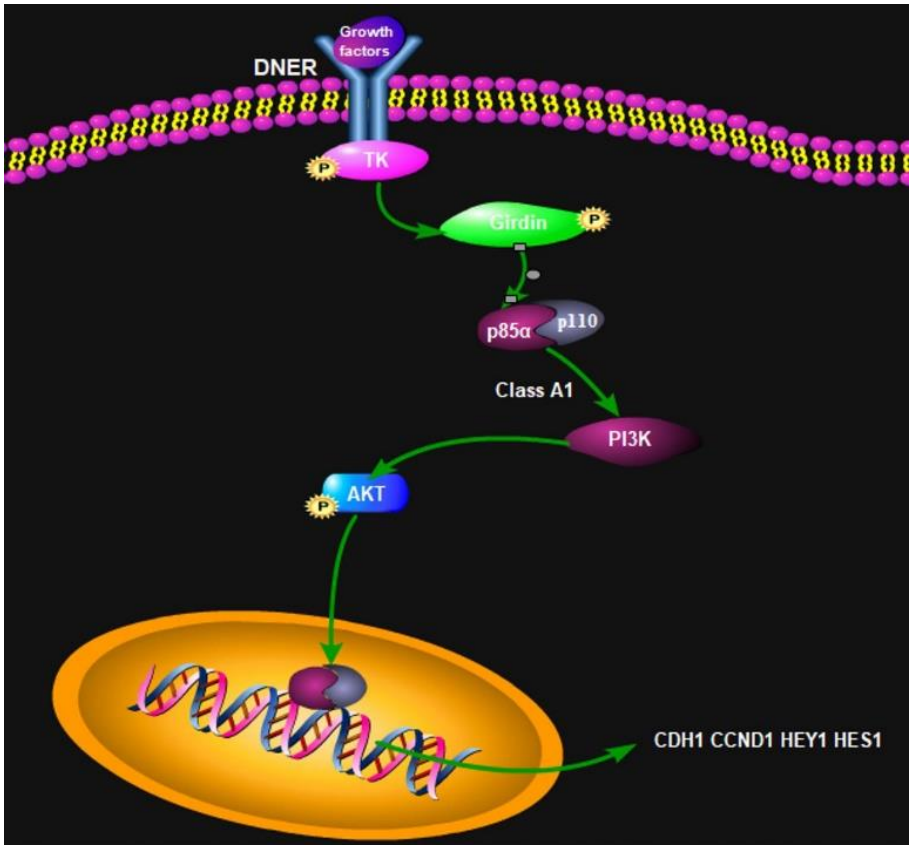

Figure 2. DNER signaling (Wang et al. 2019).

Re-differentiation and Stimulated Pluripotent Stem Cells

Another way to differentiate in vitro is to genetically reprogram using various vectors, such as viral or plasmid. Stimulated pluripotent stem cells are obtained in this way. Somatic cells, by using various viral or non-viral vectors, activate genes specific to Oct3 / 4, Sox2, klf4, c-Myc and similar embryonic stem cells, providing back differentiation (Figure 3, Figure 4) (Ullah et al., 2015). Intermediate difference is the difference of the cell that has been noticed in one direction towards another. In the Wolf repair event that takes place in the eyes of the

salamander, iris cells are noticed to form the lens by removing the lens in the eye. The concept of intermediate difference is still open to debate, as such examples are often not encountered. However, the concept of metaplasia or beyond difference in pathology can be accepted as an intermediate difference model. In metaplasia, the transformation of some of the gastric epithelial cells or stem cells into intestinal epithelial cells (intestinal metaplasia) can be considered as an intermediate differentiation model (Matur and Solmaz, 2011). One of the general features of the stem cell is its self-renewal feature. The stem cell replicates throughout its lifetime, without any specialization, and transforms into organ and tissue-specific precursor cells, if necessary. Stem cells produce the cell that will be noticed on the leading cell during the division, while also making its own backup. This event is the result of asymmetric cell division and ensures that the stem cell pool remains stable for life. Drosophila ovaries show asymmetric cleavage (Weissman, 2000).

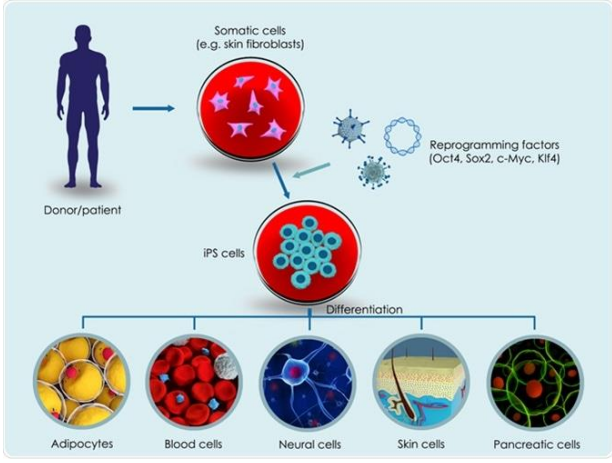

Figure 3. İPSCs reprogramming *(*https://www.news-medical.net/life-sciences/Genes-that-Control-Pluripotency.aspx*, Erişim 30.09.2020)*.

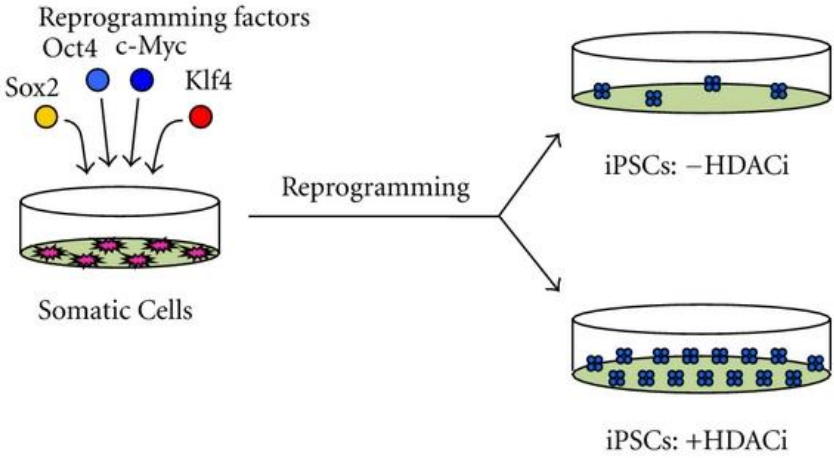

Figure 4. iPSCs by ectopic expression of the four transcription factors (Oct4, Sox2, Klf4, and c-Myc) (https://www.hindawi.com/journals /bmri/2011/835968/fig2/, Erişim: 30.09.2020).

Asymmetric cell division requires very tight control of both intracellular and extracellular factors together. The destinies of the cells in different microenvironments are also different. The extracellular matrix components that make up the niche, adjacent cells, and secretory proteins control the number of stem cells (Figure 5). For example, the axis of division of stem cells in the Drosophila ovary is determined by the niche; The mitosis shuttle is positioned at right angles to the niche. Thus, while the cell near the niche maintains its stem cell feature, the distant ones are noticeable. Asymmetry in the cell is accomplished by transferring some organelles, protein groups and RNA to only one of the offspring cells. Some studies show that DNA is also distributed asymmetrically (Figure 6). At the end of the division, the original DNA goes to one of the juvenile cells and decides, and new DNA synthesis takes place in the other cell, which will turn into a leader cell. Thanks

to this mechanism, stem cells are protected from mutations that may occur in the newly synthesized DNA and will cause accumulation and always remain intact as cells with the same genome. Although asymmetric cell division is necessary to keep the stem cell pool stable, symmetric cell division must also occur in order to meet the new cell requirement required in the development process and tissue repair of the embryo. Especially in cases of destruction of tissue functions, this mechanism turns stem cells into pioneer cells and ensures repair in a short time. In addition, stem cells divide symmetrically and form new stem cells (Biyolojisi CAKH, 2014).

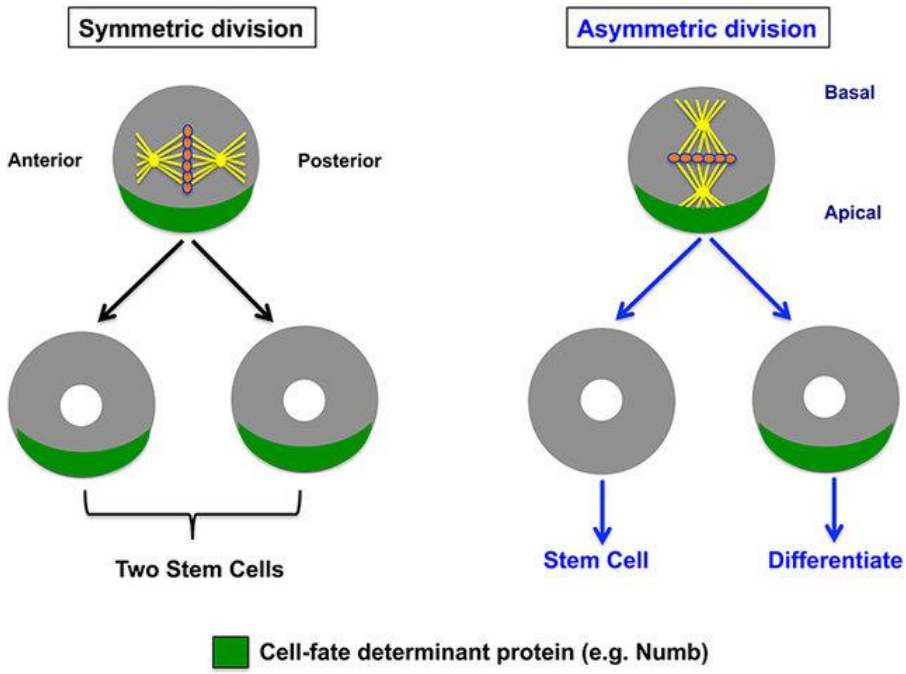

Figure 5. Symmetric vs. asymmetric cell division mammalian cells (Berika et al., 2014).

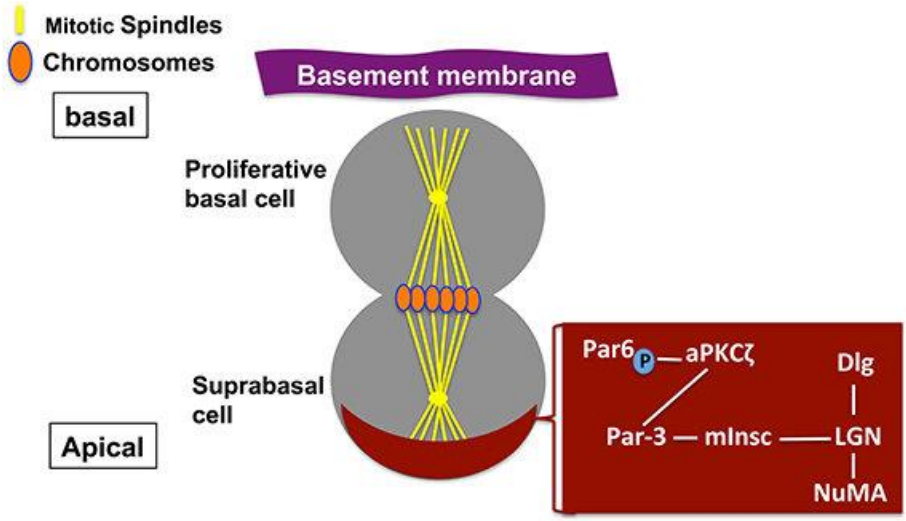

Figure 6. Asymmetric cell division in mammalian epithelia (Berika et al., 2014).

The DNA chains, called telomeres, determine the dividing capacity of the cells at the tip of the chromosomes. The longer the telomeres, the more cells can divide. The activity of the telomerase enzyme, which allows the telomeres to remain long, is very high in stem cells, so they have a large number of cleavage capacities (Matur and Solmaz, 2011). The term rootness is used to describe the cellular and molecular properties that distinguish stem cells from other cells. These features, which are accepted as the signature of stem cells, are unique gene expressions or a series of changes after translation, thanks to which stem cells retain their original structure and function regardless. The stem cell type can be determined using markers on the surface of the cells that act as signal pathways or cell-cell adhesion molecules in the cell. Many of these markers were collected under a title as clusters of differentiation CD (Clusters of differentiation). For example; the most

common CD markers CD33 and CD45 for hematopoietic stem cells; for mesenchymal stem cells, it is CD29, CD79, CD105. Several CD antigens are associated with mouse and human Embryonic root (ESC). CD9 is known to be developmentally regulated in both fee and human ESCs. Pluripotent human Embryonic stem cell CD antigens are CD24, CD30, CD50, CD90, CD133, CD200 and CD326. However, CD30 is not always expressed in human ES cells. CD133 is also a hematopoietic stem cell marker. In addition, human Embryonic stem cells express markers such as CD90 and CD117. However, CD133 and CD96 are also expressed in some tumor cells. The expression of the other set of differentiation antigens is firmly with the undifferentiated state but reflects the presence of progenitor cells in a human ES culture such as CD184 and CD87, which are considered as lineage markers. The CD147 antigen does not reflect either the differentiated or undifferentiated state, but has proven useful as a pan-human marker. Apart from difference clusters, transcription factors, enzymes or growth factors are also counted among markers (Figure 7) (Ullah et al., 2015). The classification of stem cells is done in two ways, considering the source and differentiation potential. Stem cells are named as totipotent, pluripotent, multipotent and unipotent according to their differentiation potential. In the second type classification, embryonic, fetal, placenta, cord blood, adult and cancer stem cells are defined according to the source (Matur and Solmaz, 2011). Totipotent cells are cells capable of forming an entire organism. Each blastomer totipotent cell in Morula stage can be exemplified (Figure 8, Figure 9) because each blastomer can form individual embryonic and extra embryonic structures (Brook

and Gardner, 1997). These cells are stem cells that give the embryo, post-embryo all tissues and organs, and non-embryo membranes and organs, and have the ability to unlimited differentiation and go in different directions. All blastomers up to 8 cells (Table 1) are totipotent in the early embryonal period (Karaşahin, 2012).

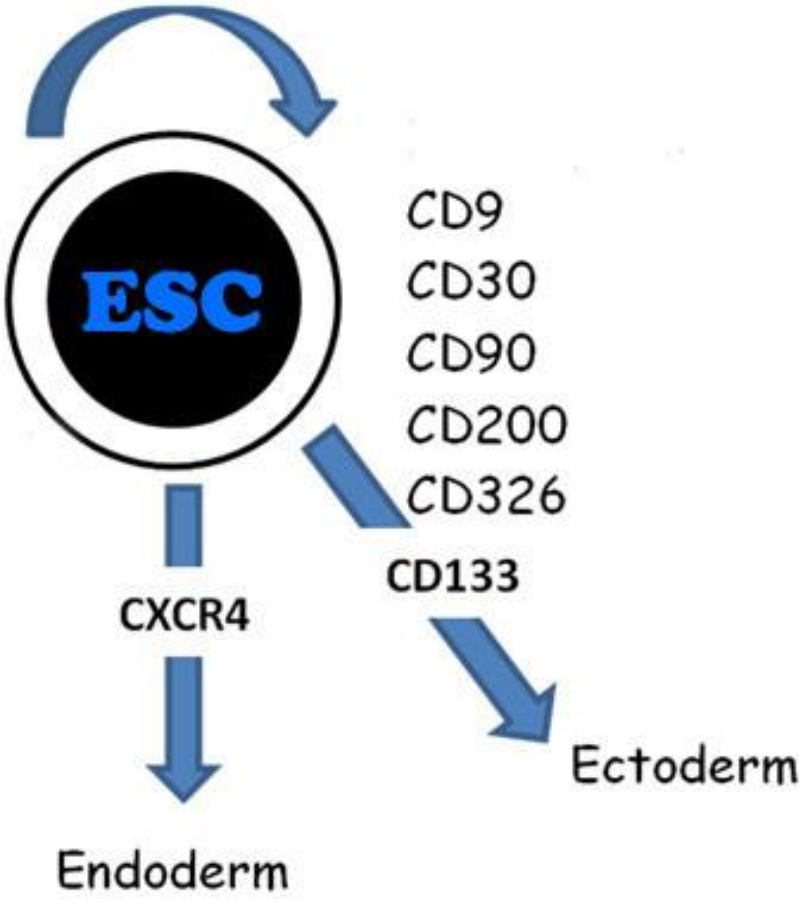

Figure 7. ESC differantiation markers *(*https://www.sinobiological.com/research/cd-antigens/cluster-of-differentiation-stem-cells, *Erişim: 30.09.2020).*

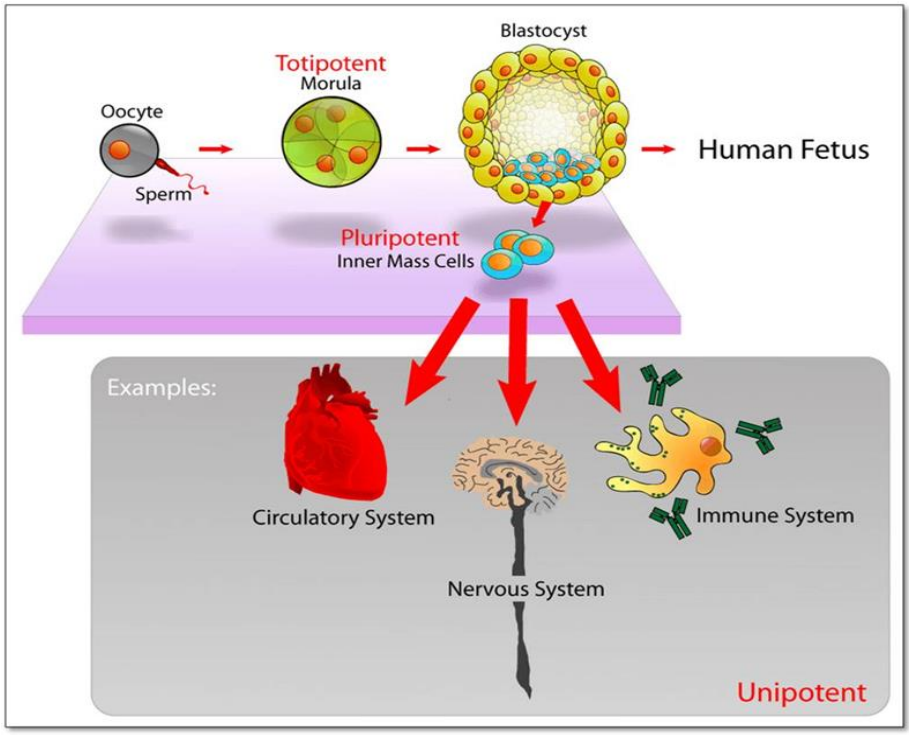

Figure 8. According to the potential of differentiation, totipotent, pluripotent and unipotent stem cells (www.koKİMKHcrenedir.com, Accessed date: 12.08.2020).

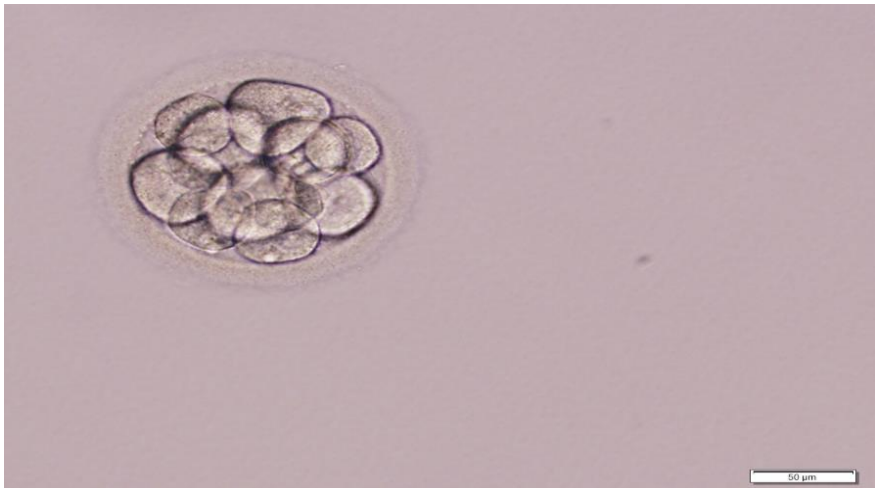

Figure 9. Fourth days embryos of morula stage (our lab obtained).

They are stem cells that cause the formation of many tissues in the organism. After compaction and blastocyst formation, cells in the inner cell mass are pluripotent and these cells have the ability to differentiate many cells of the body (Table 1, Figure 10). In the embryo, the cells in the inner cell mass of the blastocyst can differentiate into many different types of cells originating from endoderm, ectoderm, and mesoderm. Embryonic stem cells are derived from the blastocyst's inner cell mass and are pluripotent. Embryonic stem cells contain high levels of telomerase activity, no reduction in activation by cell replication. Therefore, they have unlimited proliferation capacity (Karaşahin, 2012).

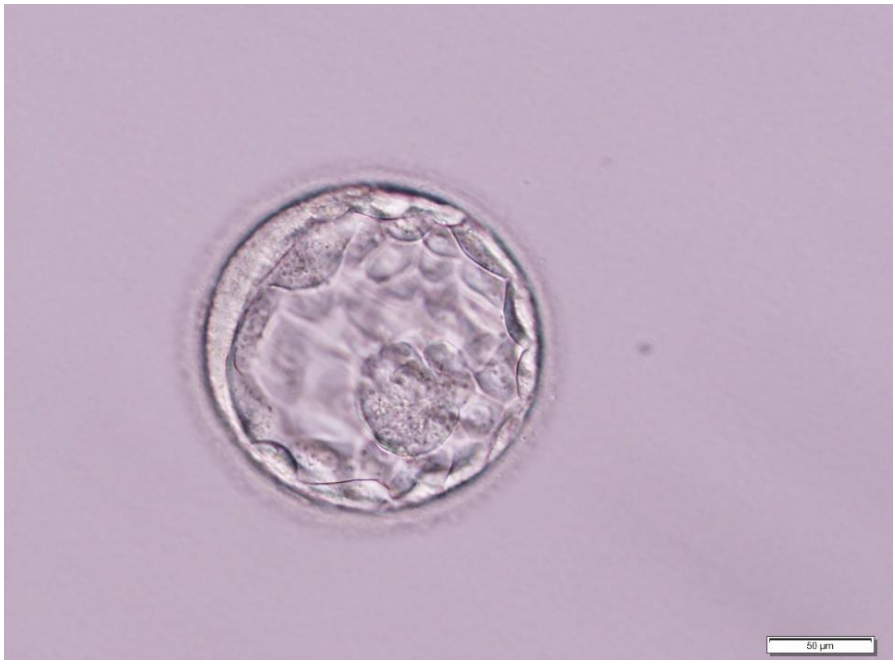

Figure 10. Compaction and blastocyst formation.

Table 1. Differentiation aspects of stem cells according to their potential to be different (Matur and Solmaz 2011).

Name	Cell type	Differentiation efficiency	Differentiation direction
ESC	Cells in the morula	Totipotent	Embryo and non-embryo tissues
ESC	Inner cells	Pluripotent	Embryo body (all somatic and germ cells)
ESC	Epiblast cells	Pluripotent	Endoderm, mesoderm and ectoderm cells
ESC	Endoderm, mesoderm and ectoderm cells	Pluripotent	All somatic cells
ASC	Specific tissue cells	Multipotent	One or more types of cells based on tissue
ASC	Resident cells in a tissue	Unipotent	One type cells

Multipotent stem cells are cells of the later stage of development and may differ in specialized cell types. Multipotent stem cells are cells that are formed by the division of these cells and have been programmed to differentiate in one direction. In later stages of development (fetal life), cells have some more specific tasks and turn into adult stem cells. These adult stem cells typically produce cell types of tissue in which they are located. Bone marrow stem cells are the best example. For example, a multipotent blood cell has the ability to transform into other specialized blood cells. Cord blood and adult stem cells are multipotent cells.

Embryonic stem cells are obtained from the inner cell mass in the blastocyst and can differ to any cell type in the organism (Thomson et al., 1998). Evans and Kaufman succeeded in obtaining embryonic stem cells from the early mouse embryo in 1981. After this study, Thomson et al. First derived human embryonic stem cell lines in the laboratory in 1998. Although these cells first appeared for reproductive purposes in in vitro fertilization methods, they were later donated for use in experimental research. In 2007, the same researchers identified specific conditions that allowed the formation of stem cell-like cells from some specialized adult stem cells by genetically reprogramming and named them pluripotent stem cells. Embryonic stem cells, which are among the stem cell types, are a stem cell group that is emphasized in tissue engineering and regenerative medicine because of its capacity to transform into all kinds of cells and tissues in living organism (Kansu, 2005). Embryonic stem cells are obtained from embryos that have reached the blastocyst stage in the early development period before implantation. An embryo at this stage consists of two different cell types. The cells called trophectoderm located on the outside form the placental structure after implantation. Cells in the form of a mass in the interior form the fetal structure. Embryonic stem cells are obtained by separating these internal cells using special immunological and mechanical methods and by incubation in environments containing special media and growth factor. Embryonic stem cells are pluripotent cells and, when stimulated with appropriate signals, they have the capacity to turn into approximately 200 cell types in the body (Figure 11). Embryonic stem cells have become the focal point of regenerative

medicine thanks to two very important features. These are the capacity to proliferate without being differentiated by the self-renewal process and the potential to form specialized cell types when they are induced for differentiation (Karaşahin, 2012).

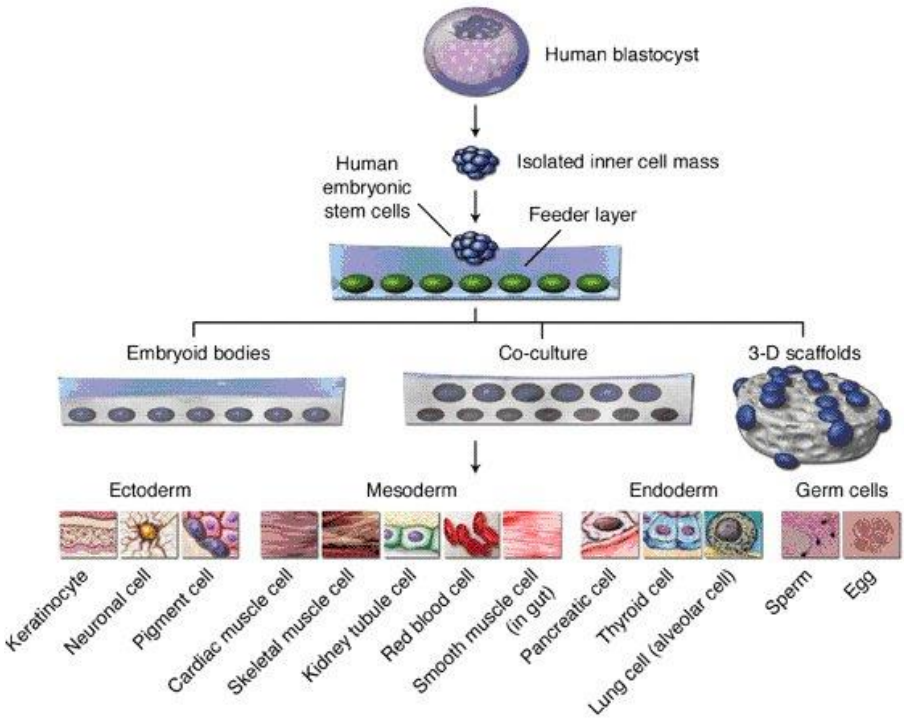

Figure 11. Derivation of a human embryonic stem cell line, and ES cell differentiation strategies (Hyslop LA., et al. 2005)

Pluripotency markers are used to identify embryonic stem cells. Of these, Oct4 and Nanog are important molecules. Embryonic stem cells from both human and mouse were found to be Sox2, CD9, CD133 positive. On the other hand, while mouse embryonic stem cells are positive for stage-specific embryonic antigen-1 (SSEA-1), SSEA-3 and 4 are TRA-1-60 and TRA-1-81 negative; In human embryonic stem

cells, SSEA-1 is negative, SSEA-3 and 4 are TRA-1-60 and TRA-1-81 positive (Figure 12). The main research topics in which embryonic stem cells are used in basic sciences are human development, toxicology, and transplantation medicine. However, studies of embryonic stem cells show that these cells are promising for many diseases that are not currently possible to treat in the near future. Thus, diseases that develop due to loss of cells that do not have the capacity to renew and repair themselves can be treated. These include Parkinson's disease, Alzheimer's disease, multiple sclerosis, accidental paralysis and other diseases caused by the loss of neurons, heart muscle failure, osteoarthritis, bone-cartilage loss, cancer and immune system diseases and diabetes. On the other hand, there are drawbacks in terms of ethical and medical practices regarding the use of embryonic stem cells. Continuous culture of human embryonic stem cells in an undifferentiated step requires animal-based material and nutrient layer. This carries the risk of cross-pathogen contamination. Human embryonic stem cells show high genomic instability and may unpredictably differentiate after long-term development. In addition, differentiated embryonic stem cells can express molecules that can cause immune rejection. It is one of the problems to be overcome before the therapy how the cells that are reproduced in a controlled manner and differentiated to a specific cell type are placed in the appropriate area in the patient and how they are adapted to the appropriate (Matur and Solmaz, 2011). As a result of the studies carried out in the late 1980s and early 1990s, it has been realized that the umbilical cord and the plesenta are a rich source for hematopoietic stem cells. This issue also

supports the development process in the fetus during pregnancy. In the blood of the umbilical cord, which provides the nutrient and oxygen requirement of the baby by providing the connection between the mother and the baby during pregnancy, in addition to the blood cells such as erythrocytes, leukocytes and thrombocytes, there are stem cells that are higher than adult blood. Cord blood, which was excreted in the old years, can now be used for therapeutic purposes or can be stored under special conditions. The only medically accepted field of use for today is blood and immune system diseases. Since cord blood is in a small volume, approximately 100 ml, the total amount of hematopoietic stem cells it contains is less than that obtained from bone marrow or growth factor-induced peripheral blood. Therefore, umbilical cord blood recipients are typically children. However, when it is realized that the blood taken from several babies can be applied to a single patient recently, it has also been used in adults. The most commonly used case in the world for the moment is the use of stem cell transplant treatment but for the treatment of patients who are not among the family members or who cannot find suitable donors. However, it is necessary to investigate whether the tissue compatibility molecules between the recipient and the donor are compatible during use. In the family, Human Leukocyte Antigens (HLA) is a fully suitable or at most one antigen incompatible donor, bone marrow / peripheral stem cell ideal donor. If no one with these features is found, non-relative donors come into play. While interpersonal transplants can tolerate an antigen incompatibility, allele level alignment should be achieved in high-resolution typing of both HLA-A, -B, -C and HLA-DRB1 regions. Otherwise, the frequency

of Graft versus host disease (GVHD) increases in one allele incompatibility, and the lifespan is shortened in more than one allele incompatibility compared to the most suitable (Gluckman, 2011). The placenta amniotic membrane, which provides the physical and functional relationship between the embryo and the mother, consists of chorion and maternal endometrium layers. Stem cells in amnion and chorion originate from the non-embryo mesoderm. Although obtained from all three trimesters, the amniotic membrane mesenchymal stem cells are mostly obtained during childbirth. The amniotic membrane has similar surface markers as mesenchymal stem cells, bone marrow and cord blood derived stem cells. However, unlike other adult mesenchymal stem cells, they also carry embryo stem cell markers. Because of these features, they have a higher potential for differentiation. 15.-18 of pregnancy There are also stem cells in the amniotic fluid taken by amniocentesis in order to make genetic diagnoses in the weeks. Approximately 1% of the cells from amniocentesis samples contain the c-kit (CD117), which is the stem cell factor receptor, while the other cells are cells that have become different and come from the fetus skin. Cells containing c-kit have been found to be capable of proliferation when separated and cultured by magnetic immune selection analysis. Amniotic fluid stem cells' self-renewal time is approximately 36 hours and does not need a nutritious cell layer. It has been observed that when appropriate signals are provided, amniotic fluid stem cells can differentiate into cells belonging to all three germ leaves. Looking at the characterization of amniotic stem cells, MHC-1

and HLA-ABC are positive for CD29, CD44, CD90 and CD105, while CD34 and CD45 show negative properties (Biyolojisi CAKH, 2014).

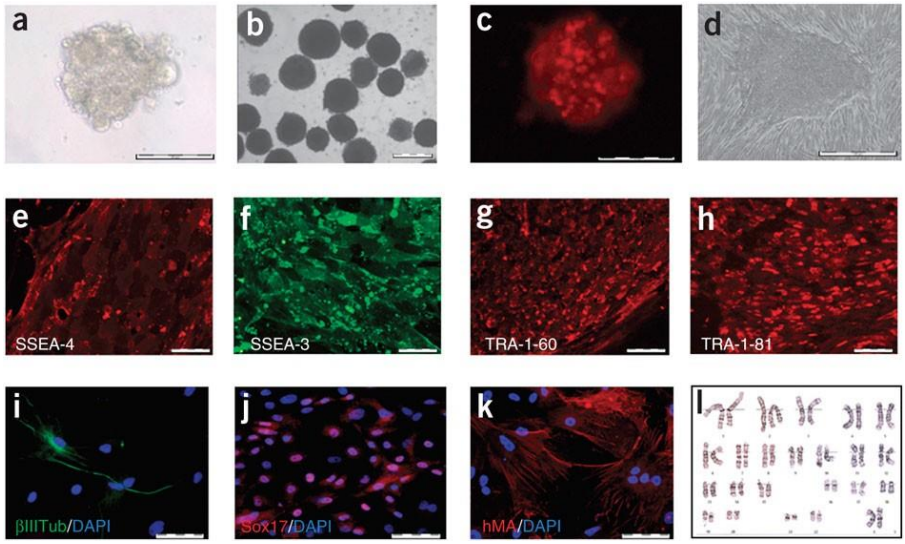

Figure 12. (**a**,**b**) An inner cell mass after transfer to suspension culture conditions (**a**), and the inner cell mass after 10 weeks of cultivation (**b**). (**c**) Fluorescence image showing alkaline phosphatase activity (**d**–**h**) After plating on feeders, the clusters gave rise to colonies with morphological characteristics of colonies of undifferentiated hESCs (**d**, phase contrast image), which were comprised of cells immunoreactive with anti-SSEA-4 (**e**), SSEA-3 (**f**), TRA-1-60 (**g**) and TRA-1-81 (**h**) (fluorescence images). (**i**–**k**) Immunostaining of in vitro–differentiated progeny, representing the three embryonic germ layers, within the outgrowth of plated embryoid bodies (β-III tubulin, (**i**); SOX-17, (**j**); human muscle actin, (**k**)). (**l**) G-banding analysis showing a normal karyotype after 10 weeks of cultivation in suspension. Nuclei are

counterstained by DAPI in **i**–**k**. Scale bars, 20 μm (**a**, **e**–**k**); 50 μm (**c**); 100 μm (**b**,**d**). HAD17 hESC line (Steiner et al., 2010).

There has been a rapid improvement in the collection and therapeutic application of these cells since the first successful cord blood transplantation in children with Fanconi anemia. The New York placenta blood program center is the largest human cord blood bank in the United States, backed by the National Institutes of Health (NIH). It currently contains about 13,000 donor samples for transplantation purposes for patients who need hematopoietic stem cells. It has started to collect cord blood since 1992 and there are thousands of cord blood units in this center for patients (Matur and Solmaz, 2011). Adult stem cells can regenerate themselves in the tissues in which they are found, in the event of cell death and tissue damage, and differentiate into specific cells of the tissue or organ in which they are located (Kørbling and Estrov, 2003). The term "somatic stem cell" is also used instead of the adult stem cell. As an organism matures, the number of stem and precursor cells decreases. Thus, tissues in adults contain few stem and precursor cells; these cells are limited to different anatomical locations. Most of the cells in a mature tissue are differentiated cells that have adapted to their environment and have certain phenotypic properties. Consequently, an organ's regeneration capacity decreases with age and in proportion to the number of stem and precursor cells that can divide effectively. With these limitations, the body has developed two major strategies for replacing and regenerating tissues. In the first way, there is the capacity to multiply in differentiated and functioning cells. Liver,

skeletal muscle and vascular endothelial cells are included in this group after migration, where mitogens are released enough to direct limited replacement of cell loss in that area, and thus cell division is stimulated. Examples include bone marrow stem cells, peripheral blood stem cells, mesenchymal stem cells, and adult stem cells located in organs. In addition, neuronal stem cells, dental pulp and stem cells originating from adipose tissue, epidermal stem cells, liver stem cells and stem cells obtained from cadaver are other stem cells located in organs (Shamblott et al., 1998). Hematopoietic stem cells (HSC) are self-renewable bone marrow or multipotent stem cells that can be isolated from blood and differentiate into different types of cells. They can develop into the bloodstream by exiting the bone marrow. They may also be exposed to programmed cell death called apoptosis. Processes such as hematopoietic stem cells to renew themselves, to remain silent in the G0 phase of the cell cycle, to adhere, to proliferate, to mature, to go into differentiation, to enter the circulation are provided in special microenvironments in the bone marrow. In this area called niche, there are osteoblasts, osteoclasts, stromal cells, extracellular matrix components, molecules, factors, cytokines, which are cells specific to bone marrow, and interactions between them ensure that hematopoietic stem cell functions and hematopoiesis remain constant. Recommended surface markers for hematopoietic stem cells; CD34 +, CD59 +, Thy1 +, CD38 ±, C-kit ±, lin--. Bone marrow is the classic source of HSC. For more than 40 years, they have performed bone marrow transplantation by pulling cells from the bone marrow, typically by piercing the hip bone with a syringe under anesthesia of the stem cell

donor (Figure 13). 1 / 10-100,000 of the cells obtained from the marrow are in the form of stem cells. Other cells are stromal cells, stromal stem cells, progenitor blood cells, mature or maturing erythrocytes and leukocytes. Bone marrow transplant application with the part directly removed from the bone, which was of extreme curiosity in the past, has now been put into practice with medical use by being thrown from the source of the hematopoietic stem cell for medical treatment. Regarding transplantation in the clinic, peripheral donor stem cell collection is used as a new method. It has been known for many years that there are few stem cells and progenitor cells in the circulating bloodstream. The researchers have found that over the past 10 years, they can inject cytokines such as granulocyte colony-stimulating factor (G-CSF) into the donor to remove a large number of cells from the bone marrow into the peripheral circulation. The procedure is started by injecting G-CSF a few days before the cells are harvested. By placing a tube in the vein by the doctors to the donor where the cell will be collected, CD34 + cell-containing leukocytes are collected by the filter system between them and the erythrocytes are returned to the donor. These collected cells are 5-10% stem cells. Thus, researches commonly prefer peripheral blood in stem cell collection. Actually; peripheral CD34 + cells are actually a mixture of different degrees of mature leukocytes, stem cells and progenitor cells. In the last 3 years, peripherally leukocytes rather than bone marrow are used for autologous and allogenic bone marrow transplantation (Bernardo and Fibbe, 2015). The first clinical uses of HSC include the treatment of blood cancers (leukemia and lymphoma) caused by the proliferation of leukocytes. In

these applications, the patient's own cancerous hematopoietic cells are destroyed by radiation or chemotherapy, then replaced by bone marrow product or HSC transplantation collected from the peripheral circulation of the compatible donor, as currently done. The compatible donor is typically a sister or brother with a hereditary similar HLA on the cell surface. Blood cancers; It includes acute lymphoblastic leukemia, acute myeloblastic leukemia, chronic myeloid leukemia, hodgkin's disease, multiple myeloma and non-hodgkin lymphomas. Although there is a significant mortality risk due to both infection and graft versus host disease after transplantation, most patients have increased their lifespan (Figure 14) (Bernardo and Fibbe, 2015). MSC is an adult stem cell type. The fact that they have a "support cell" feature in general, as they are of stromal origin, constitute the basis of the use potential of MSCs in many fields of medicine. Mesenchymal stem cells, which constitute an important part of regenerative medicine today, (Conget and Minguell, 1999) are produced by producing the cells obtained under laboratory conditions in petri dishes. They are durable cells that can be obtained from many tissues and are capable of reproduction in number (Çankırılı, 2019). The soluble factors that they secrete contribute significantly to the functions of the tissue-specific cells in which they are located due to their close relationship with the intercellular or extracellular matrix. They are of great interest because they are important components of the tissue microenvironment and mostly have suppressive properties on the immune system (Dominici et al., 2001).

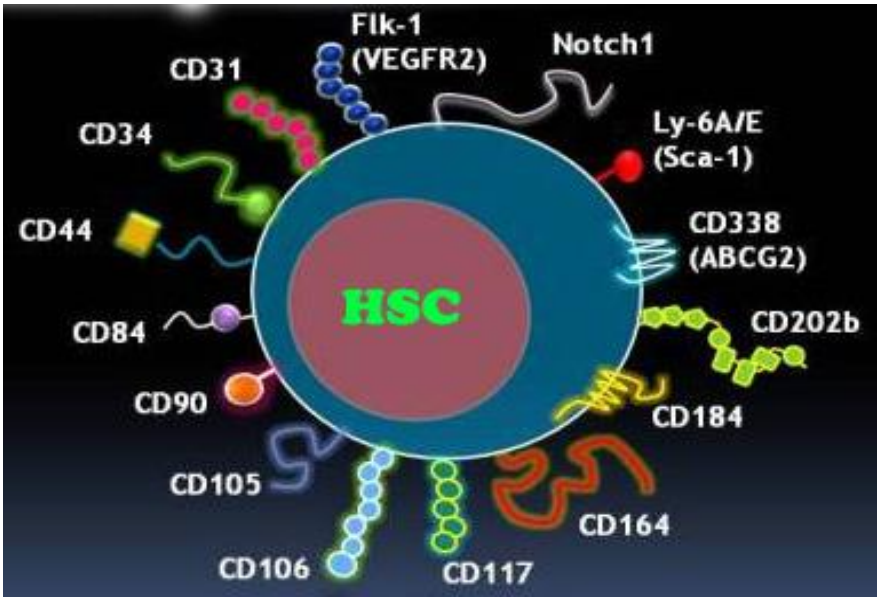

Figure 13. HSC markers *(*https://www.sinobiological.com/research /cd-antigens/cluster-of-differentiation-stem-cells*, Erişim: 30.09.2020)*.

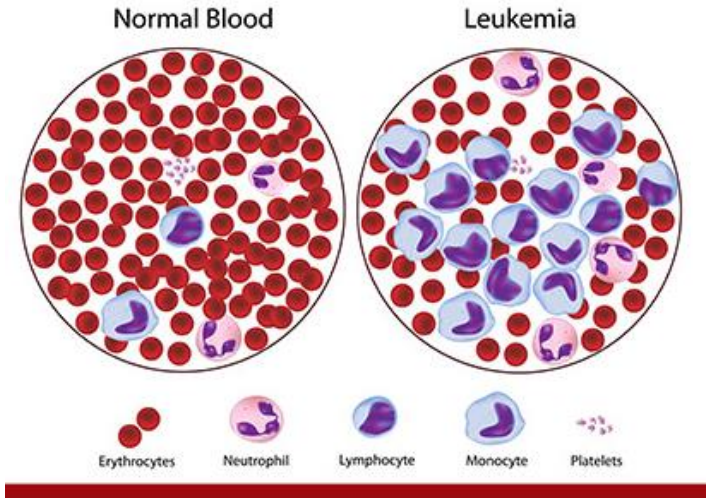

Figure 14. Blood cancer *(*https://www.oncolifehospitals.com/blog /blood-cancer-types-and-treatment-options/, Erişim: 30.09.2020).

The necessity that the mesenchymal stem cells must be replicated in vitro cell culture medium due to the very small number of tissues from which they are obtained is the main disadvantage of these stem cells in basic science research and clinical use. This situation leads to differences in phenotypic, immunological and other biological features with the effect of various stimuli and factors that cells are exposed to as a result of passages in the culture medium (Tuli et al., 2003). Since almost all of the basic studies with mesenchymal stem cells are used in in vitro culture medium, it is known that the defined properties of these cells are far from reflecting the in vivo properties, even if studied in detail. This poses a disadvantage especially for clinical applications. There is a risk of cell aging due to passage in the culture medium, cytogenetic disorder and malignant transformation, albeit low. At the same time, difficulties in establishing cell processing laboratories in accordance with internationally accepted accreditation conditions for the development of cells suitable for clinical use constitute an obstacle to the widespread use of these cells in the clinic (Tae et al., 2006). On the other hand, no serious problems related to cell use have been reported in the clinical applications of MSC, which have been increasing since the mid 2000s, but still few. MSCs are the main cells of the connective tissue. Fat can differentiate into cells such as bone, cartilage, muscle, tendon, ligament. In addition, they constitute the origin of stromal cells, which are supportive cells in all tissues (Minguell et al., 2001). These cells were first described by Fridenstein in 1 year. Fridenstein showed that bone marrow cultures using fetal calf serum (FCS) have cell colonies that show adhesion ability,

morphologically similar to fibroblasts, and have the ability to differentiate into bone and fat cells. In the studies carried out years later, it was revealed that these cells are pluripotent stem cells that are not hematopoietic, and have the ability to differentiate from cells originating from all three germ leaves. These cells, formerly called CFU-F (Colony forming unit fibroblast) and "Bone marrow stromal fibroblasts", were later identified as mesenchymal stem / stromal cells (Gregory et al., 2005).

MSCs are multipotent stem cells derived from a variety of sources. There is no specific marker to identify them; however, they are negative for hematopoietic cell markers such as CD34 and express CD90, CD73 and CD105 on their surface. Immunomodulatory effects on T cells, B cells, NK cells and dendritic cells and their interactions with T regulatory (CD4) cells. MSCs can suppress T lymphocyte proliferation caused by alloantigens, mitogens, and anti-CD3 and anti-CD28 antibodies. MSCs have a similar effect on memory and naive T cells as well as on CD4 and CD8 T cells, and this suppressive effect does not require major histocompatibility complex (MHC) restriction. Cell inhibition is believed to be due to solubl /growth factors in humans such as IFN-, IL-1, TGF-1 and hepatocyte growth factor. Its immunomodulatory activities are believed to be mediated by these growth factors and indolamine 2,3-dioxygenase and prostaglandin E2. It has been reported that secretion of HLA- G5 by MSCs is required for the following effects: suppression of T-cell and NK cell function, shift of allogeneic T-cell response to a Th2 cytokine profile, and CD4, CD25

high forkhead box P3 (FoxP3) regulatory T cells (Tregs) (Silva et al., 2003) (Figure 15).

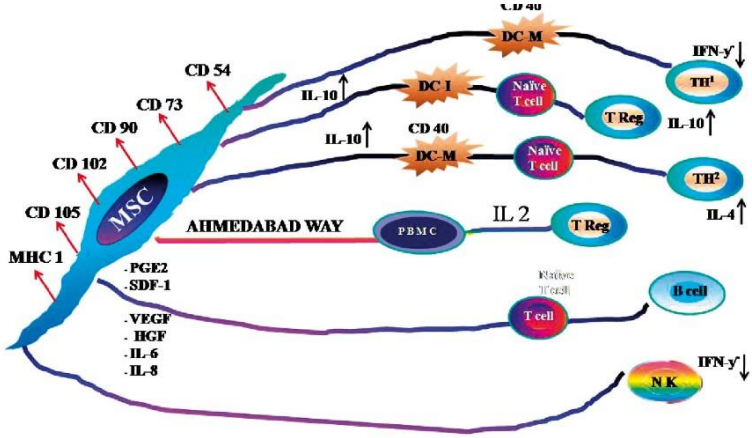

Figure 15. MSC markers *(*https://www.sinobiological.com/research /cd-antigens/cluster-of-differentiation-stem-cells, Erişim: 30.09.2020).

Stem cells can be autologous or allogenic and can be administered systemically or locally (Şahin et al., 2005). There are sometimes contradictions in identifying the typical features of mesenchymal stem cells among researchers. Many laboratories use various methods to isolate MDG and to reproduce and direct these cells to differentiation by following protocols that do not contain significant differences. MSCs with morphologically and biologically similar properties can be isolated from different tissues. However, it is reported that there are changes related to the environmental conditions under which the cells are developed in subjects such as differentiation and immunomodulatory properties of cells, and their effectiveness in vivo. For these reasons, the International Association for Cellular Treatment (ISCT, UHTD) has proposed the criteria for defining human MSCs for both basic research and pre-clinical studies. These cells were proposed

by UHTD to be called "mesenchymal stromal cells" or "multipotent mesenchymal stromal cells / MSC" instead of being called "stem cells". However, in various studies, the ability of cells to transform into different cells of endodermal and ectodermal origin besides connective tissues still causes these cells to be referred to as “MSC” by many researchers. The main features commonly used in defining MSC are; Adhesion to plastic surface (plastic adherence) is the expression of surface antigens in stromal character and the potential for multipotent differentiation (Silva et al., 2003). Bone marrow, one of the richest stem cell sources of the organism, is considered to be the main source for MSCs. In the bone marrow, there are hematopoietic, endothelium and mesenchymal stem/progenitor cells originating from mesoderm. Different studies have shown that bone marrow aspiration has an average number of MSCs ranging from 1 to 10 mononuclear cells, ranging from 2 to 100 (Colter et al., 2001). Besides bone marrow, MSC can be isolated from many tissues. Enzymatic methods are used in cell isolation from solid tissues. It is possible to separate bone and periosteal, muscle tissue, pulp and maxillofacial tissues, liver, lipoaspiration materials, cord blood, cord stroma, placenta, amniotic fluid, synovial fluid and even peripheral blood due to their adhesion properties (Alhadlaq and Mao, 2004). Mesenchymal stem cells have many features, regardless of the tissue from which they are obtained, such as adhesion to plastic tissue culture dishes, exhibiting fibroblastoid morphology, versatile differentiation, and some surface markings. These features are largely similar. However, it has been shown that there may be some changes in the differentiation capacity and

functional features depending on the type of tissue originated. Depending on the microenvironment they are in and how they are needed in the organism, there are also significant changes in the biological features and functions of MSCs. In relation to this, it has been suggested that the use of stem cells obtained from that region will have advantages for the repair of a specific tissue (Hwang et al., 2014).

The presence of MSC in peripheral blood is controversial. It has been shown that there are nonhematopoietic cells with osteogenic differentiation ability and peripheral blood. It is shown that MSC is isolated from peripheral blood in cases of bone fracture and multiorgan failure especially in cases of severe damage. Since studies with mesenchymal stem cells are always in vitro, the placement of cells in the tissues, their niche / niche regions have not been studied in detail; nevertheless, especially in recent studies, it has been reported that the cells are located in the perivascular location in the tissues, such as pericides, and coordinate the cellular functions of neighboring cells, such as maturation, differentiation or silence (Farini et al., 2014).

Physical Properties and In Vitro Reproduction

MSCs are very few in tissues, including bone marrow. In addition, there are difficulties in obtaining a sufficient number of tissues, depending on the adhesive properties. In order to reach sufficient cell numbers in both clinical practice and basic science researches, they must be reproduced in vitro. It is known that these cells are resistant cells that are suitable for reproduction in vitro and maintain their proliferation

and differentiation ability in culture. It is noteworthy that when MSCs reproduced in culture medium are examined by light or phase contrast microscopy, the cells are spindle-shaped and form fibroblast-like cell assemblies. It is observed that when cells are cultured at low concentrations, they tend to colony formation, but at higher cell density, they multiply in groups of cells arranged next to each other instead of forming a colony (Zou et al., 2014).

Stem Cell Microenvironment

The differentiation capabilities of stem cells are regulated under the influence of indoor genetic pathways and external signals. Stem cells need an environment that supports them and allows these regulatory signals to be transmitted. This microenvironment, called "stem cell niche", contains the cellular and molecular factors necessary for the regulation of cells and control of their functions. In some tissues (like skin), this microenvironment has a regulatory effect on both stem cells and their precursors (Figure 16) (Zhang and Li, 2008).

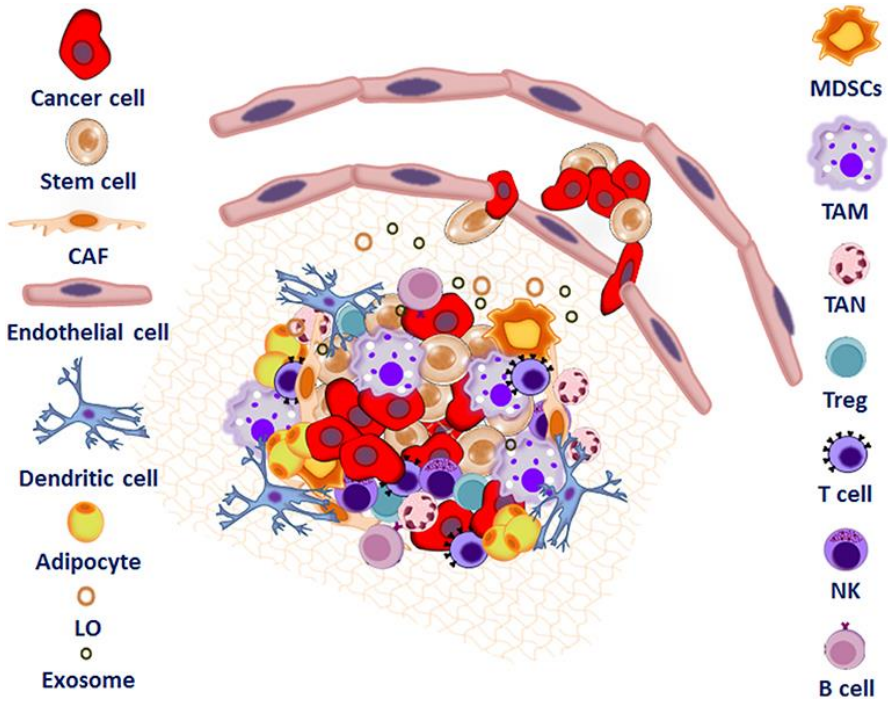

Figure 16. Stem Cell Microenvironment (Ingani et al., 2019).

Stem cell niches provide support for stem cells, create an environment suitable for their lives, regulate their proliferations and direct their differentiation. Studies on this subject provide very important information. For example; each niche system uses special molecules such as Notch, which provide physical interaction and cause the asymmetric or symmetric division of the stem cell. Versatile stem cells can be found in a niche. Most stem cells divide acidmetrically to form a son cell that will remain in the niche and a son cell that will leave the niche to differentiate. However, symmetrical division also takes place. Symmetric division of stem cells can ensure the formation of all stem cell lines and the number of stem cells remains unchanged. This is because any decrease is offset by an increase. Stem cells, when tissue

damage develops, leave their microenvironment and migrate to the area where the damage develops. Therefore, balancing the number of stem cells in the microenvironment is very important (Conway and Schaffer, 2014).

MESENCHYMAL STEM CELL (MSC)

MSCs are adult stem cell types. They are the main cells of connective tissue. They can differentiate into cells such as fat, bone, cartilage, muscle, tendon, and ligament. It was first described by Friedenstein in 1976. This researcher reported that when culturing bone marrow using fetal calf serum, there were colonies of fibroblast-like cells that had the ability to adhere and that they had the ability to differentiate into fat and bone cells. These cells are also called mesenchymal stromal cells or multipotent mesenchymal stromal cells. Its properties such as adhesion to plastic, expression of stromal surface antigens and the potential for multipotent differentiation enable the cells to be defined as mesenchymal stem cells (MSCs). They are found at a ratio of approximately 2-100/1x10^6 MSCs/mononuclear cells. Apart from bone marrow, it is found in bone, muscle, dental pulp, liver, cord stroma, placenta, amniotic fluid, peripheral blood (NIH 2011; Odorico et al., 2005).

Apart from mesenchymal origin tissues such as osteoblastic, chondrogenic and adipogenic lines, it has been shown in recent years that MSCs can also be differentiated into the cell lines of non-mesenchymal tissues such as neuronal or cardiomyogenic lines

(Rastegar et al., 2010). They have common features such as showing morphology, multi-directional differentiation, and carrying some surface markers (more than 95% CD105, CD73, CD90 markers), but there may be some differences in differentiation potential and functional properties depending on the originated tissue type. Therefore, it would be correct to take stem cells from that area for the treatment of a specific area. In addition, they are very few even in bone marrow and due to their adhesive properties, there are difficulties in obtaining sufficient numbers from the tissue they are found in. They must be reproduced in vitro for use in clinical applications and experimental studies. They are resistant cells suitable for in vitro propagation and retain their proliferation and differentiation ability in culture. When MSCs reproduced in the laboratory are investigated a light and phase contrast microscope, it is known that they are spindle-shaped and form fibroblast-like communities (Figure 17, 18). Mesenchymal stem cells, thanks to their various properties, contribute to the repair of tissue damage. These features include their ability to fuse with damaged cells, release of bioactive substances and soluble factors (such as growth factors, cytokines, chemokines), their ability to reach the tissue thanks to their migration properties, and their immunomodulatory, anti-inflammatory, antiapoptotic and angiogenic effects. Soluble factors such as stromal origin factor-1 (SDF-1), monocyte chemoattractant protein (MCP-1) released from the damaged area provide stem cells from their niches and migrate to the damaged area (Odorico et al., 2005, Rastegar et al., 2010; Hass et al., 2011).

MSC Isolation/Characterization

The biggest advantage of MSCs is that they can be taken directly from patients and therefore there is no rejection or immune reaction that may occur. Although MSC studies have been carried out in the field of basic development and cell therapy, the self-renewal mechanisms, proliferation, and multineage differentiation of these cells are still unknown and they are open to research. It is understood from the studies that these cells must be increased to certain cell numbers in order to be used in treatment. MSCs are generally obtained from bone marrow. They are also derived from the human pelvis iliac crest or from the tibia and femurs used as other sources, or from the thoracic and lumbar spine. Stem cell sources with mesenchymal potential other than marrow; periosteum (Fukumoto et al.2003), trabecular bone (Tuli et al., 2003), adipose tissue (De Ugarte et al., 2003), synovium (De Bari et al., 2001) are skeletal muscle (Wakitani et al., 1995), liver (Noort et al., 2002), and the deciduous part of the tooth (Miura et al., 2003).

In Vitro Cultures of MSCs

When MSCs are released into culture, they stick to the bottom of the plastic container.

Growing cells are removed with 0.25% trypsin-EDTA and inoculated into new culture dishes.

One fresh medium is added every 3 days.

It continues until the desired number is reached, it can be frozen for backup.

Non-adherent cells are removed within 24-48 hours.

However, it should be kept in mind that with the increase in the number of passages, symptoms of stress appear in over-manipulated cells and it is known that these may cause deviations from the in vivo state of the cells. Since there are situations such as cytogenetic disorder, telomere shortening, actin accumulation and reduction in adherence in the following passages, it would be more correct not to passage more than 3 times (Rastegar et al., 2010; Hass et al., 2011).

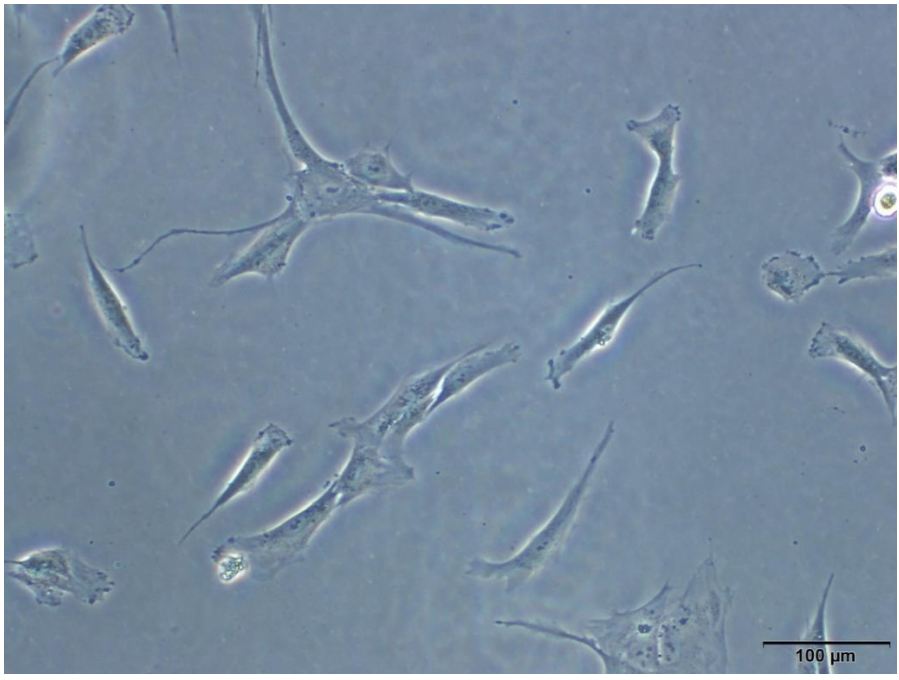

Figure 17. *Images of cells in the 4th passage that have settled and multiplied in the culture medium of ADSCs (Ozturk et al., 2019).*

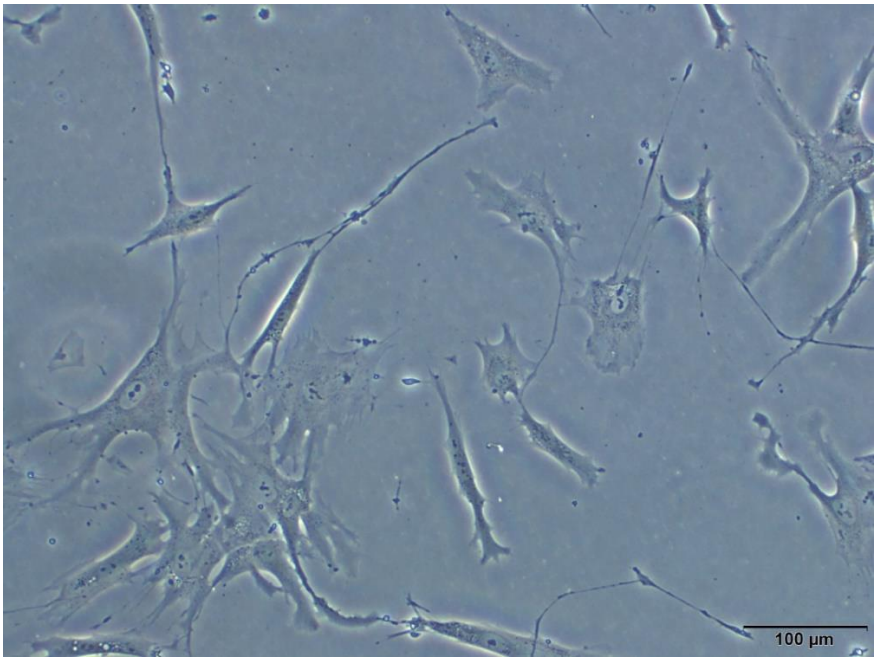

Figure 18. *Images of cells in the 4th passage that have settled and multiplied in the culture medium of ADSCs (Ozturk et al., 2019).*

MSC Identification Methods

Although MSCs are morphologically similar to fibroblasts, the most important feature that distinguishes them from fibroblasts is that they have a symmetrical nucleus. Differential diagnosis of MSCs from other cells in the marrow in the culture medium, such as macrophage and plasma cells, is that they can adhere to the culture container. These cells can attach to the culture dish and can be propagated by passages. At the same time, the insensitivity of the cell membranes to extracellular elements such as adenosintriphosphate (ATP) ions allows them to be separated from other cells (Alhadlaq and Mao, 2004). Although the telomere lengths of MSCs are short, they have high telomerase activity

and they do not lose their normal karyotype and telomerase activities despite their high growth capacity in vitro (Pittenger et al.1999, Minguell et al., 2001). At the same time, MSCs in the culture medium synthesize high levels of cytokines and growth factors; these are: stem cell factor (c-kit ligand), interleukin-7 (IL-7), IL-8, IL-11, transforming growth factor (TGF-β), cofilin, galectin-1, laminin-receptor-1, cyclophilin A is matrix metalloproteinase-2 (MMP-2) (Silva et al., 2003, Ahadlaq and Mao, 2004). A very small proportion of MSCs tend to proliferate actively, while the other vast majority wait in the G0 / G1 phase of the cell cycle (Conget et al., 1999). After a large number of passages, MSCs show wide but unstable spread. It was reported that they did not spread over 30-40 folds. The reason for this depends on many factors: the procedure of marrow retrieval, the frequency of mesenchymal progenitor cells (MPH) in the marrow product (2-5 MPH versus 1x106 mononuclear cells), age of the donor, and genetics. After advanced subcultures, senescence and apoptosis are observed in cells (Conget et al., 1999).

MSC Surface Markers

Various marker are used in defining MSCs. Of these, cell surface antigens and peptides are: CD105, CD90, STRO-1, other adhesion molecules and cytokine growth factor receptors: CD166, CD54, CD102, CD121ab, CD123, CD124, CD49. On the other hand, MSCs also include the endothelial cell marker CD31, macrophage / monocyte cell marker CD14, lymphocyte cell marker CD11a / LFA-1, leukocyte cell marker CD45, and other hematopoietic cell markers CD3, CD14,

CD19, CD34, CD38. They are negative for CD66b (Maleki et al., 2014) (Figure 19, 20, 21).

Table 2. Basic features of bone marrow mesenchymal stem cells (Minguell et al.2001)

Marker type	Markers
Spesific antigens	SH2, SH3, SH4, STRO-1, alfa-SMA, MAB1740
Cytokine and growth factors	Interleukins: 1 α, 6, 7, 8, 11, 14 and 15, LIF, SCF, Flt-3 ligand, GM-CSF, M-CSF
Cytokine and growth factor receptors	IL-1R, IL-3R, IL-4R, IL-6R, IL-7R, LIFR, SCFR, G-SCFR, IFNR, TNFIR, TNFIIR, TGF-IR, TGF-betaIIR, bFGFR, PDGFR, EGFR
Adhesion molecules	Integrins: α1, α2, α3, α4, α5, β1, β2, β3, β4, ICAM-1, ICAM-2, VCAM-1, ALCAM-1, LFA-3, L-selectin, Endoglin, CD44
Extracellular matrix	Kologen type I, III, IV, V, Fibronectin, Laminin, Hyaluronan, Proteoglycan

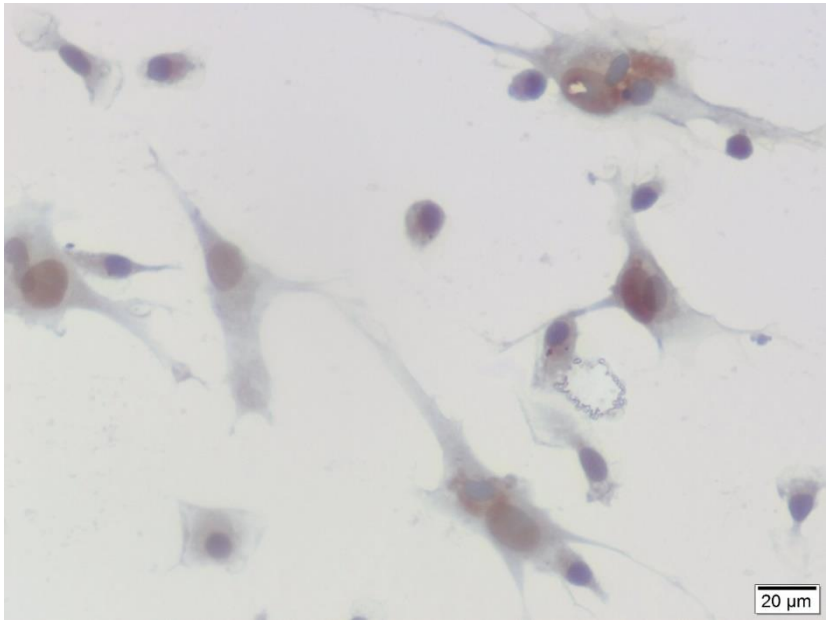

Figure 19. BMDSC's were identified with positive immunohistochemistry images stained with c-kit antibody, x400.

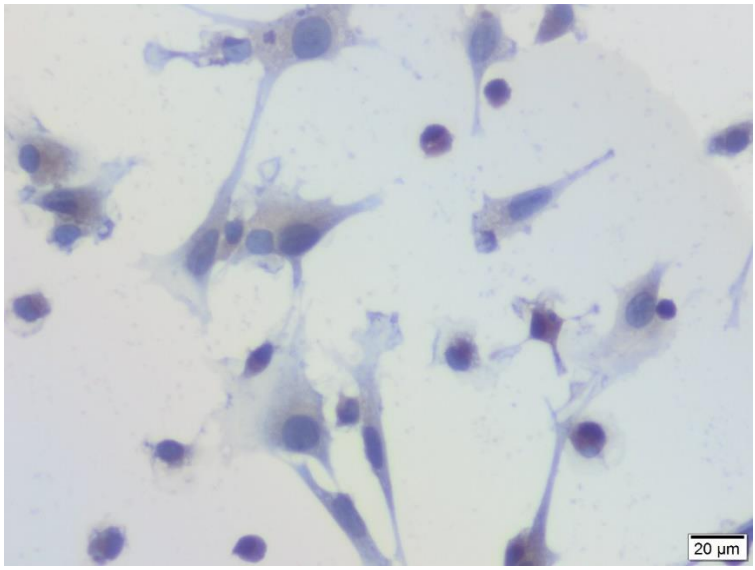

Figure 20. BMDSC's were identified with positive immunohistochemistry images stained with stro-1 antibody, x400.

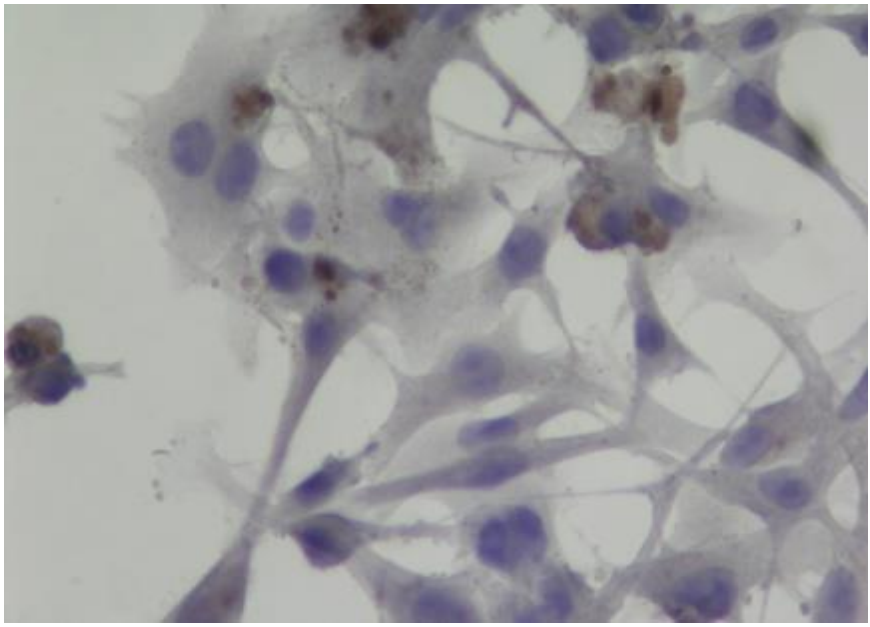

Figure 21. BMDSC's were identified with positive immunohistochemistry images stained with CD-90 antibody, x400.

MSC Differentiation

In Vitro Osteogenic Differentiation

The differentiation of MSCs into osteoblasts in vitro occurs when cells that have spread as a single layer are kept alive for approximately 2-3 weeks in a medium containing ascorbic acid, dexamethasone and β-glycerophosphate. Cells that differentiate into osteoblast precursors show cuboidal morphology and express alkaline phosphatase, osteocalcin and mineralized nodules (Dominici et al. 2001). Bone morphogenic proteins (BMPs) come first among other inducing agents used for osteogenic differentiation. However, when BMPs are applied at high concentrations (such as 100 ng / ml), they prevent the induction

of alkaline phosphatase/calcium deposition and increase Msx-2 expression (Gregory et al.2005) (Figure 22, Figure 23). This is a transcription factor Msx-2 that inhibits the differentiation of osteoprogenitor cells. A positive wingless (Wnt) signal has been indicated to inhibit osteogenic differentiation of MSCs, but it is an inducing agent under alternative conditions (Gregory et al. 2005).

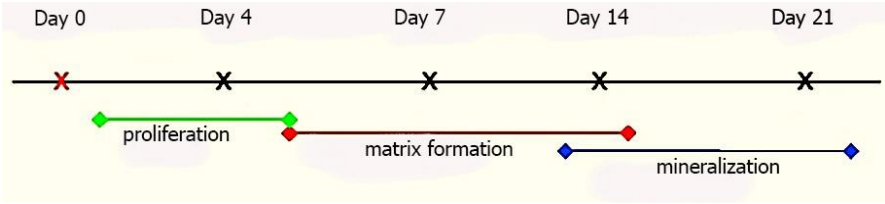

Figure 22. Time-dependent changes as a result of osteogenic induction (Kulterer et al.2007).

In Vitro Chondrogenic Differentiation

In the early stages of differentiation, chondrocytes synthesize type I collagen. Mature chondrocytes, on the other hand, synthesize the characteristic collagen type II and IX. TGF-β induces chondrogenesis through protein kinases (which are extracellular signal regulator kinase 1, p38, protein kinase A, protein kinase C, Jun kinase) (Tuli 2003). TGF-β mediated kinase activation stimulates Wnt expression, thus increasing the expression of N-cadherin, the adhesion molecule (Tuli et al. 2003).

In Vitro Adipogenic Differentiation

This differentiation occurs with the stimulation of a hormone mixture consisting of dexamethasone, isobutyl methyl xanthine (IBMX) and indomethacin. It causes an increase (up-regulation) in the production of IBMX protein kinase A. Protein kinase A activity results in increased production of hormone sensitive lipase (hormone sensitive lipase, HSL). HSL converts triacylglycerol to glycerol and free fatty acid. Indomethacin is known as the ligand of peroxisome proliferator activated receptor (PPAR) α / γ and is the initial transcription factor key for adipogenesis (Sekiya et al. 2004). Suppression of Wnt signals is necessary for cells to undergo adipogenesis, which is achieved by rapid degradation of β-catenin in the proteosome, PPAR PP (Liu, 2004). Inhibition of Wnt signals for the realization of adipogenesis provides the realization of adipogenic rather than osteogenic regulation by MSCs. This indicates that there is a link between the activated PPAR γ and the Wnt signal; The control of whether MSCs differ in bone or fat is thought to depend on these two events.

In Vitro Myogenic Differentiation

Myogenic differentiation induced by MSCs was first performed by Wakitani et al. (1995). In long-term cultures, MSCs express α-smooth muscle actin, metavinculin, calponin and myosin heavy chain, which are muscle differentiation markers (Galmiche et al. 1993). In the studies conducted, marrow stromal cells were treated with 5-aza and bFGF and it was observed that the cells produced myotubes and myosin. Tomita

et al. In their study, they observed that rat MSCs treated with 5-aza are capable of forming myotubes and express cardiac troponin I and cardiac myosin heavy chain from myocardial specific proteins. It was also found that the cells in the culture medium treated with Amphotericin B also exhibited the same effect (Prockop et al. 1997).

Immunological Profile of MSCs

MSCs have the ability to both increase and suppress immunological reactions. It act as antigen presenting cells through an autocrine interferon-gamma (IFN-gamma) dependent pathway, increasing immunological reactions. However, if the INFgamma level rises above a certain level, then antigen presentation is directly suppressed and they suppress the immunological reaction. This regulation of immune activity is thought to be for the protection of mesenchymal stem cells against foreign antigens as well as for limiting the damage caused by excessive immune response. In addition, cells do not express HLA-DR and costimulatory molecules on the surface and have immunosuppressive HLA-G expression. Tissue group compatibility is not necessary for in vivo use as they can escape the immune reaction. This provides an advantage in therapeutic use (Rastegar et al., 2010; Hass et al., 2011).

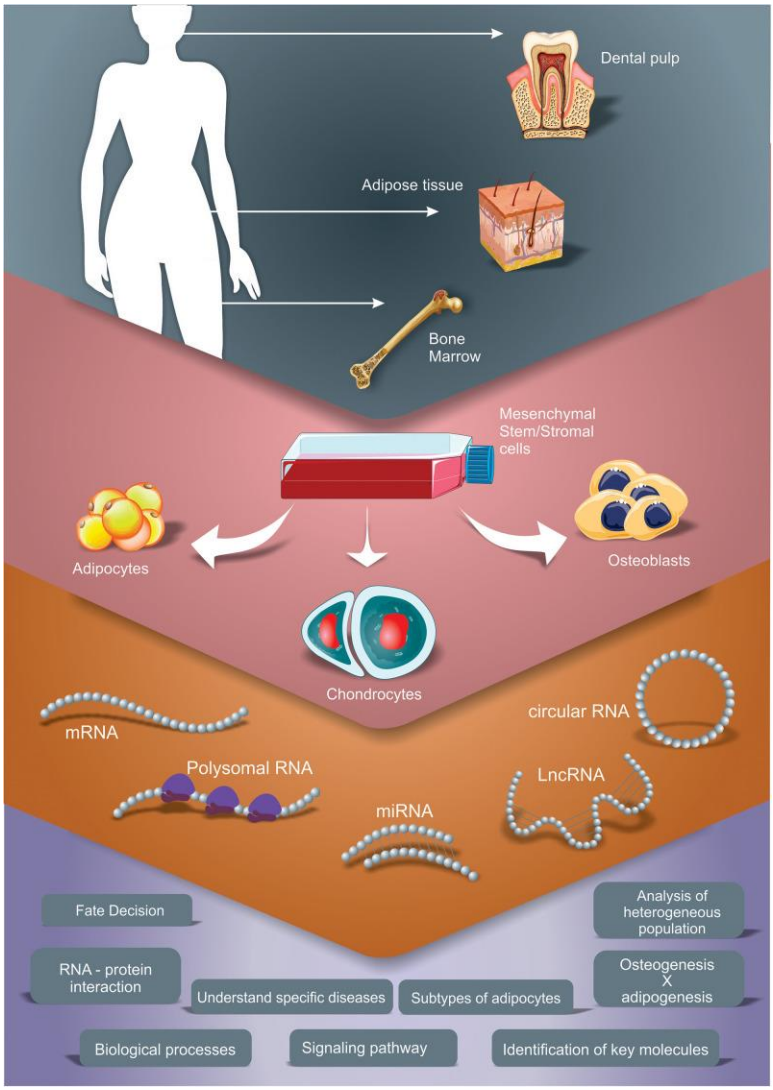

Figure 23. Different transcriptomic approaches to study gene expression profile during adipogenic, chondrogenic and osteogenic differentiation of MSC. Different RNA types were analyzed, as mRNA (by total mRNA, polysome profiling and/or ribosome footprint profiling analysis), microRNA (miRNA), long non-coding RNA (lncRNA) and circular RNA (circRNA) (Robert et al., 2020).

Immunomodulatory Effects of MSCs

Although the exact mechanism of action is not known, the immunomodulatory effects of MSCs are important in immune therapy. As mentioned before, MSCs show immunosuppressive effect and suppress T lymphocyte alloreactivity stimulated by non-specific mitogens or mixed lymphocyte culture. Whether MSCs suppress lymphocyte response created by memorized antigens is controversial. It is estimated that MSCs have T lymphocyte suppressing effect against both natural and recalling T lymphocytes. This situation does not cause an immunological restriction. This effect occurs as a result of either autologous stimulation of MSCs or their interaction with lymphocytes or other interactions. Because of these features, MSCs to be used in allogeneic stem cell transplants support the idea that it is not necessary to obtain only from hematopoietic stem cell donors. The immunosuppressive effect of MSCs is dose dependent (Aggarwal and Pittenger, 2005). High dose application of MSCs to mixed lymphocyte culture suppresses lymphocyte proliferation, while it is interestingly increased when applied at low doses. T lymphocytes that encounter MSCs are not apoptotic and anergic. Because when MSCs are removed from the environment, T lymphocytes can be stimulated again. MSCs decrease CD4 + activation markers, CD25, CD38 and CD69 expressions in phytohemagglutinin stimulated lymphocytes. They increase regulatory T lymphocytes. In fact, the suppressive effect of MSCs on T lymphocyte stimulation occurs with different mechanisms. For example, MSCs increase the transcription of IL-2 and soluble IL-2

receptors in mixed lymphocyte culture, and decrease it in the presence of phytohemagglutinin stimulated lymphocytes. Suppression of T lymphocytes probably occurs before IL-2 secretion. T lymphocytes stimulated by concanavalin-A suppress MSCs. However, with the addition of IL-2, MSC's suppressive effect on T lymphocyte stimulation is reduced. In addition to these effects, the addition of activated dendritic cells decreases TNF-α secretion while increasing IL-10 secretion (Krampera et al., 2006). When effector T lymphocytes and NK lymphocytes are placed in the culture medium together with MSCs, IFN-N decreases and IL-4 secretion increases. It causes an increase or decrease in IL-10 secretion depending on the kinetics of MSCs in mixed lymphocyte cultures. These in vitro variability of MSCs can be partially explained by their immunosuppressive effects. Although its immunosuppressive effects have not been fully explained, some mechanisms have been suggested. It is mediated by some previously mentioned soluble factors, which are formed as a result of the interaction between MSCs and lymphocytes. The proof of this is; While the suppressive effect occurs with the supernatants obtained from the environment in which human MSCs and lymphocytes are cultured, the alloreactive effect does not occur only with the supernatants obtained from the environment where the lymphocytes are cultured. However, it only needs intercellular interaction for alloreactive effect with MSCs obtained from mice. The most important soluble factors responsible for interaction are HGF and TGF-β. Preventing the suppression of lymphocytes when HGF and TGF-β antibodies are added to the environment in the presence of MSCs proves this effect. In one study,

it was stated that PGE2 formed by MSCs suppresses lymphocyte proliferation. In another study, inhibition of the conversion of indoleamine 2,3-dioxygenase-mediated tryptophan to kynuren by MSCs creates T lymphocytes suppressive effect. However, in contradiction with all these studies, another study emphasizes that the immunosuppressive effect does not occur with the suppression of IL-10, TGF-β, PGE2 and indoleamine 2,3-dioxygenase mediated tryptophan formed by MSCs (Sotiropoulou et al., 2006; Le Blanc et al., 2007; Fibbe et al., 2007).

Of all these contradictory results; Obtaining MSCs in in vitro studies with different techniques, applying different stimuli, using different culture media, variability of application amounts, kinetics, use of different lymphocyte groups in the study are held responsible, and these variations cause different chemokine and cytokine secretions. Another important problem is that there is no specific marker or group of markers that fully defines MSCs. When these problems are minimized, the immunomodulatory effects of MSCs will be better understood.

STRO1

Rat IgM monoclonal antibody STRO-1 defines a cell surface antigen secreted by human bone marrow stromal stem cells. Stro-1 antibody binds to CD 34+ cells, which are a subpopulation of MSCs. STRO-1 does not bind to hematopoietic precursor cells. Studies have shown that STRO-1 does not bind to cells such as T and B lymphocytes, myeloid cells, macrophages or megakaryocytes (Paul J. Simmons and Beverly

Torok-Storb, 1991). The STRO-1 + bone marrow derived MSCs are that have the ability to differentiate into adipocytes, chondrocytes and osteoblasts (Shen et al., 2012). The fully pure primary strains of mesenchymal stem cells express many surface antigens, including STRO-1. When defining the phenotype of mesenchymal stem cells, in addition to examining the expression of surface receptors such as STRO-1, CD105 / endoglin, integrin alpha 1, nerve growth factor receptor (NGFR), and to distinguish them from hematopoietic ones, CD45, CD34, CD14 or CD19 and HLA-DR surface molecules should not be expressed either (Quirici N, et al. 2002).

MSCs USE OF CLİNİC

The ability of stem cells to renew themselves, differentiate, reproduce, create tissues and organs can be used as an alternative to organ transplants or in organ transplantation to drugs with negative side effects and in the treatment of many diseases. Today, stem cells can be obtained from many sources for therapeutic purposes and the most studied sources are bone marrow containing embryonic stem cells, hematopoietic and mesenchymal stem cells. Studies for the treatment of human embryonic stem cells, such as Parkinson's, diabetes, heart failure, medulla spinalis injuries, osteogenesis imperfecta and Purkinje cell degeneration are still at the experimental stage; Because embryonic stem cells must be differentiated to target tissue cells before transplantation. However, in experimental treatment studies with stem cells, their use in the clinic is suspicious due to the difficulty of

monitoring and determining the behavior and cellular mechanisms of these cells (Jiang et al., 2006).

Injury of the heart muscle as a result of obstruction of the coronary artery vessels leads to significant losses in regional heart functions. Transferring cardiomyocytes that will provide cardiomyocyte in the damaged myocardium seems to be promising for patients without treatment options other than heart transplantation. By-pass surgery and reperfusion therapy increase the risk of death, although there are limited treatment options for these diseases. With mesenchymal stem cell therapy, it has been shown that healing can occur due to tissue regeneration as a result of these cells migrating to damaged heart tissue. In addition, the fact that this method does not require a surgical procedure is another reason why it is preferred. Studies are planned to use MSC in degenerative diseases such as developmental anomalies, bone infections, trauma, osteoarthritis and osteoporosis related to skeletal system and tumors. It is considered more appropriate to use mesenchymal stem cells in osteoarthritis caused by trauma and aging in injuries requiring cartilage and bone repair (Chanda et al., 2010).

MSCs are also seen as an important alternative in the treatment of meniscus. While treatment applications focus on cartilage tissue cells, a lot of experimental research is ongoing for MSC applications for bone and muscle tissue. Because of its immunosuppressive and immunomodulatory effects, MSCs are promising in the treatment of autoimmune disease. Although there are experimental researches on this subject, clinical practice experience is extremely limited today

(Ben-Ami et al., 2011). However, these cells can be used. Today, treatment methods that provide full recovery in a significant part of human nervous system diseases have not been developed. These include multiple sclerosis, neurodegenerative diseases and traumatic nerve cuts (Uccelli et al., 2011). Effective regenerative treatments in spinal cord injuries have been shown in the near future.

Cell-based therapies offer a promising therapeutic approach with specific soluble mediators and biomaterial combination to heal damaged tissue and allow the use of new treatment strategies in regenerative medicine. Adipose tissue consists of fat cells arranged in lobules. More than 90% of the tissue volume is highly complex tissue consisting of preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes / macrophages, lymphocytes, and ADSCs (Zuk et al., 2002). Adipose tissue such as bone marrow is obtained from mesenchyme and contains a supporting stroma that can be easily isolated. Therefore, adipose tissue has a source of stem cells that can have far-reaching effects in various fields. Stem cells in adipose tissue can be obtained by lipoaspiration and exhibit stable growth and proliferation kinetics in culture. The multilineage differentiation capacity of these cells led us to predict that a multipotent stem cell population comparable to MSCs could be isolated from human adipose tissue. (Figure 24) (Zuk et al., 2002; Schipper et al., 2008).

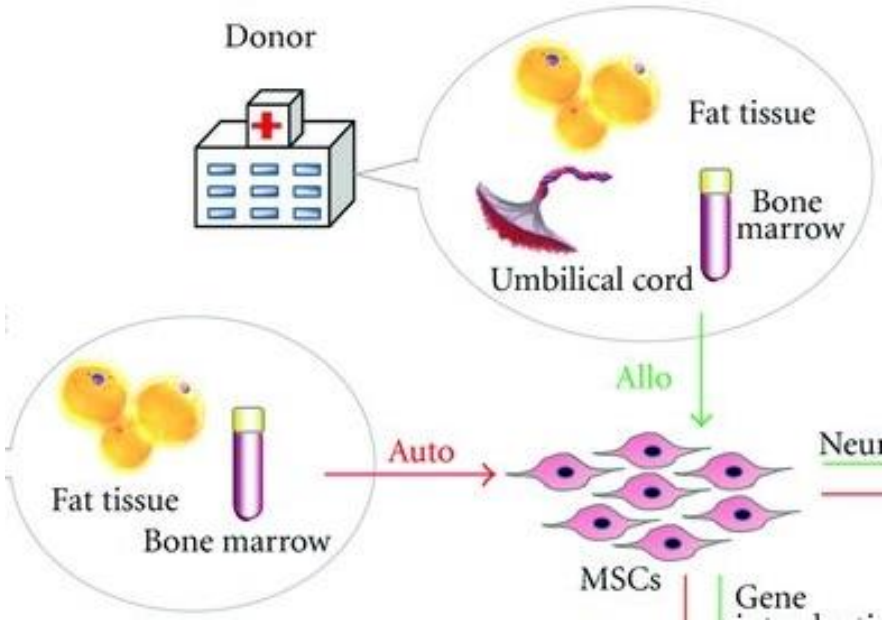

Figure 24. MSCs can be obtained from fat tissue or bone marrow aspirates (Kitada et al., 2012).

Embryonic stem cell research began with the discovery of mouse embryonic stem cells in 1981 by two independent groups at the University of Cambridge and the University of California San Francisco (Martin, 1981). Thomson et al. reported on human embryonic stem cells from the University of Wisconsin in 1998 (Thomson et al., 1998). In 2006, Takahashi and Yamanaka came up with a method for reprogramming differentiated adult stem cells to act as embryonic-like stem cells, and these altered cells were termed induced pluripotent stem cells. (Takahashi and Yamanaka, 2006). Stem cells possess the ability to self-renew and differentiate into defined cell types. These cells are the main cells that make up of all the tissue and organs in our body, and they regenerate themselves by division, keep their numbers constant

and are presumed to be used for replacement and reconstruction (Evans and Kaufman, 1981). Stem cells are generally examined in two groups as those obtained from embryo called embryonic stem cells (ESC) and those derived from adult tissues called adult stem cells (ASC). Embryonic stem cells have two main advantages over adult stem cells; first, their ability to proliferate over a long period of time, and second, their ability to differentiate into a larger cell type (Volarevic et al., 2011). Also, due to the ethical contradictions surrounding ESCs, their use in pre-clinical and clinical research has been limited and is therefore not seen as a reliable, therapeutic approach in the near future. Therefore, MSCs have become the main focus of current research, and recent discoveries have proven that this cell type exhibits greater self-renewal and differentiation potential than originally anticipated. Adult stem cells, stem cells obtained from umbilical cord blood, and embryonic stem cells are the three basic stem cells known today. Adult stem cells can be found in many tissues and organs in the body, and if the cells in that region are damaged, they repair the damaged part by multiplying.

One of the most important qualities that differentiates stem cells from pioneer cells is that while stem cells produce the cell that would become the pioneer cell, they also create their reserve during divisions. This happens due to asymmetrical cell division and enables stem cell pool to always remain constant. Extra-cellular asymmetry of stem cells is performed by the niche that is outside of the cell. Extracellular matrix components that form the niche, neighboring cells and secretion proteins keep the number of stem cells and the state of the cell under

control. Stem Cell Niches fall into two classes: stromal niche and epithelial niche. The stromal niche stabilizes the stem cell activity and is defined by localized anatomical locations containing specialized support cells for it. Stem cells attach to the niche thanks to cell adhesion and extracellular matrix molecules, and efficient communication is provided between cells thanks to secreted cytokines and growth factors (Kiefer, 2011). The vascular wall has become more and more important today with ADSCs, HSCs, and neural stem cells. In contrast to all this, the epithelial niche does not contain the system of support cells, but rather resides on the basement membrane and is located directly adjacent to the mature cell. An example of the epithelial niche are stem cells derived from muscle.

The ability of MSCs to differentiate into different cell lines (adipocytes, chondrocytes and osteoblasts), repair tissues, migrate to the injured area, and control the damaged area by modulating a number of effective immune / inflammatory mechanisms are of significant interest in the field of regenerative medicine for biomedical researchers (Dominici et al., 2006; Galipeau and Sensebe 2018). To date, as many as 969 clinical reports of MSCs with high potential as cellular therapy for various immunological and non-immunological diseases have been reported (https://ClinicalTrials.gov). If this versatile cell is a suitable candidate for cellular therapy, its properties include isolation from multiple accessible tissues tertiary, low ex vivo culture expansion suitability, low immunogenicity, and documented safety properties. The International Association for Cellular Therapy sets minimum criteria

for home MSCs, including the expression of CD105, CD73 and CD90 and the absence of human leukocyte antigen (HLA) -DR, CD11b, CD14, CD19 and CD34, a general consensus is that the differences and effect of MSCs. it still has its shortcomings. These shortcomings are largely due to heterogeneity in primary MSC phenotypes in different tissues and ex vivo culture conditions (Galipeau and Sensebe 2018; Moll et al., 2019).

Among its functional properties, MSCs inhibit the proliferation of helper (CD4 +) and cytotoxic (CD8 +) T cells and indirectly dendritic cell (DC) mediated antigen presentation (Lukomska et al., 2019). It is known that MSCs are provided by an additional mechanism that can exert both short and long-term effects on antigen-specific T cell responses and contributes to this mechanism by stimulating regulatory T cells (T-reg) (Lukomska et al., 2019). In 2008, Di Ianni et al. demonstrated that when human T cell subpopulations are co-cultured with MSCs, the frequency of T-reg increases and T-reg suppressive activities are maintained for a long time (Di Ianni et al., 2008). As we explain later in this article, a relatively wide range of experimental studies have been published to validate this phenomenon and add mechanical details (Engela et al., 2012), and the topic continues to attract public interest (Dai et al. 2019; Kadle et al., 2018; Guo et al. ., 2019). In addition, ex vivo expanded MSCs and T-reg have been shown to be a potent immunomodulator in various animal disease models and have safe and viable potential for human autoimmune diseases in clinical studies (Galipeau and Sensebe 2018; Perico et al., 2018;

Sharabi) and others, 2018). Therefore, it seems imperative to understand how the mechanisms underlying MSC-mediated induction of T-reg or combined MSC / T-reg cellular therapy can be successfully brought into the clinic.

Experimental animal model studies have documented increases in T reg numbers after MSC administration. For example, in the collagen antibody-induced arthritis model, Nam et al. Reported that MSCs in mice induce differentiation of CD4 + T cells into T-reg in vitro, and FOXP3 expression was upregulated in arthritis-induced mice after MSC infusion (Nam et al., 2018). Similar findings were reported by Roux et al, who observed functional CD4 + FOXP3 + T-reg induction when co-cultured with human pluripotent stem cell-derived MSC (hu - iPS - MSC) in vitro. These findings were confirmed in vivo following administration of hu - iPS - MSC to mice (Roux et al., 2017). In a rat model of high-risk corneal allo-transplantation, Lohan et al. Performed intravenous administration of third-party allogeneic MSCs before transplantation in graft-draining lymph nodes (Lohan et al., 2018). Bai et al reported that T-reg counts increased following administration of IL-17A-treated MSCs to mice with renal ischemia-reperfusion injury, which was associated with greater protection against acute kidney injury and was dependent on COX2 / prostaglandin E2 (PGE2). Bai et al., 2018). Groups investigating the interactions of MSCs with T-reg in vitro also reported T-reg induction by MSCs derived from different sources (Engela et al., 2013; Prevosto et al., 2007). As can be seen from the literature summarized so far, a wide variety of in vitro and in vivo

studies have documented the potential of MSCs to induce, extend, or preferably promote survival of T-reg in human and experimental animal species. Also, the kinetic and mechanical details of this phenomenon are not fully understood and will likely be complex and context dependent. In Figure 25 and in the sections below, there is evidence for four basic mechanical models for MSC effects on T-reg.

With the advent of studies in stem cell biology and the improvement of techniques in cell culture, stem cell applications have started to be seen to provide hopeful results in the treatment of such things as cancer, organ deficiencies, metabolic, rheumatic, cardiologic diseases, bone diseases, and nerve damages and nervous-system diseases. Recently, what has become a current issue is using stem cells in the treatment of various diseases that require organ or tissue transfer, and diseases in which existing medical treatments fall short. Although adipose tissue is not practical, recent studies show that this tissue provides a huge amount of adult tissue source. ADSCs can show osteogenic differentiation quite fast. This, in return, suggests tissue engineering and the use of adipose stem cells in broad bone defects. In the field of tissue engineering, many studies have been conducted in order to obtain bone marrow stem cell. However, not enough cells can be obtained from the bone marrow. Nevertheless, adipose tissue makes it possible to obtain many stem cells without morbidity. In bone tissue engineering, osteogenic differentiation is achieved by using scaffold materials, and it is believed that they will be used in vitro models in the future.

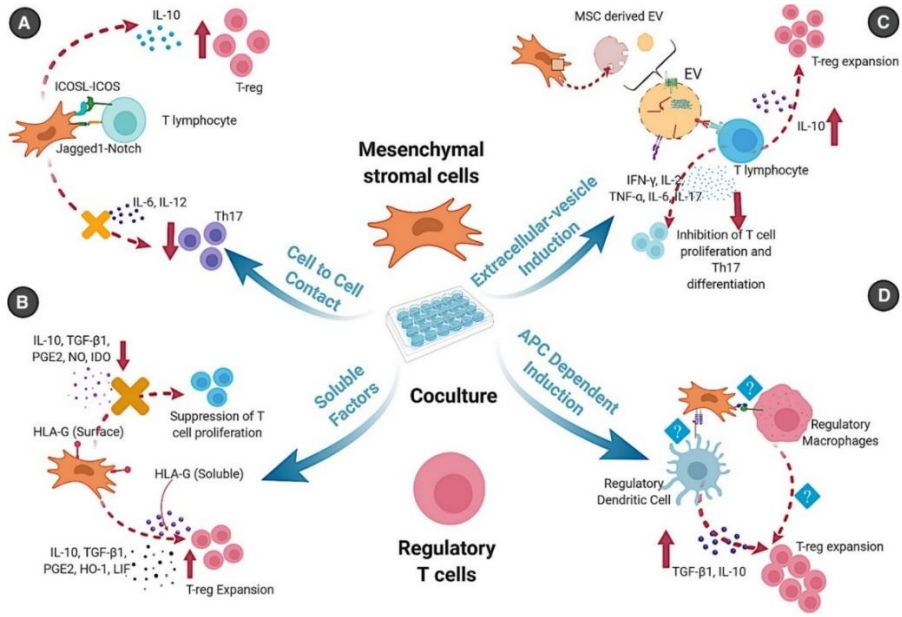

Figure 25. Schematic representation of different mechanisms used by mesenchymal stromal cells (MSCs) for regulatory T cells (T-reg) induction. (**Biorender.com**).

The therapeutic applications of autologous and allogeneic MSCs have been actively followed up with many experimental and clinical trials in the last decade on the basis of their low immunogenicity, genetic stability, easy availability and various immunosuppressive / anti-inflammatory properties (Moll et al., 2019; Lukomska et al. , 2019; Naji et al., 2019; Weiss and Dahlke 2019). The main factor underlying MSC activity in autoimmunity, transplantation and other inflammatory diseases is their potential to induce or functionally develop specific populations of innate and adaptive immune cells with regulatory/suppressive functions. (Zheng et al., 2019; Le Blank 2012).

ADSCS CLINICAL APPLICATIONS

Mesenchymal stem cells obtained from fat and their regenerative properties Zuk et al. They discovered the induction factors of cells differentiating into adipogenic, chondrogenic, myogenic and osteogenic cells in vitro by performing suction-assisted lipectomy (liposuction) of human adipose tissue under general anesthesia (Figure 26). They can also be obtained from bone marrow, but this is an extremely painful procedure that yields very little cells. In fact, the number of these cells in adipose tissue is 100 to 500 times higher than in bone marrow (Casteilla et al., 2011). For this reason, the oils in the subcutaneous and internal organs are the simplest and most practical places to obtain these cells. Zuk et al. They reported that with a minimally invasive surgical procedure, subcutaneous and visceral fat extraction can be achieved without the need for larger tissue and the procedure is quite simple (Housman et al., 2002). Although ADSCs have proven to exist in adipose tissue, it has been more difficult to determine exactly where these cells are located. But now we know that these cells reside in a stromal stem cell niche. More specifically, research has revealed that they are located in a microenvironment of the adipose vasculature (Lin et al., 2009). Depending on the particular environment in which these cells are found, their potential extends from vascular smooth muscle and endothelial cells to adipose tissue and other mesenchymal cell types. Once obtained, adipose tissue must be digested using a collagenase to dissolve structural components of the tissue and separated from stem cells, also known as stromal-vascular fraction (SVF). Next, the stem cells must be classified according to their

specific cellular markers. Initially identifying these cellular markers was difficult due to the differentiation potential of these stem cells. Therefore, different studies have isolated ADSCs of different potential and reactivity. Characteristic markers commonly used for sequencing include CD29, CD34, CD73, CD90, and CD105 (Figure 27) (Baer, 2014). To demonstrate that cellular pluripotency surpasses many other stem cell types, ADSCs have been cultured in a variety of media to induce their differentiation into endothelial, smooth muscle, and neuronal cells (Ning et al., 2009). Among the many types of mesenchymal stem cells, they are considered the most promising fat-derived mesenchymal stem cells in cell therapy for various reasons. It has been shown that these cells play a role in repairing damage to appropriate tissues and organs, and can transform into neurons and myocytes as well as chondrogenic and adipogenic changes like other mesenchymal stem cells in vitro. Alternatively, mesenchymal stem cells are being modified in tissue engineering and investigated for use in various regenerative treatments.

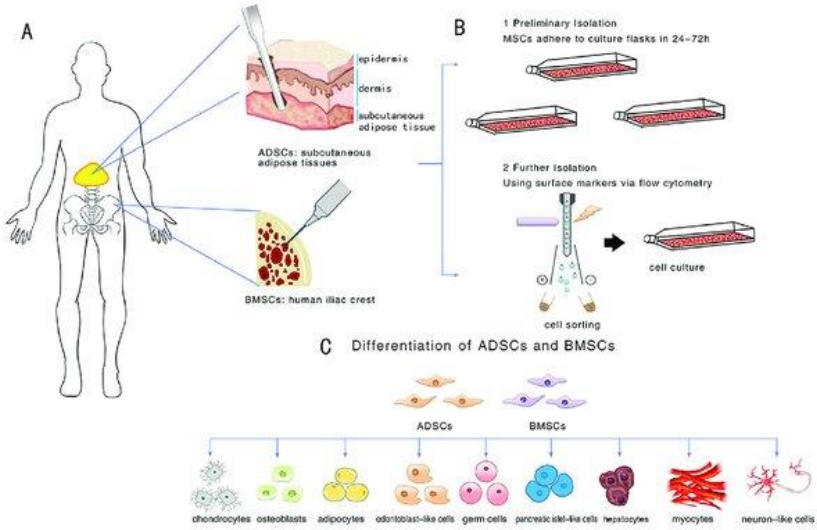

Figure 26. Overview of isolation method and differentiation of ADSCs and BMSCs (Li et al., 2018).

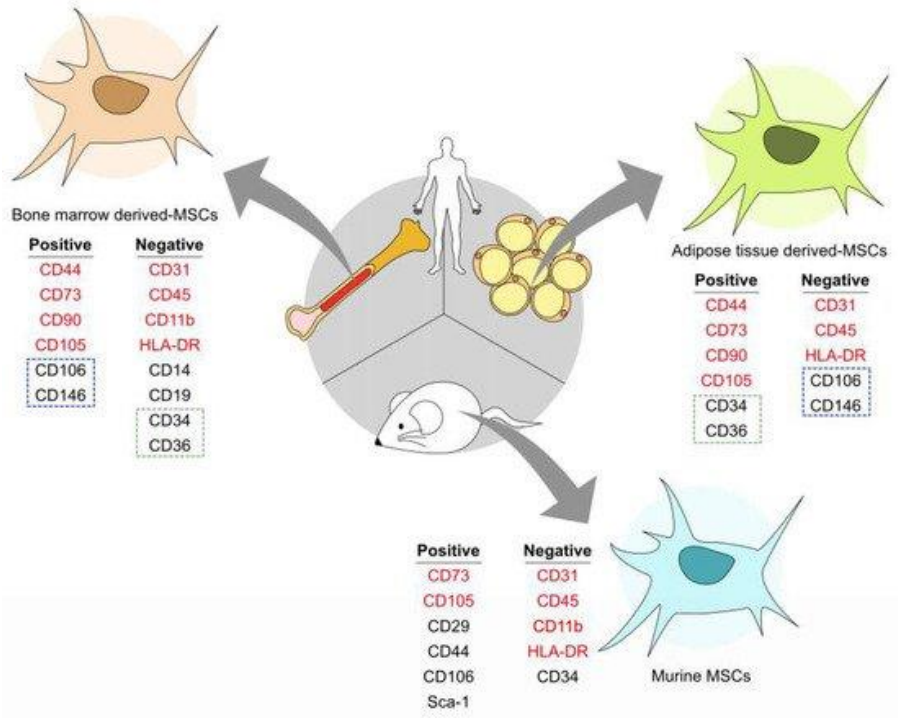

Figure 27. Cell surface markers of vary between mesenchymal stem cells/multipotent stromal cells (MSCs). Human bone marrow-derived (BM)-MSCs share most of the markers such as CD44, CD73, CD90, and CD105 with adipose-derived (AD)-MSCs. CD106 and CD146 (Jiang et al., 2019).

Regenerative medicine stands out as the most compelling field for stem cell treatment. Potential dangers of using these cells without optimizing cell preparation methods and without sufficient clinical experience for burn treatment, for neurological disease, for osteoporosis treatment, for myocardial infarction, for implants in dentistry, and especially for the treatment of diseases which already have alternative treatment methods have been noted. Therefore, reported studies on this fields are limited.

Cells therapy promises great hope for difficult to heal wounds. In the new methods using cell molds in tissue engineering, mesenchymal stem cells are placed on these molds and carried to the wound area. In the studies in which mice are applied singular or triple molds, which were obtained from human adipose stem cells, it was observed that there is a faster and more effective healing in the experiment group compared to the control group. In the method which aims to increase angiogenesis in wound area, different cell types and allogenic cell molds are also examined. Recent studies have shown that autologous fat grafting and fat-derived cells have great benefits in treating burns and scars they cause. In patients with deep burns, these treatments can be useful in the acute and chronic stages of wound healing. Some researchers have obtained isolated cells that show more noble differentiation from burned surgical waste. Several mouse studies have shown that healing occurs successfully when these cells are used in deep wounds. One human study described the use of these cells placed under skin grafts in patients with deep excision by spreading into collagen-elastin scaffolds, resulting in 90 percent viability. This emerging therapy is promising as these cells may be a source of autologous multipotent cells that can help patients burn wound healing without increasing their morbidity by performing additional fat harvesting procedures (Condé-Green et al., 2016). The hope for staying young has always been fresh for human beings. Many drugs against aging have been manufactured. However, none of them can significantly slow the aging process. Stem cells provide highly important advantages in this respect. Even when we become adults, stem cells can turn into the organs that are in their

program and can be constantly regenerated. Our skin is one of such organs that are constantly regenerated. With growth factors applied to the skin, it can regenerate itself faster, and this indeed is made possible with the use of stem cell technology by which the transfer of regenerative products onto the skin is accelerated by means of stem cells. Neovascularization, a key process for wound healing and elimination of ischemia, plays an important role in the survival of transferred cells (Gurtner et al., 2008). ADSCs secrete a large number of growth factors that directly affect angiogenesis and wound healing. ADSCs are high levels of epidermal growth factors, basic fibroblast growth factor, keratinocyte growth factor, PDGF, insulin-like growth factor, VEGF, TGF-Beta, hepatocyte growth factor (HGF), brain-derived neutrophilic factor (Kilroy et al., 2006) secrete. . These growth factors promote wound healing by inducing migration and proliferation of ECs, increasing the vascularity of the wound bed, granulation tissue thickness, and collagen deposition (Ebrahimian et al., 2009). HGF is considered to be the main angiogenic factor secreted by ADSCs as its suppression impairs angiogenic and regenerative effects (Cai et al., 2007). The in vivo results demonstrated the efficacy of using ASC in reducing the time required for full recovery from 21.4 days to 17.2 days and with the addition of ADSCs to 14.6 days. It demonstrates that cryopreserved ADSCs accelerate complete wound closure by increasing skin maturation and blood perfusion, and their therapeutic benefit in the context of wound healing (Lee et al., 2011). ADSCs have the potential for myocardial regeneration and have reported that transplantation of these cells following myocardial infarction (MI) in

animal models led to modest improvements in cardiac function. In another study, ADSCs treated with ISX1 (3,5-disubstituted isoxazoles) reported significant increases in neovascularization and significant improvement in cardiac function in the transplanted heart. These findings suggest that myocyte differentiation has a strategy that facilitates healing as an alternative to drugs, and also increases the persistence of exogenously transplanted ADSCs in vivo to improve cardiac function and consequently increase vascularization in the tissue (Burchfield et al., 2016). Cardiac myogenesis with fat stem cells which can alter to myocardial cells were done not only in vitro but also in vivo. In myocardial infarcts, it was shown that these cells both alter to myocardial and endothelial cells and they decrease infarct scale. However, several problems may occur during transplantation. It becomes rather difficult for fat stem cells which are very similar to stem cells to survive after implantation. Thus, scientists have been searching new way to eliminate this problem. For instance, they have tried to use ADSCs by combining them with hemooxygenase-1 transduction. However, BMSCs and ESCs are more advantageous at this. Recent experiments are yet at animal level, but it is for certain to have human applications soon.

Clinical applications of MSCs are emphasized due to their potential for multiple alteration. They can especially provide tissue regeneration in cases of bone damage, heart attack, and nervous system damages. A group of researchers proved that these cells adapted to xenogeneic bone material as well. Moreover, studies have reported that MSCs which can

repair defects and damages can eliminate cartilage damage without induction and that they trigger osteogenesis in osteonecrotic tissue whose vascular microstructure has been damaged.

It has been determined that if ADSCs are cultured with β-mercaptoethanol, they tend to shape into neuron-like cells and they express early markers of neuronal line, neuronal nuclei and neuro-specific enolase. However, mature neuron markers could not be found during long-term culturing. This may be due to the restrictions of the used mediums. Recent studies show that peripheral nerve damage is repaired by ADSCs. In animal models, subjects who were given peripheral nerve damage via cell transplantation have shown functional recovery. On the other hand, it was reported that they can induce to Schwann-like cells and repair myelination damage. Although their mechanisms have not been fully understood, it is commonly believed that these mechanisms are related to paracrine secretion.

In order to become different cells in the body, stem cells require growth factors. Growth factors provide the necessary nutrition for stem cells to grow and form new cells. Factors that play an important role in the repairment process when there is any sort of damage and facilitate paracrine mechanism in tissue growth are released from the cells. Paracrine factors include growth factors and citokynes. Their functions are to enable blood flow to ischemic tissues, to prevent apoptosis, to regulate inflammation, and to facilitate regeneration of damaged tissue. Studies show that VEGF and HGF are important paracrine factor and that angiogenesis and arteriogenesis are regulated by these enzymes.

Moreover, it has been reported that VEGF and HGF levels in the tissue increase when MSCs are used in treatment of heart attacks. In the studies on human adipose-derived stem cells, it has been shown that Rehmania glutinoa oligosaccharide increases proliferation capacity and cell vitality by enabling the release of paracrine VEGF and HGF.

ADSCs are key regulators of new blood vessel formation and have been extensively studied for their role in tissue regeneration and tumorigenesis. However, the cellular and molecular mechanisms by which ADSCs regulate angiogenesis are not well understood. The aim here was to demonstrate the functional contribution of ADSC-mediated extracellular matrix (ECM) remodeling to endothelial cell invasion. In one study, ADSCs were embedded in 3-D collagen type I hydrogels and pre-cultured for 7 days to show the effect of ECM remodeling; controls were not pre-cultured. A composite monolayer of human umbilical vein endothelial cells (HUVECs) was seeded on top and invasion into the underlying hydrogel analyzed. Without pre-culture, ADSCs stabilized the endothelium, inhibiting vascular germination. In contrast, 7-day pre-culture of ADSCs significantly increased the invasion of HUVECs. This effect is thought to be largely mediated by proteolytic ECM degradation by matrix metalloproteinases (MMPs) derived from ADSC rather than vascular endothelial growth factor (VEGF), as the results show that blocking MMPs inhibits endothelial sprouting, not VEGF. All these data will provide new treatment possibilities for the angiogenic capacity of ADSCs, pro- and anti-angiogenic therapies (Song et al., 2016). It is known that many cytokines related to

angiogenesis are released from ADSCs. Most frequently seen ones are TGF-β and VEGF. It is reported that they inhibit inflammation of TGF-β in xenogenic bone materials. Moreover, they increase the growth of TGF-β fibroblast, osteoblast and Shwann cells. It was reported that they are significant in providing the revascularization of TGF-β, bFGF, and VEGF expressions through cell transplantation. All of these show that ADSCs alter to vascular endothelial cells in ischemic or necrotic damages or they help revascularization directly through paracrine secretion. Thus, the area with lesion can get more nutrition via revascularization, and this shows that a nutrition source is needed for tissue regeneration. However, although ADSCs moves are not yet fully understood, there is an assumption that micro-frame can be changed by stimulating the secretion of such factors as VEGF and TGF-β. Moreover, it is thought that VEGF and TGF-β secretion can be achieved from ASCs through their activation.

It has been reported that diet increases the incident of many diseases such as prostate cancer and hepatic cancer. This makes ASCs suspect, because in some studies it was observed that ASCs in the fat tissue which was formed due to overeating surprisingly strengthen prostate cancer cells by increasing tumor vascularization via FGF-2. In later studies, it was found out that ASCs eliminate or reduce the latent period of tumor cells that promote melanoma growth, and that exosomes released from tumor cells turn ASCs into myofibroblastic phenotype by activating intercellular signal pathway. All these negative aspects notwithstanding, it was also found out that ASCs can inhibit tumors in

many different ways. Because of ASCs surprisingly capacity for migrating against malignant glioma, there are studies in order to enable them to play a therapeutic role in carrying the oncoliytic myxoma virus in vitro and in vivo. Moreover, these cells not only help carry the virus, but they also enable the replication of it. Thus, these replications are promising in shedding light on the treatment of malignant glioma. Additionally, it was reported that ASCs inhibit the growth of pancreatic ductal adenocarcinoma and stop the cellular cycle of tumor cells.

In another study, animals with urinary incontinence were transplanted ADSC isolating it. Before and after the transplantation, urethra tissue and tissue around urethra were subjected to urodynamics test and morphological evaluation. Urodynamics measures were done when bladder was full, and pressure value was measured. It was observed that in animals that were given ASC local urethral muscle was stronger and the sphincter improved both morphologically and functionally. While there are several treatment options available for erectile dysfunction treatment, in diabetics, postprostatectomy patients, and those with Peyronie's disease, erectile dysfunction may be more severe in degree and less likely to respond to traditional medical treatments. One of the most impressive treatment strategies in the preclinical for treating this disease is stem cell transplantation. Depending on the cell type, recent research has shown that by transplantation these stem cells can exert a paracrine effect on the surrounding penile tissues and differentiate into smooth muscle, endothelial and neurons. Preclinical studies using animal models for various disease processes responsible for this disease

have provided evidence supporting stem cell differentiation and cavernosal tissue incorporation (Gökçe et al., 2016). In experimental animal studies, ADSCs have been reported to have positive effects on erectile function in subjects with erectile dysfunction. In acute animal models, such as erectile dysfunction due to cavernous nerve injury and chemically induced Peyronie's disease, vaccination and differentiation were not observed and stem cells were believed to interact with host tissue in a paracrine manner, while in chronic disease models some evidence suggests that both vaccination and paracrine factors can support improved function. . While clinical research is now investigating the therapeutic efficacy of cellular therapy, the first safety studies in humans have been recently published (Soebadi et al., 2016).

In standard approaches, pure tissue reconstruction is done with autologous tissue flaps, fat transplantation, and alloplastic implants. All these approaches prove to be at a disadvantage in terms of implant migration, absorption, being foreign to the body, and donor-site morbidity. ASCs are used in many fields, especially in plastic surgery, and they resolve such limitations. Autologous free fat transfers have many applications in plastic surgery. They can be used in reconstruction and breast reshaping without oncologic resection. They can be used effectively in HIV lipodystrophy, face reshaping, hand and face rejuvenation operations. Moreover, fat tissue is used in correcting the asymmetrical structure in Poland Syndrome and Parry-Romberg syndrome, and in repairing defects resulting from hip fractures. Their appropriateness for vascular tissue and valve transplants. Recently, they

have begun to be used in orthopedic surgeries, laryngology, nerve surgeries, general surgeries, and vascular surgeries.

Periodontal tissue regeneration with ASCs has been reported in experimental animal models. The interaction between ASC's and PRP (Platelet- rich plasma) promotes tissue regeneration and neovascularization. Tobita et al studied the combination of ASC's and PRP with the later used as a cell vehicle/carrier in animal models (Tobita and Mizuno, 2010). In a study using rat periodontal tissue defect model, green fluorescent protein labeled rat ASC's were implanted with PRP and evaluated 2,4,and 8 weeks after implantation (Tobita et al., 2008).

Advances in stem cell biology have yielded promising results in vitro and in vivo suggesting that future applications in regeneration may be achievable. Because large quantities of adipose derived stem cells can be harvested from adipose tissue, it might be possible for clinical use. The ability of the ASCs to differentiate into several tissues makes it particularly attractive type of adult stem cell for periodontal regeneration and tissue engineering. The periodontal microenvironment may induce ASC to grow and differentiate into periodontal tissues and the ASCs themselves might secrete various cytokines that stimulate resident progenitor cells. Further preclinical and clinical studies are needed to determine whether ASC based therapies can fulfill expectations and can be used successfully in periodontal regeneration (Mizuno et al., 2015). In addition to this, it was also seen that salivary glands damaged due to radiation exposure are partly regenerated, that

radiation is gone, and blood flow is increased after ADSC transplantation.

CANCER STEM CELLS

In various types of cancer cells, the cells have been shown to have surface markers also found in stem cells. The similarity of cancer cells with stem cells suggests whether they originated from them. Similarities between cancer stem cells and normal stem cells; Asymmetric cell division features are used for self-renewal, such as Wnt, Sonic Hedgehog (SHH), Notch expressing similar transcription factors (such as Oct-4, Nanog, Sox2, Nodal, Klf4), resistance to drugs, metastasis and common surface receptors (CXC4, CD133, etc.) c-kit can be summarized as having c-met). When the self-renewal and asymmetric division properties of the stem cells are combined with the increase in telomerase activity in the cancer cell, an infinite division occurs. The qualities such as the cells acquiring a malignant character and migrating (metastasis), preventing apoptosis, changing the carrier mechanisms in the cell membrane and growing without attachment become evident, and these are the characteristics of cancer tumor cells. In addition, it has been revealed that some cell signaling pathways known to exist, especially in embryonic stem cells, and the proteins involved in them have reached excessive or disrupted in some types of cancer (Öktem et al., 2009; Wend et al., 2010).

Cancer Metastasis and Invasion

Because stromal cells contribute to the development of a variety of tumors, it is important to consider the effect of implanted/administrated mesenchymal stromal/stem cells on cancer development before these cells can be used clinically in regenerative medicine. Stromal cell compartments contain a variety of cell types such as fibroblasts, pericytes, myofibroblasts, vasculature, and macrophages, which together form a microenvironment that tightly controls the proliferation and differentiation of epithelial cells (Bissell et al., 2002). During the initiation and progression of breast cancer, the tumor cells reorganize the tissue microenvironment to support their proliferation and invasion into the surrounding tissue (Pupa et al., 2002). Tumors recruit stromal fibroblasts in a process referred to as a desmoplasmic reaction. These tumor-associated fibroblasts are reprogrammed to produce growth factors, cytokines, and extracellular matrix-remodeling proteins (Orimo et al., 2005).

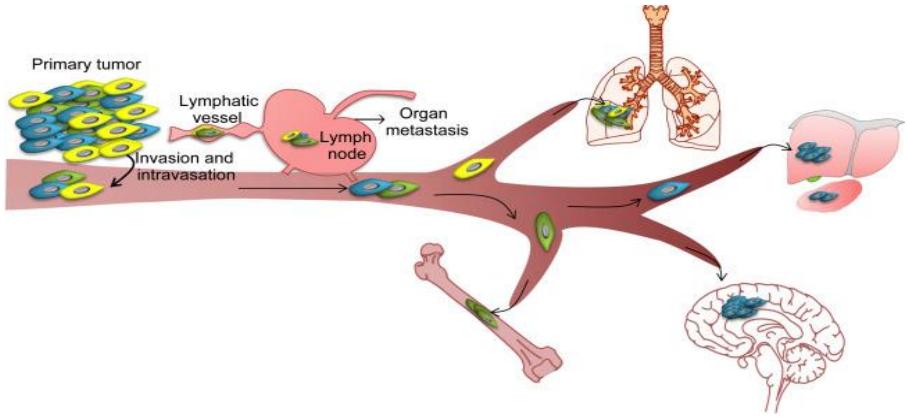

Figure 28. An overview of the cancer metastasis (Zubair and Ahmad 2017).

Recent studies have demonstrated that BSCs recruited by breast carcinomas promote breast cancer metastasis and invasion. BSCs produce chemokine ligand 5 (CCL5), which promotes breast tumor progression in direct co-cultures. Compared with BSCs, ASCs are tissue-resident stem cells that occur locally adjacent to breast cancer cells, and interactions between adipocytes and breast cancer cells have been described previously (Iyengar et al., 2003). Recent studies have linked white adipose-derived cells to cancer development. An *in vivo* murine model demonstrated that ASCs home to tumor sites when injected intravenously, and the stromal-derived growth factor-1 (SDF-1)/CXC receptor 4 (CXCR4) axis plays an important role in mediating the tumor-promoting effect of ASCs. This study reported that IL-6 secreted by ASCs is related to the migration and invasion of breast tumor cells. IL-6 is a critical growth factor for several types of cancer such as multiple myeloma (MM) and prostate cancer. Furthermore, an *in vivo* study showed that SDF-1 secreted by ASCs promoted the invasion and metastasis of breast cancer. Another study reported that human ASCs produce CCL5. Significant amounts of CCL5 were detected in conditioned medium from human ASCs after co-culture with MDA-MB-231 breast cancer cells. However, the effect of ASCs against cancer tumor cells is controversial. Several studies have reported that implanted ASCs inhibited breast cancer metastasis and growth in a murine model. Reciprocal interactions between breast tumor cells and stromal cells are mediated by inflammatory cytokines and chemokines, and may affect tumor development and progression. Therefore, the molecular basis of the effects of adipose tissue on the

behavior of tumor cells should be carefully examined before the future clinical application of stem cell therapies (Zubair and Ahmad, 2017).

CONCLUSION

ADSCs are under investigation for a variety of therapeutic applications. These cells are known to home to some tissues such as injured tissue. Although the mechanisms underlying the migration of ADSCs remain to be determined, clarification of the roles of chemokine receptors and adhesion molecules on ADSCs may lead to the development of therapeutic strategies to enhance the recruitment of cultured ADSCs to injured or damaged tissue.

Because human adipose tissue is a promising alternative source of stem cells, autologous ASDCs will lead to novel clinical applications in various medical fields. However, a greater understanding of the mechanisms of interactions among ASDCs, growth factors, and biomaterials on tissue regeneration is needed to advance the clinical utility of this therapy. Because chemokines derived from ASDCs may also affect cancer metastasis or invasion, additional findings are necessary to address the safety of ASDCs in the field of clinical tissue regeneration.

The adipose tissue microenvironment may induce ADSC to grow and differentiate into adipose tissues and the ADSCs themselves might secrete various cytokines that stimulate resident progenitor cells. Further preclinical and clinical studies are needed to determine whether

ADSC based therapies can fulfill expectations and can be used successfully in tissue regeneration.

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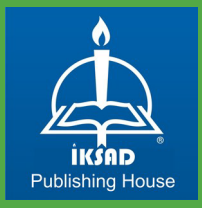

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