DAVETLİ DERLEME (Invited Review)

The Biology and Plasticity of Stem Cells: Progress and Promise

Kök Hücre Biyolojisi ve Plastisitesi: Gelişmeler ve Umut

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Abstract
Stem cells are undifferentiated cells that can renew themselves through and into a diverse range of specialized cell types by a process to as plasticity. In recent years the properties of regenerating themselves or producing specialized cell types make stem cells appealing for scientists seeking to create medical treatments that replace lost or damaged cells. Especially repairing the vulnerable and normally non- regenerative tissues and organs such as heart tissue and neurons by stem cells are going to be promising treatment modalities in the future. In this review the significance and biological features of stem cell types and recent developments in stem cell characteristics are described.

Key Words: **Cell Differentiation; Stem Cells.**

Özet
Kök hücreler mitotik bölünmeyle kendi kendilerini yenileyebilen ve plastisite işlemiyle pek çok özelleşmiş hücre tipine dönüşebilen farklılaşmamış hücrelerdir. Kaybedilmiş veya hasar görmüş hücrelerin yerini alabilecek medikal tedavilerin geliştirilmesiyle ilgili araştırmalarda son yıllarda kök hücreler kendilerini onarabilme veya özelleşmiş hücrelere dönüşebilme özellikleri nedeniyle bilim adamlarının ilgisini çekmektedir. Özellikle normal şartlarda hassas ve kendi kendini yenileme özelliği olmayan, nöronlar ve kalp kası gibi dokuların kök hücre uygulamaları ile tamirinin mümkün olması gelecekte umut vaadeden tedavi uygulamaları arasındadır. Bu derlemede kök hücrenin önemi, biyolojik özellikleri, kök hücre tipleri ve kök hücrelerle ilgili son gelişmeler anlatılmıştır.

Anahtar Kelimeler: **Hücre Farklılaşması; Kök Hücre. , Kök hücre tipleri, Plastisite.**

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Introduction

Until recently, basic information about stem cells was limited to hematopoietic stem cells which have also been used in treatment for a long period of time. However, there has been a rapid increase in information regarding stem cell types, basic biological features and their differentiation capacity into different cell types (plasticity) within the last 15 years. The point that has been reached in stem cell research today is extremely promising for the future. Difficulty in finding an appropriate donor is the most important factor precluding the chances of treatment in diseases in which the only treatment alternative is organ and tissue transplantation. If advances in stem cell research continue in the desired direction, organ differentiation from only one stem cell may be achieved and an unlimited tissue source can be generated for patients in need for transplantation. Moreover, the potential of restoration the functions of tissues and organs that have been impaired due to aging, disease or trauma has recently led to the emergence of a new medical field called "regenerative medicine" which has become a popular matter of discussion.

Stem cells

Stem cells are cells which have not been differentiated yet, and they can divide for long periods of time, can regenerate by themselves and thus can transform into different cell types. While somatic cells such as muscle, heart, liver, skin cells form cells that are identical to themselves when they are divided, stem cells do not have such a target. Therefore, stem cells can transform into different cell types depending on the signals they receive. Genes located in the cell nucleus and external stimuli are the most important determinant factors for this process. When death or damage occurs in any cell group within the body, stem cells transform into those cells that are needed by the body.

In vivo, stem cells can contribute to undifferentiated generations even if there is no tissue damage. Other criteria to define the stem cell are as follows (1).

Stem cells have the ability of dividing for a long period of time and renewing themselves. One of the factors determining the continuation of cell division for a long period of time is the DNA chains located on both endings of the chromosomes called telomeres (2). These consist of TTAGGG hexanucleotide sequences repeated for thousands of times. Telomeres function in maintaining the structural integrity of chromosomes by preventing the chromosome endings from disintegration, distribution or attachment of with the other chromosomes (3-6). The ability of DNA polymerase to synthesize DNA only in the 5'›3' direction and removal of short RNA primers during replication leads to the incomplete replication in the lagging strand and causes a reduction in telomere length in every cell division. As a result, the telomere length is approximately 100 base pairs shortened in every division of a normal cell. Telomerase is an enzyme of ribonucleoprotein structure that balances this loss at the chromosomal endings by attaching the hexomeric pieces (TTAGGG) it synthesizes using its own RNA as a template, to the chromosomal endings (7, 8). Shortly, it is the telomerase enzyme that preserves the length of DNA chains called telomeres. The telomerase activity of the cell determines the extent of telomere length maintenance. Telomerase activity is very active in the stem cell and accordingly the telomere chains are long. Consequently, stem cells can divide and copy themselves for long periods of time. This enzyme which is active in embryonic and adult stem cell, is not detected in the normal somatic cell, and is reactivated in the indestructible cancer cells (9). Telomeres in the somatic cells are structures that become shorter in every cell division causing cell division to stop at the point when their lengths reach a certain limit. Telomeres function as biological clocks of the cells playing role in the regulation of cellular life. Telomere length is 20 kb in the sperm and egg cells, and 10 kb in the cells of the newborn. Telomeres play role in chromosomal replication, chromosomal stability, gene expression, cell division, tumor formation and aging (10, 11). Mean life span of children with "progeria syndrome", a very rare disease also known as rapid aging disease, is around 12 years and these children have the facial features of a 60 to 70 year-old human being. The facts that somatic tissue telomeres of elderly individuals have been found to be shorter when compared to young individuals, and telomeres of children with progeria, an early aging syndrome, have been found to be shorter when compared to age-matched controls, support the relationship between aging and telomere length.

The first mammal that has been cloned from an adult cell was a female domestic sheep, "Dolly" (12). The cell which Dolly was cloned from was obtained from a healthy 6 year-old sheep. Dolly lived for approximately 6 years, while normal life span of a sheep is 10 to 12 years. Investigators have attributed this early death to the shorter telomeres of the cloned mammal compared to other age-matched mammals (13).

Stem cells are not specialized. A stem cell cannot work together with neighboring cells to pump blood to the body as in a heart muscle and cannot transport oxygen into the tissues as in erythrocytes. It can only act as a source for differentiating into specialized cells.

Stem cells can act as a source for non-specialized cells. This phenomenon is called differentiation. Stem cells can differentiate into more than one cell type. The best example for this can be observed starting from the zygote. The zygote that is formed after fertilization starts to divide by mitosis. The cells that are formed are called blastomeres. The zygote has the genetic information and power to generate the whole organism all by itself. This first embryonic cell that has the potential to differentiate into all cells in the body is called "totipotent", which means "capable of doing everything". All blastomeres up to 8 cells in the early stage are considered to be totipotent. At about the fifth day of fertilization, these cells transform into fluid filled cell groups called the "blastocyst". Inner cell mass within the blastocyst (embryoblasts) can differentiate into very different cells (nearly 250 types) originating from endoderm, ectoderm and mesoderm. Cells with these characteristics are termed as "pluripotent" cells. Human embryonic stem cells are acquired from the inner cell mass within the blastocyst and they are pluripotent. In later stages of development (fetal life), cells have more specific functions and transform into adult type stem cells. These adult type stem cells typically produce the cell types of the tissue they are the part of. Stem cells in the bone marrow are best examples for this, and these more specialized cells are termed as "multipotent" cells. Progenitor cells that tend to differentiate only into certain cell lines are called "unipotent" cells. For example, erythroblasts only generate erythrocytes.

Stem cells can regenerate the recipient tissue functionally after they have been transplanted into the damaged recipient tissue. In experimental studies, stem cells of bone marrow origin have been injected into the damaged heart tissue and it has been demonstrated that the previously damaged heart tissue function normally after regeneration, via the differentiation of the injected stem cells of bone marrow origin into heart muscle cells (14, 15).

Various stem cells have been isolated from embryo, fetal tissues and umbilical cord blood. Stem cells have also been isolated from adult mammalian tissues including bone marrow, brain, skin, dental pulp, eye, heart, kidney, lungs, gastrointestinal system, pancreas, liver, breast, ovary, prostate and testis (16).

Embryonic Stem Cells (ESC)

Embryonic stem cells are obtained from early stage mammalian embryo stem cells and have the unlimited capacity to multiply without differentiation any limit in vitro.

Isolation of embryonic stem cells and information related to how these cells can be replicated without differentiation under laboratory conditions dates back to nearly 30 years ago.

Two independent study groups (Evans and Kaufman, Martin) shared the success of extracting embryonic stem cells from inner cell mass of three and half days old mouse blastocysts in 1981, for the first time (17, 18). It has been possible, in several studies, to replicate embryonic stem cells for long periods of time before differentiation. In later years, a prominent database related to the biology and culturing of these cells was formed by studies of embryonic stem cells extracted from mouse blastocysts. Experience from mouse embryonic stem cells has provided clues in culturing human embryonic stem cells and methods used for their differentiation. Finally in 1998, Thomson et al. (19) have used, obtaining written permission from the families, fresh or frozen embryos generated by in vitro fertilization (IVF) method for clinical purposes and donated for investigational purposes. Thomson et al. have used these embryos in order to obtain human embryonic stem cell series. As a result of a series of studies, 14 inner cell masses were isolated from cultured human embryos until the blastocyst stage and out of these, 5 embryonic stem cell series were acquired.According to Thomson et al, the absolute characteristics of embryonic stem are listed below:

a) These cells have to be obtained from preimplantation stage embryo,

b) They must have the characteristic of long-term replication without differentiation,

c) They must have the potential to steadily generate derivatives of all three embryonic germ layer even after being kept in culture for long periods of time.

Acquisition and replication of embryonic stem cells

Human embryonic stem cells are acquired from the inner

cell mass that will form the embryo by removing trophoectoderm, the outer layer of blastocyst. The remaining intact inner cell mass is separated from this layer by complement mediated destruction method using antibodies. The isolated inner cell mass is then cultured over mitotically inactivated mouse embryonic fibroblasts which is used as a feeder layer. This layer primarily provides an attachment surface for the cells obtained from the inner cell mass and also provides the nutritional and growth factors required by the embryonic stem cell, removes the negative effects of the media and most importantly, assists in the replication of embryonic stem cells without differentiation. By performing sub passages, millions of embryonic stem cells, which provide an unlimited stem cell source, are achieved from the initial 30-70 cells of inner cell mass approximately in 6 months. Recently, instead of a feeder layer, the cytokine, leukemia inhibitory factor (LIF), has been used in the culture medium. While LIF inhibits the differentiation of mouse embryonic stem cells (ESC), it can not do so for the human embryonic stem cells. Moreover, the use of embryonic stem cells cultured from animal cells for treatment purposes is equivalent to xenografts. In addition, there are some dangers associated with this type of *in vitro* culture media, such as transmission of foreign viral and infectious agents to humans. Therefore, investigators have attempted to provide some alternatives to the use of mouse fibroblasts in the acquisition of embryonic stem cells. In one of the studies designed for this purpose and suggested positive results, investigators have succeeded to culture human embryonic stem cells without differentiation for long periods of time by using a nutritive layer formed by human fetal and adult fibroblast cells instead of mouse embryonic fibroblast cells (20).

Human embryonic stem cells express pluripotent and undifferentiated cell markers CD 9, CD 24, octamer binding protein (Oct-4), Nanog, LIN 28, Thy-1, Tra-1-60, Tra-1-81, SSEA-3 and 4, and they also show high telomerase and alkaline phosphatase activity and normal diploid karyotype (21). When LIF or feeder layer is removed from the culture medium, the cells spontaneously come together in aggregates and form buds called embryoid bodies. It is possible for these buds to differentiate into more specialized cell lines by the use of more specific growth factors and cytokines.

For example, embryonic stem cells can differentiate into all three embryonic germ layers by nerve growth factor and hepatocyte growth factor; however, they can differentiate into progenitors expressing ectodermal and mesodermal markers by fibroblast growth factor (FGF-2), retinoic acid and bone morphogenetic protein (BMP-4) (22).

The best way to show the pluripotency of the embryonic stem cells (ESC) is the formation of a teratoma containing all 3 germ layers 7 to 8 weeks after the injection of human ESC to a severe combined immunodeficient mouse.

Fields of Embryonic Stem Cell Application

Human and mouse embryonic stem cells represent strong instruments for many basic and applied aspects of cell biology.

Scientific researches on embryonic stem cells help to identify gene target of a new drug.

The goals of scientific research conducted by embryonic stem cells include:

- Identification of gene targets to determine new drugs and their toxicities

- Identification of teratogenic and toxic compounds in developmental biology

- Gene therapies

- Understanding the mechanisms underlying the formation of malignancies

- Production of more mature cells and tissues to be used in cell-based therapy (23).

Human embryonic stem cells that can be used in scientific research are achieved from 3 sources: They can be acquired from unused and frozen embryos in Test Tube Baby Units by permission of their families. Also they can be obtained from fetuses of pregnancies terminated due to various reasons. However, because these are in later stages of development, they have a more limited replication potential. Finally they can be obtained by therapeutic cloning.

Diseases that can be treated by using human embryonic stem cells include cancer, nervous system disorders and injuries (Alzheimer, Parkinson, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, spinal cord injury), diabetes, heart diseases, organ failures, rheumatic diseases, infertility, hearing losses, bone diseases (24).

However, several problems need to be solved before the application of therapeutic approaches associated with embryonic stem cells enter into clinical practice (24). There problems are follow:

a) Acquisition of the preferred cell type in adequate numbers and purity

b) Understanding which cell type should be replicated under what conditions in order to treat a known pathology

c) It seems that, development of several *in vitro* models, understanding the stem cell outcome, on site evaluation of the differentiation and assessment of their effects on functions are needed for the evaluation of cell-based therapy.

d) Cell-based therapy should be discontinued in case of an undesirable condition.

e) It should be determined whether embryonic stem cells will be influenced from mutations that may arise in the long run

f) Teratoma formation

g) Development of strategies to prevent rejection of the donated cell by the immune system

Adult Stem Cells

Adult stem cells are undifferentiated cells within a tissue or an organ. These cells can renew themselves and can differentiate into cell types of the tissue or organ they are the part of. The main function of adult stem cell in the organism is to repair the tissue in case of damage and to maintain the tissue continuity.

Adult stem cells have been detected in organs such as bone marrow, heart, kidney, pancreas, liver, lungs, breast, ovary, prostate and testis. The special environment in which adult stem cells and their supporting cells are located within the tissue or organ is called the microenvironment or the stem cell niche (25). Adult stem cells can stay at rest inside the niche for short or long time periods, and then can proliferate or differentiate into more mature and tissue-specific cell types following the changes within the niche (26). Despite adult stem cells express high telomerase activity within the niche, their differentiation capacity is limited compared to embryonic stem cells, and they cannot differentiate into all cell types. For example, nerve, muscle and liver cells can be obtained from adult type blood cells. Also, blood and muscle cells can be achieved from brain stem cells. However, the use of embryonic stem cells in the treatment of various diseases or repair of the damaged tissue has several disadvantages, including ethical dispute, and causing immune reactions seen in organ rejection (27). Therefore, research has concentrated on adult stem cells in recent years.

The plasticity potential of adult stem cell has been first discovered by the demonstration of donor cells in nonhematopoietic tissues of cases receiving bone marrow transplantation. A new concept explaining adult stem cell plasticity has been developed in recent years. According to this concept, the stem cell can acquire the phenotype of a stem cell of a different tissue or organ and, in certain conditions mesoderm, ectoderm and endoderm cells can differentiate into each other (28). The new "stem cell plasticity" concept has been explained by 5 different mechanisms:

- Transdetermination: Transformation of the stem cell into a stem cell which belongs to a different tissue.

- Transdifferentiation: A differentiated cell acquiring the differentiated cell phenotype of a different tissue. Formation of the esophageal skeletal muscles from smooth muscles during normal mammalian development is a good example for this phenomenon (29).

- Dedifferentiation: Differentiation of a mature cell into a more immature cell belonging to another cell-line which then differentiates into cells belonging to another cell-line.

- Cell Fusion: The best example for this is the injection of the nucleus of a mature cell into an ovum which has been enucleated during cloning. The cell becomes involved in a completely new programming process (12).

- Development of multipotent tissue specific cells from totipotent stem cell.

Hematopoietic Stem Cells

Stem cells acquired from hematopoietic tissues are considered to have the plasticity potential since 1961, with the concept of bone marrow transplantation (30). Hematopoietic stem cells are present in bone marrow, umbilical cord and peripheral blood. Surface markers of

hematopoietic stem cells in humans are often CD38–, CD34+ and CD133+ (31-33). Recently, stem cells in both hematopoietic and nonhematopoietic tissues have been found to be able to generate cells belonging to different tissues, other than the tissue they originally belong (34). Skeletal muscle, myocardium, epithelium, endothelium, neurons and hepatocytes can be generated from stem cells found in hematopoietic tissues (35). The richest tissue in hematopoietic cells is the bone marrow. Stem cells of the bone marrow are thought to involve at least 3 primitive stem cell populations (36-38):

- Hemangioblasts: Precursors of hematopoietic and blood vessel endothelial cells.

- Mesenchymal stem cells: Muscle, bone, cartilage, neural, adipocyte cells of mesodermal origin can be formed which support the hematopoietic stroma.

- MAPC (Multipotent Adult Progenitor Cell): Can generate ectodermal, mesodermal and endodermal cell series. Many different tissue cells can be developed from bone marrow stem cells on account of the ability of these cell series to transform into one another.

Bone marrow derived cells (BMDC) have been transferred into mouse brain tissue and these cells have been detected in various regions of the brain, including cortex, cerebellum and thalamus (39). This experiment proves that BMDC cells migrate into different regions and transdifferentiate into neural, glial cells. A similar finding has been reported in humans after bone marrow transplantation. Bone marrow cell transplantation from a male donor has been performed to a female patient and male donor cells containing Y chromosome have been detected in the patient's brain tissue (40). Although most of these donor derived cells possess hematopoietic cell features, a small portion have the features of brain tissue cells such as oligodendrocyte, astrocyte, and microglia. This study has demonstrated that hematopoietic stem cells can differentiate into brain tissue cells and may be used for regeneration purposes in brain damage. Although it is yet unknown whether or not these cells have cognitive functions, it has been suggested that they could be used in the treatment of ischemia, convulsion and paralysis. Furthermore, they provide a light of hope for the treatment of yet-untreatable diseases such as Parkinson, Alzheimer, multiple sclerosis, amyotrophic lateral sclerosis.

Cardiomyocytes are very sensitive to hypoxia and ischemia, and their regeneration capacity is limited (41). They are the first cells to die after myocardial infarction. The potential of hematopoietic cells or BMDC cells to transform into cardiomyocyte, endothelial and smooth muscle cells have been investigated in splenectomized mice. BMDC cells proliferate in the infarction area when directly injected to the infarction site after the infarction. It has been observed that these cells expressed surface markers of cardiac smooth muscle cells and led to improvement in cardiac functions. This experiment is the first animal study to show that hematopoietic stem cell can be used in the treatment of cardiovascular diseases (14). BMDC cells have been successfully used to stimulate angiogenesis in ischemic heart in humans, as well, and intracardiac injections of BMDC (CD34/CD133+) cells has been shown to be safe in humans (42-44).

It has been demonstrated in several studies in rodents and humans that stem cells of mesodermal origin, i.e. hematopoietic cells, can transform into endodermal cell series (44, 45). Differentiation of hematopoietic cells into hepatocytes has also been shown. Petersen et al. (46) have transplanted bone marrow cells from a male mouse to a female mouse and shown engraftment of these cells in the liver of the female mouse. One of studies best demonstrating stem cell plasticity has been conducted by Lagasse et al. in 2000 (47). A mouse model of Type 1 Tyrosinemia (fumarylacetoacetate hydrolase-FAH deficiency) has been created in this study. Liver failure in FAH deficient mice has been treated by intravenous administration of bone marrow cells of a healthy mouse. This is an important study proving that BMDC cells could regenerate hepatocytes. Formation of new hepatocytes by fusion of bone marrow stem cells with recipient hepatocytes have been shown in recent studies (48). Engraftment of bone marrow derived stem cells in the liver has also been shown in clinical studies. Alison et al. (49) have detected Y chromosome and cytokeratin 8 positive cells in 4 to 40% of the liver cells of female recipients getting bone marrow transplantation from male donors. It has been proven in recent studies that apart from the recipient liver, donor derived bone marrow cells were also present in gastrointestinal epithelium and skin (50). As a result of all of these animal studies and clinical studies in humans; adult bone marrow stem cells have been shown to differentiate into hepatocytes. However, the mechanism underlying this phenomenon is not clearly known. The latest opinion for explanation is cell fusion.

It is still not known whether this fusion event takes place between donor-donor or donor-recipient cells.

Conclusion

In order for a better understanding of stem cell biology, the mechanisms directing the self–renewal, differentiation and proliferation events of the stem cell needs to be elucidated. New advances in stem cell biology have revolutionary potential from both scientific and sociological perspective for the history of humankind. Thanks to the abilities of the stem cell of self-renewal and differentiation into different cell types; the physicians will not remain desperate in certain clinical problems, including wound healing, autoimmune diseases, cancer, degenerative diseases and infertility. The plasticity potential of the adult stem cells is not yet fully understood. However, embryonic stem cells are totipotent and can differentiate into every direction. Therefore, they can be used in a wide spectrum both therapeutic and organ generation purposes. As future advances in stem cell biology will be integrated into clinical studies, the humankind will come one step closer to immortality, the greatest dream of all times.

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