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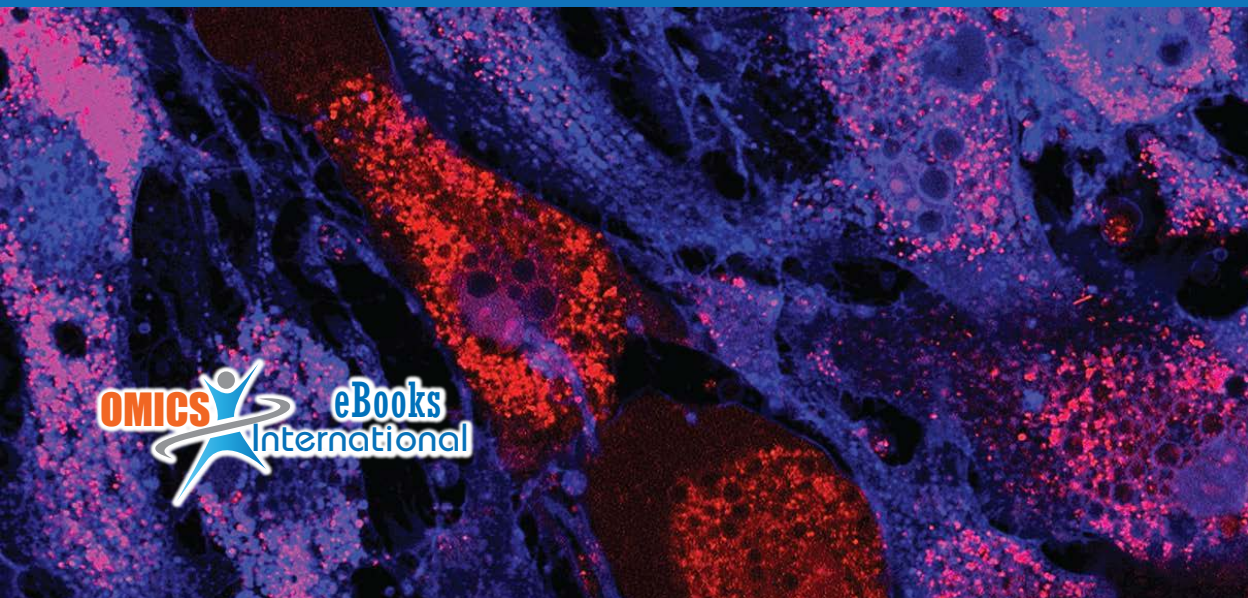

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Stem Cells in Cell Therapy and Regenerative Medicine

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PREFACE

Umbilical cord cells constitute the most important stem cell source for collection, storage and use. The cord blood obtained from this source can be used in a variety of fields for regenerative medicine applications. Apart from this stem cell source, there is also the availability of alternative stem cell sources, and these also provide a great advantage in that regenerative medicine can be used. By using tissue engineering techniques, regenerative medicine can be utilized in the treatment of common diseases such as neurodegenerative diseases, cardiac diseases and diabetes mellitus.

The role of cancer stem cells, which have a restrictive effect in cancer treatments and affect the prognosis negatively, and the managing these cells are gaining importance. The data obtained from cancer stem cell research provide significant added value for the development of new therapeutic strategies based on regenerative medicine. These therapeutic strategies bring renewed hope to cancer patients.

In this prepared book, the above mentioned topics are explained in detail and presented to the use of the scientific community.

Dr. İdil ÇETİN

Dr. Mehmet Rıfkı TOPÇUL

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We would like to express my gratitude to the many people who saw us through this book; to all those who provided support, talked things over, read, wrote, offered comments, allowed us to quote their remarks and assisted in the editing, proofreading and design.

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Grateful and deepest thanks are also extended to Istanbul University Advanced Stem Cell & Biomolecular Technology Research Team, friends and students for their caring and support. They have been wonderful supporter and we would not be here today if it were not of them.

Last and not least: We beg forgiveness of all those who have been with me over the course of the years and whose names we have failed to mention.

Chapter 1

Umbilical Cord Stem Cells and Their Regenerative Potential

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Abstract

The stem cells, which are the source of the tissues and organs in the organism, regenerate the damaged and diseased tissues as the organism ages. There are different types of stem cells with self-renewal and differentiation ability in the umbilical cord blood located in the umbilical cord and placenta.

Introduction

The human umbilical cord (UC) in itself contains distinct anatomical regions consisting of an umbilical vein, two umbilical arteries, cord lining, and Wharton's jelly. This is jelly-like tissue surrounds the blood vessels and plays the functional role in supporting the vessels [1].

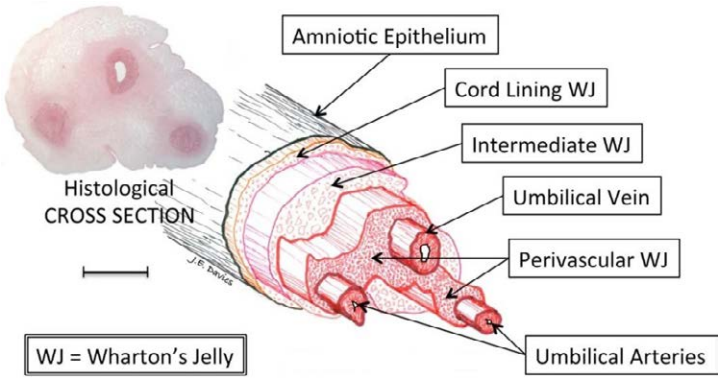

Figure 1: Structure of umbilical cord [2].

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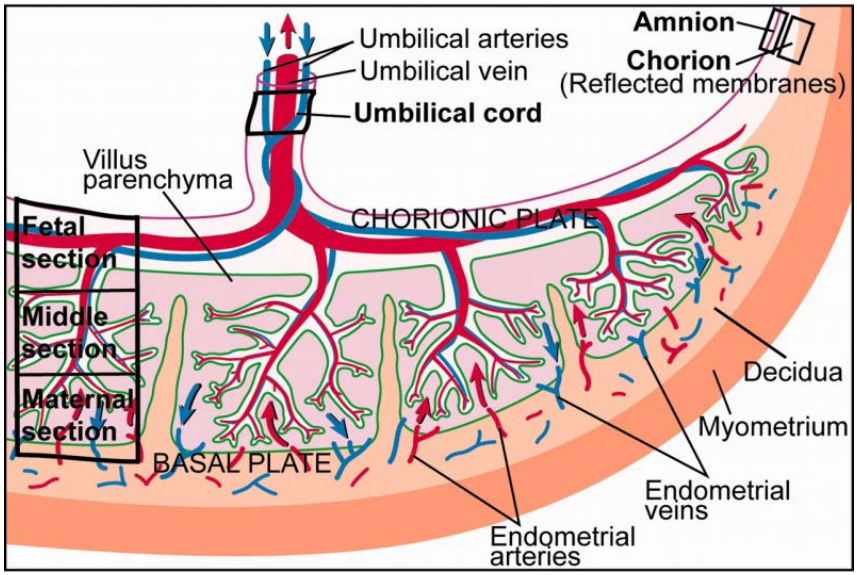

Figure 2: Structure of placenta [3, 4].

The placenta is the organ that connects the developing fetus via the umbilical cord to the maternal uterine wall carrying out nutritive, respiratory, and excretory functions [4, 5]. Similar to the umbilical cord, the placenta originates from the same zygote as the fetus. It begins to develop during implantation of the blastocyst into the maternal endometrium and grows throughout pregnancy [4, 6]. Anatomically, the placenta has a dark maroon color and round flat appearance. It averages around 20 cm in diameter and 2.5 cm in thickness at the end of gestation [4] (**Figure 1 & 2**).

Because UCB is a highly enriched stem cell source [7, 8], it is thought to be a helpful treatment for a number of genetic diseases, blood malignancies, and immune deficiencies. UCB may be also of medical use for a sick sibling or relative. Banking UCB is thus a way to preserve potentially life-saving cells that are usually discarded after the interruption of the blood supply from the umbilical cord to the newborn infant. Prior to collection, UCB donors are required to sign an informed consent form. At this time or alternatively up to 7 days before or 7 days after birth of the child, they are also tested for infectious diseases and microbial sterility. The precise timing for clamping and extracting the residual cord blood is important because umbilical vessels tend to collapse, according to Burton's theory [8, 9].

As ethnic diversity increases in developing countries, it is imperative to find alternative stem cell sources when an adult-matched unrelated donor cannot be identified. At present, there are three alternative options: a partially HLA-mismatched unrelated donor, a haploidentical related donor, and a UCB stem cell product [8].

Cord blood has recently been used in a variety of regenerative medicine applications [10]. Work done by McGuckin and colleagues [11-13], Rogers and colleagues [14]. Kucia and colleagues [15], Harris and colleagues [14, 15] has shown that cord blood contains a mixture of pluripotent stem cells capable of giving rise to cells derived

from the endodermal, mesodermal, and ectodermal lineages [13].

Thus, cord blood appears to be a practical substitute for embryonic stem cells and readily available for use in tissue engineering and regenerative medicine [13], Recently, clinical trials have begun using cord blood stem cells to treat type 1 diabetes, cerebral palsy, and peripheral vascular disease among others [16, 18].

Cord Blood-Derived Stem Cells

In terms of ontogeny, CB-derived stem cells are at the intermediate point between embryonic and adult life [19, 20]. CB stem cells also exhibit longer telomeres associated with high levels of telomerase activity and a high proliferation potential [20-23]. It appears that CB stem cells are relatively tolerant and are less likely to react immunologically against the host [24-26].

It is generally accepted that UCB contains mesenchymal stromal cells (MSCs) [27, 28], endothelial progenitor cells (EPCs) [26, 29], unrestricted somatic stem cells (USSC) [26, 30], very small embryonic-like stem cells (VSELs) [26, 31], multi-lineage progenitor cells (MLPCs) [26, 32], and neuronal progenitor cells [33].

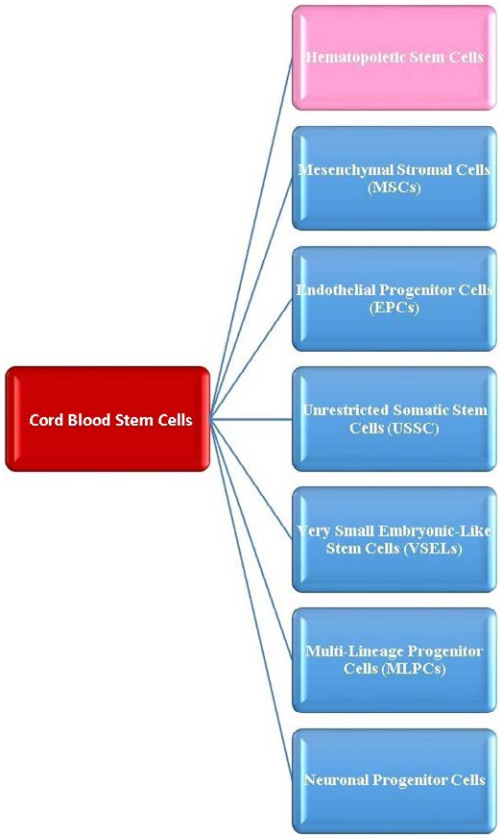

Figure 3: Different types of cord blood stem cells.

Hematopoietic Stem Cells

HSCs possess the ability of both multipotency and self-renewal [34]. Multipotency is the ability to differentiate into all functional blood cells. Self-renewal is the ability to give rise to HSC itself without differentiation [35].

Hematopoietic stem cells are of therapeutic interest to the clinicians and researchers due to their promising assistance in management of malignant and inherited hematological conditions [36]. Umbilical cord blood collected from the postpartum placenta and cord is a rich source of Hematopoietic Stem Cells (HSCs) and is an alternative to bone marrow transplantation] [4, 37]. Characteristic feature of hematopoietic stem and progenitor cells is the presence of CD34 antigen [20, 38].

Several investigators have demonstrated that UCB-derived Hematopoietic Stem/ Progenitor Cell possess higher expansion and proliferation potentials than their BM counterparts [4, 39-47].

Hematopoietic progenitors from umbilical cord blood are enriched for *in vivo* long-term repopulating stem cells. Compared to adult cells, umbilical cord blood hematopoietic stem cells produce larger hematopoietic colonies *in vitro*, have different growth factors requirements, and are able to expand in long-term culture *in vitro*, engraft SCID-human mice in the absence of additional human growth factors, and have longer telomeres [48].

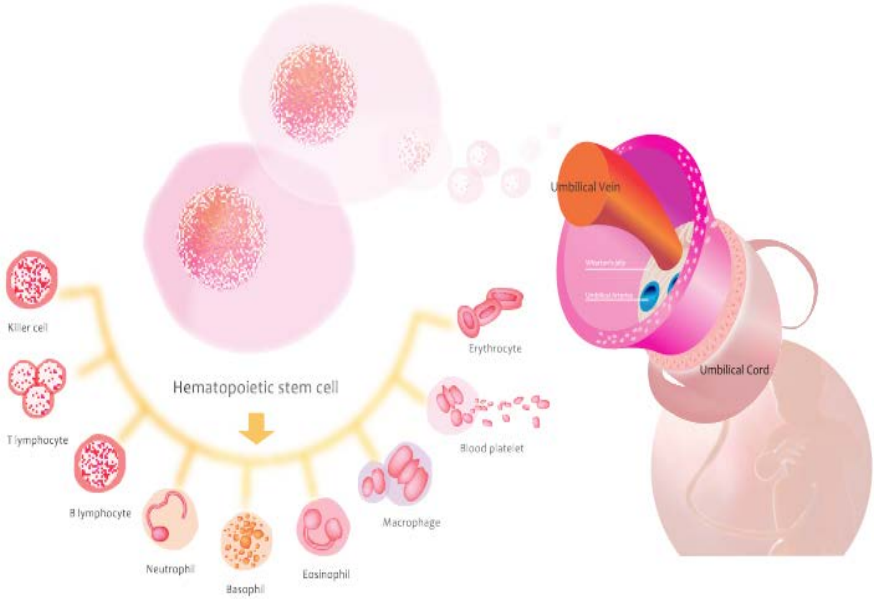

Figure 4: Umbilical cord hematopoietic stem cells [49].

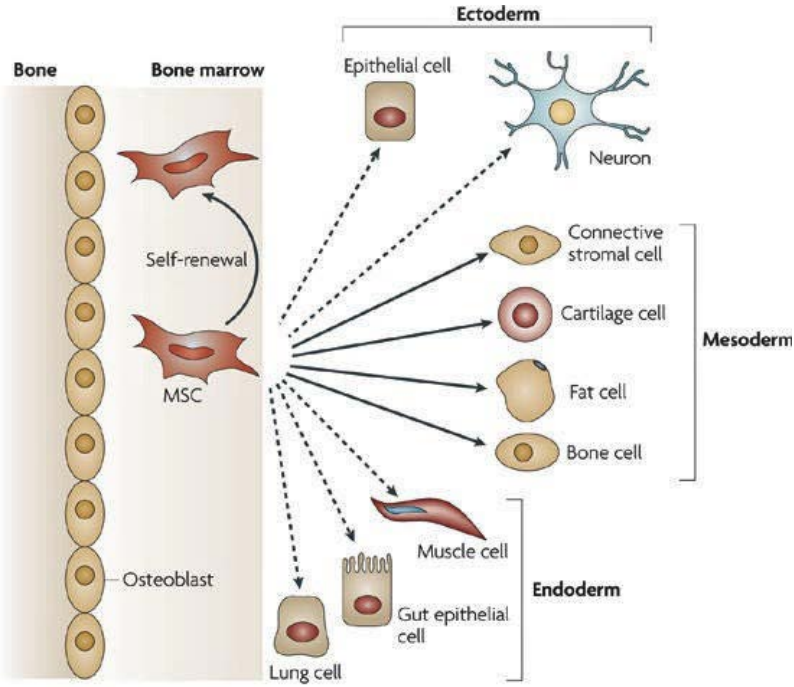

Figure 5: Differentiation of mesenchymal stem cells [50, 51].

Phenotypically, these non-hematopoietic cells are characterized by their negativity of the hematopoietic cell markers, CD34 and CD45, and for expressing the MHC class I, but do not express MHC class II [52, 53]. IL-1, IL-6, IL- 7, IL-8, IL-11, IL-12, IL-14, IL-15, LIF, SCF, FLT-3 ligand, GM-CSF, G-CSF, and M-CSF [54-56]. MSCs also express receptors for some cytokines and growth factors such as: IL-1 R (CD121a), IL-3 R (CD123), IL-4 R (CDw124), IL-6 R (CD126), IL-7 R (CD127) [54, 57, 58], LIFR, SCFR, G-CSFR [54], VCAM-1 (CD106) [53, 54, 57], ALCAM-1 (CD166) [52, 54, 57, 59-65], LFA-3 (CD58), TGF(1R, TGF(2R, IFN(R (CDw119), TNF1R (CD120a), TNF2R (CD120b), bFGFR, PDGFR (CD140A), EGFR [54, 57] and CXCR-4 [57, 66-72] are numerous proteins secreted by MSCs.

Cord Blood-Derived Endothelial Progenitor Cells (EPCs)

Endothelial Progenitor Cells (EPCs), first identified in adult peripheral blood [73, 74] but present in significantly higher numbers in UCB [75-77], form vascular networks *in vivo* [78-81], a characteristic that has created motivation for developing new EPC-based vascularization therapies.

The cultured EPCs characterized by endothelial Cell-Colony Forming Units (CFU-ECs) express not only endothelial markers CD31, CD105, CD144, CD146, vWF, UEA-1 and KDR, but also monocyte/macrophage markers CD14, CD45, and CD115. In addition, the cultured EPCs possess myeloid progenitor cell activity, differentiate into phagocytic macrophages, and fail to form perfused vessels *in vivo* [26, 81].

Recently, Ingram et al. [26, 75] have identified other EPCs with blood vessel-forming ability, termed Endothelial Colony-Forming Cells (ECFCs), which are also referred to as blood outgrowth endothelial cells [26, 74], from human peripheral blood and UCB. ECFCs express endothelial markers CD31, CD105, CD144, and CD146, but not hematopoietic cell markers CD45 and CD115. ECFCs are characterized by robust proliferative potential and by their ability to form perfused blood vessels *in vivo* when transplanted with collagen fibronectin matrix into immune deficient mice [26, 75, 81].

ECFCs are enriched in UCB compared to adult peripheral blood. In addition, UCB-derived ECFCs have greater proliferative activity and enhance vessel forming ability compared to adult peripheral blood-derived ECFCs [26, 75, 78]. Thus, UCB-derived ECFCs may more effectively contribute to vascular regeneration [26].

Recent studies have shown that EPCs are a potential tool for therapeutic angiogenesis in the treatment of patients suffering from severe limb ischemia or myocardial infarction [82]. EPCs have been identified as contributors to vessel development in both normal physiological processes such as wound healing and pathological processes such as cancer [83].

New evidence accumulated over the past decade demonstrates that umbilical CB provides distinct advantages over other EPC sources and has the potential to be therapeutically applied across a wide range of pathological conditions [20]. Therefore, as a legitimate resource of stem cells, CB became an attractive choice for tissue engineering and regenerative medicine [20].

Unrestricted Somatic Stem Cells (USSC)

In addition to MSCs, human CB contains Unrestricted Somatic Stem Cells (USSCs) [30, 84]. USSCs are considered a precursor to MSCs and can be distinguished from MSCs by their higher expansion capacity, broader differentiation ability, and differential expression of genes including D-like 1/preadipocyte factor 1 (DLK1) and the Homeobox (HOX) gene clusters [84-87]. USSCs have the potential to differentiate *in vitro* to osteoblasts, chondrocytes, and hematopoietic and neuronal cells and *in vivo* to bone, cartilage, hepatocytes, hematopoietic cells, myocytes, etc. USSCs constitutively express a series of cytokines including stem cell factor, leukemia inhibitor factor, Vascular Endothelial Growth Factor (VEGF), Stromal Cell-derived Factor (SDF) 1, etc., and have strong hematopoietic stimulating activity [84, 88]. Similar to MSCs, USSCs lack expression of immunorelevant adhesion and costimulatory molecules. However, immunosuppression by USSCs is conditional and dependent on Tumor Necrosis Factor-a (TNF-a) and Interferon-g (IFN-g) [84, 89].

Administration of USSCs in multiple animal disease models has resulted in the promotion of bone healing and recovery from neural injury and myocardial infarction [84, 90-93].

Human umbilical cord blood contains a subset of stem cells that can differentiate into cells representative of all three germline layers [30, 94-96]. The first to describe the multilineage capacity of these cells *in vivo*, in calling them “Unrestricted Somatic Stem Cells” (USSCs) [26, 30].

Although USSCs are rare compared to haematopoietic stem cells in cord blood, they can be expanded rapidly to yield large numbers of cells for study or transplantation [97].

Pluripotent Unrestricted Somatic Stem Cells (USSC) from human umbilical cord blood reside in an early differentiation state, can be propagated to high cell numbers, and on treatment with appropriate stimuli display broad differentiation capabilities *in vitro* and *in vivo* [30, 86]. They thus represent promising candidates for regenerative and cell replacement therapies [98].

Very Small Embryonic-Like Stem Cells (VSELs)

Recently researchers worldwide they found stem cells isolated from umbilical cord blood that expressed early transcription factors found typically in the embryonic stem cells [15, 99]. These cells are first described by Kucia et al. 2006, in a fraction of murine bone marrow stem cells [31, 99], and named Very Small Embryonic Like Stem Cells (VSELS). VSELS are very small (2-4 μm) CD34 and CD45 negative stem cells that strongly express CXCR4 Sca-1+ antibody and embryonic transcription factors as OCT and Nanog. These transcriptions factors are considered as markers of mouse and human embryonic stem cells playing a basic role in stem cell pluripotency [99-102]. VSELS are smaller than erythrocytes and larger than platelets. They can be distinguished from large platelets not only based on different surface markers, but also because they contain nuclei. Interestingly, VSELs despite their small size posses diploid DNA, contain numerous mitochondria and high telomerase activity. They do not express MHC-1 and HLA- DR antigens and are CD90− CD105− CD29− [103-105].

Multi-Lineage Progenitor Cells (MLPCs)

A Multipotent Cell (Multilineage Progenitor Cells [MLPC]) potentially representing a new subset of stem cell was recently identified in UCB as a CD45+/CD34+/CD9+/ nestin+ plastic adherent population [106]. These cells have demonstrated extensive expansion capacity, while maintaining normal genetic stability [107], as well as the ability to be differentiated into cells representing all three germinal layers. These cells are thought to bridge the span between pluripotent ES cells and adult-source stem cells by demonstrating extensive plasticity without teratoma potential [28, 108].

Neuronal Progenitor Cells

Subpopulations of CB isolated according to the expression of hematopoietic stem cells markers such as CD34+, CD133+ or CD45+ were induced *in vitro* to differentiate towards neuronal-like phenotype [14, 109-112]. Subsequently, CD34- CD45- non-hematopoietic stem cells, and MSC and Unrestricted Somatic Stem Cells (USSCs) were identified as origins of the neuronal-like cells [30, 86, 113-117]. Umbilical cord blood stem cells have demonstrated efficacy in reducing lesion sizes and enhancing behavioral recovery in animal models of ischemic and traumatic Central Nervous System (CNS) injury [118, 119].

Advantages of Cord Blood Stem Cells

Autologous MSC derived from BM have been applied for cell-based therapies, including the treatment of osteogenesis imperfecta, intracoronary transplantation in patients with acute myocardial infarction, and support of haematopoiesis [120-125]. However, the harvest of BM is a highly invasive procedure, and the possibility of donor morbidity as well as the number, differentiation potential and maximum life span of human BM-derived autologous MSC significantly decline with the age of the donor [125-128].

UCB is rapidly gaining attention for its therapeutic value for several reasons. An attractive alternative source of MSC, UCB can be obtained by a less invasive method, without posing harm to the mother or infant. Cells from UCB have many advantages because of the immature nature of newborn cells compared to adult cells. Moreover, UCB cells provide no ethical barriers for basic studies and clinical applications [129-130].

First, UCB has more primitive HSCs per volume than bone marrow [51, 131]. Second, there is a lower incidence of rejection after UCB transplantation [51, 132-134]. Third, unlike bone marrow transplants, UCB transplantation does not require perfect antigen matching [51, 132]. Fourth, UCB transplantation has been useful for the treatment of inborn errors in metabolism [51, 135]. Finally, the methods for collecting, storing, and freezing human blood were developed in the 1940s, so no new technology is needed to save the mononuclear cells from UCB. This has led to the establishment of cord blood banks and the increased use UCB for transplantation [51, 136, 137].

As compared to other sources of HSCs, like peripheral blood and BM, the UCB offers numerous logistic and clinical advantages such as: (1) practically unlimited offer, (2) immediate availability of cryopreserved units in public UCB banks, and which decrease an average 25-36 days the wait for transplantation as compared to BM, (3) extension of the pool of donors due to the tolerance of up to two mismatches in the HLA system, (4) lower frequency and severity of the Graft Versus Host Disease (GVHD), (5) lower risk of transmission of latent infections such as cytomegalovirus and Epstein Barr Virus, (6) absence of risk to the donor, and (7) higher incidence of rare haplotypes than those found in the records of BM donors [56, 138].

Umbilical cord blood can be stored and cryopreserved in cord blood banks for later uses in transplantations applications [119, 139-142].

Regenerative Potential of Cord Stem Cells

Self-renewal and differential capacity make stem cells as potential tools for regeneration, restoration or replacement therapies in a variety of disease conditions [30]. Stem cell based therapies are increasingly being utilized with promising results in both malignant and non-malignant disorders [136, 143]. Three sources of cells have been used for haematopoietic reconstitution Bone Marrow (BM), Peripheral Blood (PB), and Umbilical Cord Blood (UCB) [20, 136, 144, 145].

Umbilical cord blood stem cell populations are promising source of stem cells for research and clinical applications because of their abundance, accessibility and

differentiation potential [19,119, 139]. Compared with stem cells obtained from adult bone marrow harvests, UCB stem cells have greater proliferative potential and longer telomeres [146]. CB stem cells are capable of giving rise to hematopoietic, epithelial, endothelial, and neural tissues both *in vitro* and *in vivo* [13]. CT stem cells are capable of giving rise to various mesenchymal lineages, including bone, cartilage, and fat [147]. Thus, cord blood and cord tissue stem cells are candidates to develop stem-cell-based therapies for a wide variety of diseases, including cardiovascular, ophthalmic, orthopaedic, neurological, and endocrine diseases [13, 148].

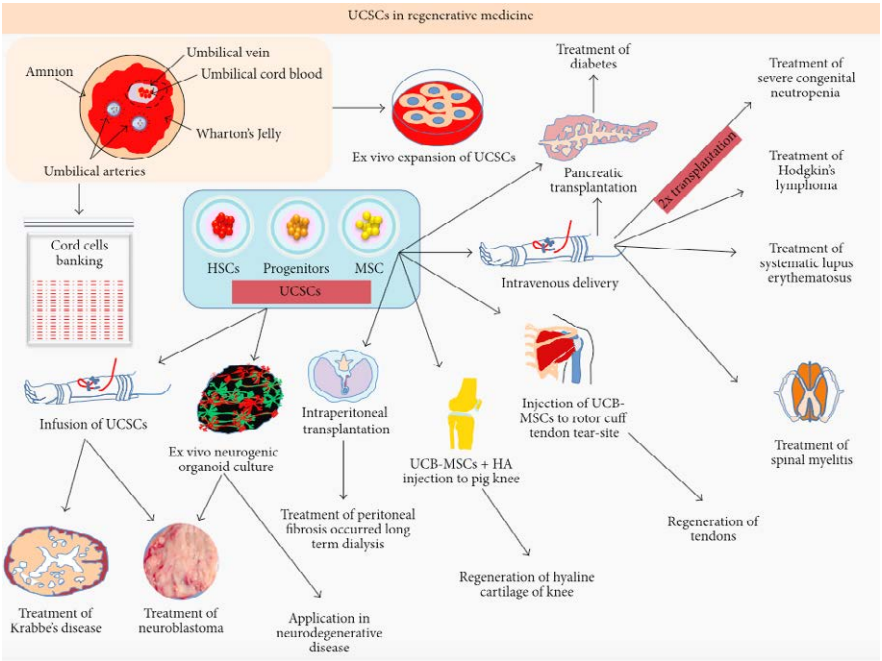

Figure 6: Application of umbilical cord stem cells [149, 150].

Leukemia, anemia, sickle cell disease are some hematopoietic conditions that can be treated with cord blood stem cells. Ongoing studies are shown that a number of inherited metabolic disorders, including Hurler Syndrome, Scheie Syndrome, Hunter Syndrome and many others also can be treated these stem cells [151, 152]. Umbilical cord stem cells have also been proved promising in possible treatment of several diseases and conditions such as diabetes [153], certain diabetic wounds [154], and brain damage associated with neonatal hypoxia [155], stroke [156], autism [157], acute liver failure [158], cerebral palsy [159, 160] and Alzheimer's[161].

Discussion

Stem cells can be used for the routine treatment of more than 80 diseases especially hematopoietic and oncological diseases. Advanced applications in stem cell studies may be hopeful for many diseases in the future. The cord blood stem cells collected during birth can be used for the baby himself/ herself and his/her

brothers, or even for other family members as long as the tissue is compatible. Although cord blood can only be collected at the beginning of life, it can be used even after many years. In the near future perhaps everyone will be offered to keep healthy stem cells. This stem cell source may be cord blood or it may be another stem cell source. In the treatment of many deadly diseases such as heart disease, stem cell therapy appears to be a revolutionary new treatment option.

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Chapter 2

Induced Pluripotent Stem Cells

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Abstract

Although there are various stem cell sources, embryonic stem cells have unique features for the treatment of various diseases. These cells have several limitations in addition to being a promising source. To overcome these limitations, stem cells which are unproblematic and posses embryonic stem cell features are generated. In this context, in this chapter Induced Pluripotent Stem Cells (ipsc) were discussed.

Introduction

In cell biology, the definition of pluripotency has come to refer to a stem cell that has the potential to differentiate into any of the three germ layers: endoderm, mesoderm or ectoderm. Pluripotent stem cells can give rise to any fetal or adult cell type. However, a single cell or a conglomerate of pluripotent cells cannot develop into a fetal or adult animal because they lack the potential to organize into an embryo [1, 2]. Pluripotent stem cells derived from the blastocyst as embryonic stem cells; from epiblast as epiblast stem cells; from primordial germ cells as embryonic germ cells; from gametes as spermatogonial germ stem cells at different stages of embryonic development [3].

Consistent with their origin from the inner cell mass, ESCs express a core set of transcription factors consisting of Oct4, Nanog, Sox2, and Tcf3 that provide maintaining the pluripotent state of ESCs and they exist in a pre-X-inactivation state with both X chromosomes active in female cells [4-7]. However, distinct biological and molecular characteristics distinguish ESCs from their *in vivo* counterparts of the inner cell mass. For example, cells of the inner cell mass are not self-renewing, and they are characterized by a genome that is globally hypomethylated [3, 8]. In contrast, ESCs have unlimited proliferation potential, and their genome is highly methylated [9].Clinical applications of embryonic stem cells are expected to range from being used as tools for *in vitro* investigation of cellular processes and drug discovery, to being a source of cells for tissue generation and cell replacement therapies. Their unique characteristics include the ability to grow *in vitro* indefinitely, while retaining their capacity to differentiate into specialised somatic cell types [10, 11].

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Human Embryonic Stem Cell (hESC) research is ethically and politically controversial because it involves the destruction of human embryos [12]. Due to ethical objections to the use of human ES cells, many investigators and legislative bodies examined the alternative ways for producing ethically, scientifically and therapeutically acceptable pluripotent stem cells [13].

Alternative Source of Embryonic Stem Cells

Organismically Dead Embryos

One definition for an organismic death of an embryo is cessation of "continued and integrated cellular division, growth, and differentiation" [14]. When this happens, as is the case for many embryos derived via *In vitro* Fertilization (IVF), the embryo would not develop any further *in vitro* and would not be viable following uterine transfer. Most IVF embryos are cultured to the 2-10 cell stage (2-3 days old) or up to the blastocyst stage (5-6 days old), and then transferred into the uterus. At the 2-8 cell stage, each component cell, called a blastomere, is totipotent. However, by 5-6 days following blastocyst formation, the inner cell mass-composed of the cells that are usually extracted to derive hESC lines-has formed and no individual cell is capable of full embryonic development. In other words, there are no longer any totipotent cells present [15].

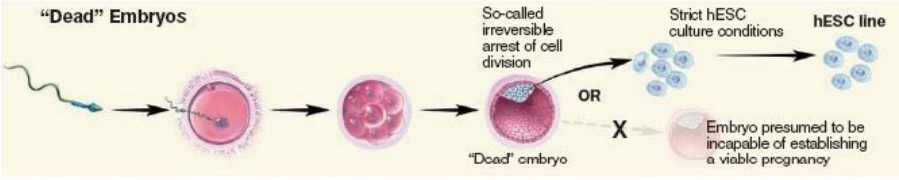

Figure 1: Organismically dead embryos [16].

As many as 60% of IVF embryos produced by infertility clinics are judged to be incapable of developing to live birth, due to abnormal appearance or failure to divide appropriately, and are not used by the infertile couple. Although failure to divide is often caused by genetic abnormalities and might seem to eliminate any prospect of using these embryos even for research, several studies suggest that some normal cells may be obtained from such organismically dead embryos and may be useful in creating stem cell lines [17] (**Figure 1**).

Biopsied Single Blastomer

Preimplantation Genetic Diagnosis (PGD) is a form of prenatal diagnosis that is performed on early embryos created by *In vitro* Fertilization (IVF) [18]. A single cell (or cells) is removed from each embryo of an *in vitro*-developing cohort, on which a diagnostic genetic test is carried out. Up to three of the embryos that are unaffected are transferred to the patient in the hope of establishing a pregnancy. Only embryos that are shown to be free of the genetic disorders are made available for replacement in the uterus, in the hope of establishing a pregnancy [19]. PGD embryos diagnosed as affected by monogenic diseases such as myotonic dystrophy type 1 (DM1), Cystic Fibrosis (CF) and Huntington Disease (HD) have been used for derivation of new hES cell lines [20] **(Figure 2**).

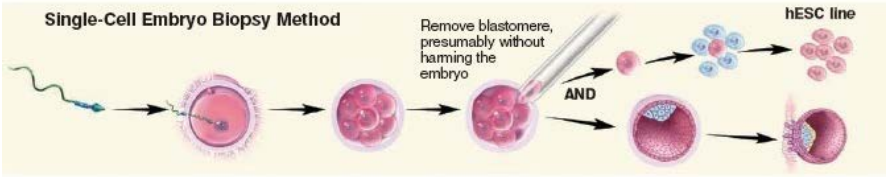

Figure 2: Biopsied single blastomere [16].

Somatic Cell Nuclear Transfer (SCNT)

Somatic Cell Nuclear Transfer (SCNT) takes advantage of a unique property of the oocyte cytoplasm that allows somatic nuclei to be reprogrammed to a pluripotent state [21]. In this case, the nucleus of a somatic cell is transferred into an enucleated oocyte. The somatic nucleus is then reprogrammed and partial development to the ICM stage can occur in culture, followed by either transplantation into a prepared uterus in order to generate cloned animals, or harvesting the ICM to generate ESC lines [22, 23] (**Figure 3**).

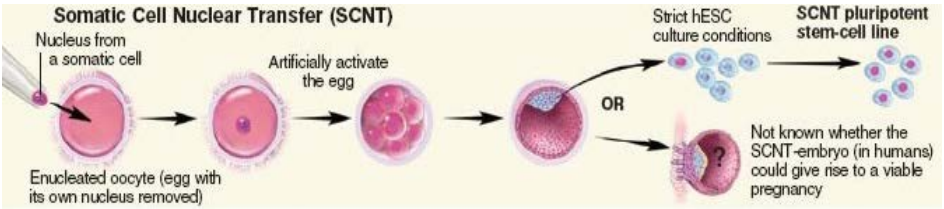

Figure 3: Somatic cell nuclear transfer (SCNT) [2, 16].

Until recently, SCNT was the only technique to accomplish complete nuclear reprogramming and was used not only to clone live animals, such as Dolly (reproductive cloning) but also to establish SCNT-derived ES cell lines from cloned murine [24] and recently, primate blastocysts [25] for the purpose of therapeutic cloning [26, 27].

Somatic Cell Nuclear Transfer (SCNT) products have histological compatibility with the nuclear donor, which circumvents, in clinical applications, the use of immunosuppressive drugs with heavy side effects. While the goal of reproductive cloning is the creation of a person, the purpose of therapeutic cloning is to generate and direct the differentiation of patient-specific cell lines isolated from an embryo not intended for transfer in utero. Therapeutic cloning, through the production of these autologous nuclear-transfer Embryonic Stem Cells (ntESC), offers great promises for regenerative and reproductive medicine, and in gene therapy, as a vector for gene-delivery [27].

Altered Nuclear Transfer (ANT)

Altered Nuclear Transfer (ANT) technique is a variant of SCNT where the transferred nucleus is altered so that no blastocyst develops [28]. Alteration of nucleus is carried out through silencing a gene such as Cdx2 (**Figure 4**).

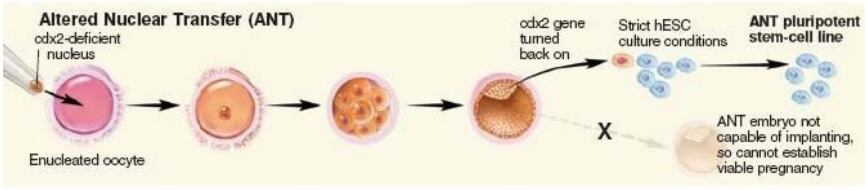

Figure 4: Altered nuclear transfer (ANT) [16].

Cdx2 is essential for trophectoderm formation in mouse. Blastocysts with disabled Cdx2 lack trophectoderm and can not implant but can serve as a source of normal ES cells after removal of a transgene producing the Cdx2-interfering RNA [29, 30]. The need for removing and reinserting genes in the process of ANT could produce genetic errors and such ES cells may not be useful for scientific or therapeutic applications [26].

Induced Pluripotent Stem Cells (iPSCs)

iPSC technology is a novel and reliable method for generating pluripotent stem cells. In contrast to other methods for generating pluripotent stem cells, such as the derivation of ESCs from the inner cell mass at the blastocyst stage, or nuclear transfer and fusion of somatic cells with ESCs, this method can directly convert somatic cells into pluripotent cells, regardless of the availability of embryonic cells (**Figure 5**). In the future, iPSCs may replace the use of human ESCs in various applications,including as cellular models in the study of human diseases and for drug research and development [31]. This iPSC technology may also speed the search for better ways to induce pluripotent cells to differentiate into desired cell types, because reproducible chemical recipes to differentiate most cell types from human ESCs are still elusive [32].

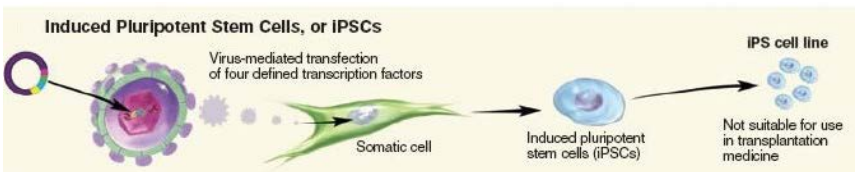

Figure 5: Induced pluripotent stem cells (iPSCs) [2, 16].

In order to find the minimal combination of “stemness” factors capable of inducing an ES cell-like phenotype in somatic cells, Shinya Yamanaka’s group initially screened a group of 24 gene candidates known to be critical for pluripotency. They found that just four genes Oct4, Sox2, Klf4 and c-Myc are sufficient for reprogramming of mouse embryonic fibroblasts into, so called, Induced pluripotent stem (iPS) cells when transduced with retroviral vectors [33]. Further analysis shows that three of these genes, Oct4, Sox2, and Klf4, are critical to the process and that c-Myc functions to enhance reprogramming efficiency [34-37].

In the absence of Oct4, embryos die at the time of implantation because of a lack of pluripotent ICM cells [38, 39]. Oct4 is therefore considered a master regulator for the initiation and maintenance of pluripotent cells during embryonic development.

Interestingly, the precise expression level of Oct4 is a critical determinant of ESC fates, and their pluripotent potential can be sustained only when the Oct4 expression level is maintained within a normal range [39-42].

Depletion of Sox2 by either gene-knockout or RNA interference considerably compromises the pluripotent state of both mouse and human ESCs as shown by the changes in cell morphology, loss of pluripotent marker expression and their differentiation primarily into trophectoderm [43-45].

As a key factor in reprogramming, Kruppel-like factor 4 (Klf4/GKLF/EZF) functions as both a transcriptional activator and repressor to regulate proliferation and differentiation of different cell types [37, 46]. In Embryonic Stem (ES) Cells, Klf4 has been shown to be important to activate Lefty1 together with Oct4 and Sox2 [37, 47]. Klf4 interacts directly with Oct4 and Sox2 in iPS and ES cells [37].

There are two types of methods for the delivery of reprogramming factors into the somatic cells can be used [31]. These are integrating viral vector systems including retroviral, lentiviral and inducible lentiviral systems and non-integrating methods including viral vectors, plasmid DNA, recombinant proteins and synthetic mRNA [31].

Therapeutic and Scientific Potential of iPS Cells

Cell Replacement Therapy

Embryonic Stem Cells (ESCs) and induced Pluripotent Stem Cells (iPSCs) have the capacity to differentiate into any specialized cell type of the human body, and therefore, ESC/iPSC-derived cell types offer great potential for regenerative medicine [48].

Regenerative medicine aims at helping the body to form new functional tissue to replace lost or defective ones [49]. The promise of stem cell biology for the development of novel therapeutics has fueled a veritable explosion in studies aimed at using these cells in “regenerative medicine,” an emerging field of biomedicine focused on the “repair, replacement, or regeneration of cells, tissues or organs” [50]. The advantages of iPSC are as follows: Autologous cells, which suppress the risks of rejection and infection, could be used; diseases caused by single gene defects could be addressed by made-to-order gene replacement in cells and allogenic cells from healthy people could be used [51].

iPSCs have been used in treating a number of injuries and degenerative diseases [2]. The various conditions that can be treated are Hematopoietic disorders, Musculoskeletal injury, Spinal cord injury, liver damage by the generation of specific cells with the help of iPSCs [31, 52-55].

In vitro Disease Modeling

Research into the pathophysiological mechanisms of human disease and the development of targeted therapies have been hindered by a lack of predictive disease models that can be experimentally manipulated *in vitro* [56].

Recent advances in stem cell research, especially the development of induced pluripotent stem cell (iPSC) technology [33, 57, 58], provide new opportunities which

may overcome many of the challenges and shortcomings associated with disease modeling and drug screening [58-62].

Tissue culture of human cells is today largely limited to tumor cell lines or transformed derivatives of native tissues. With the iPS cell technology it is possible now to derive permanent cell lines from patients with a variety of genetic diseases with either Mendelian or complex inheritance. Tissue-specific cells resembling those in diseased organs can be differentiated *in vitro* from iPS cells and used for studying the disease pathophysiology, development of new drugs and, eventually, autologous cell replacement therapies (**Figure 6**) [37]. In addition, iPS cell lines from patients with monogenic diseases could be used for repairing gene defect ex vivo prior to transplantation [63]. Many complex genetic diseases have familial and sporadic forms. iPS cells derived from patients with complex sporadic diseases would have the unique advantage of carrying the precise patient- specific constellation of genetic factors responsible for the disease in that person [26].

First, hiPSCs can be generated from patients with genetic diseases and, therefore, the derived target cells thus possess the same genetic background as the patient. This is important because an individual's genetic makeup can profoundly influence disease progression, its severity, as well as the elicited drug response [64].

To date, many patient-specific iPSC lines have been established and used for disease modeling, and are expected to facilitate studies on rare diseases [65].

Patient-specific iPSCs with described disease phenotypes are Mucopolysaccharidosis type IIIB [66], Parkinson's disease, familial [67-72], Polycythaemia vera [73], Pompe disease [74], Prader-Willi syndrome [75], Retinitis pigmentosa [76], Rett Syndrome [77-81], Schizophrenia [82, 83], Sickle cell disease [84], Spinal muscular atrophy [85, 86], Timothy syndrome [87, 88], Werner syndrome, atypical [89], Wilson's disease [90].

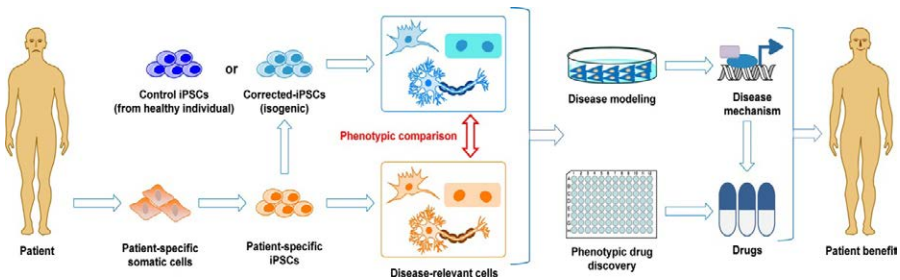

Figure 6: *In vitro* disease modeling, drug screening and toxicological analyses [91].

Drug Screening and Toxicological Analyses

A newly discovered drug or therapy must be tested on human cells or human test models itself. These reasons make it more important to be able to use the systems closer to humans. Moreover, these studies need to be done in a system where the results could be directly extrapolated to humans. These studies include steps such as prediction/identification of a potential drug molecule followed by its synthesis,

generation of iPSCs, their differentiation to specific somatic cells, and testing for toxic or non-toxic effects of the synthesized drug on the somatic cells. For toxicity studies, iPSCs from normal and diseased cells are used to generate neurons, hepatocytes, cardiomyocytes etc. Toxicity and potential side-effects are often most common cause to rule out most of the therapeutic molecules [31].

An ideal drug screening platform would provide reproducible and quantifiable disease-relevant phenotypes in a scalable cell population. iPSC-derived cells have advantages over primary cells and immortalized cell lines because they can provide inexhaustible, scalable, and genetically relevant sources for cell-based drug screening [58, 92, 93].

Currently, toxicological testing is based on the established immortal cancer cells lines containing chromosomal abnormalities, primary explanted somatic cells, and laboratory animals. Immortalized cell lines, showing several features reminiscent of cancer, mimic neither the normal physiological status nor the diseased state of the organism *in vivo*. The heterogeneity of primary explant cultures leads to inconsistent results and low reproducibility in toxicity testing. Using live animal models for toxicity testing may not mimic the human physiology, can raise ethical/animal welfare concerns, and is rather expensive. Research on ESCs and iPSCs promises to enhance drug discovery and development by providing simple, reproducible, and cost-effective tools for toxicity testing of drugs under development and, on the other hand, for studying the disease mechanisms and pathways [94-97]. Modeling human disease in standardized cell culture and the opportunity for high throughput drug screening are potential advantages of using iPSCs [94, 97]. Patient-specific iPSCs could improve the efficiency of drug discovery by helping the identification of drugs effective in specific patient populations [97].

Conclusion

iPS cells are similar to embryonal stem cells in the transcriptional and epigenetic level, this is also available as a functional similarity. iPS cells during differentiation constitute a very valuable resource so as to ascertain the epigenetic changes. Reprogramming deletes tissue-specific DNA methylation by epigenetic modification and returns the cell specification process by creating embryo-like methylome again. After reprogramming during the re-differentiation of the cell it has been demonstrated that normal cells are better differentiated to the adult issue on which they originate.

Although iPS cells have similar properties to embryonic stem cells, iPS cells are thought to be more useful than embryonic stem cells due to they have the same genome with the patients in cell and tissue transplantation. In the future iPS cells will be promising for patients awaiting organ because these cells will form form a rich source of organs factories.

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Chapter 3

Stem Cells in Regenerative Medicine

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Abstract

Although conventional therapy of disorders due to loss of organ and tissue is organ and tissue transplantation, currently the term of regenerative medicine has emerged. This is an interdisciplinary field of research and clinical applications focused on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function resulting from any cause, including congenital defects, disease, trauma and ageing. Embryonic and mesenchymal stem cells are promising source for this field.

Introduction

Organ transplantation remains a mainstay of treatment for patients with severely compromised organ function. Despite initiatives to increase the availability of transplant organs, however, the number of patients in need of treatment still far exceeds the organ supply, and this is expected to worsen as the global population ages. In the last two decades, as a response to the needs of these patients, scientists have attempted to grow native and stem cells, engineer tissues, and design treatment modalities using regenerative medicine techniques for virtually every tissue of the human body [1].

Regenerative medicine is an interdisciplinary field of research and clinical applications focused on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function resulting from any cause, including congenital defects, disease, trauma and aging [2].

Overall, regenerative medicine is a multidisciplinary field that requires expertise in a wide variety of scientific disciplines, including cell and molecular biology, physiology, pharmacology, chemical engineering, biomaterials, nanotechnology, and clinical sciences. Although modest clinical success has been achieved in specific areas, the field is still in its infancy. Long-term studies are still essential to assure safety and efficacy before these technologies can have widespread clinical application [3].

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There are three basic measures of stem potency: Totipotent, pluripotent and multipotent stem cells [4]. A fertilized egg is the ultimate stem cell as it contains the potency to differentiate into all cells of the three embryonic germ layers and extraembryonic cell types. Fertilized eggs are accordingly a totipotent cell. ES cells are pluripotent and can differentiate into all cell types of the three germ layers, but they have lost the ability to differentiate into cells of the extra-embryonic tissue. Most SSC cell types are multipotent and produce usually cells restricted to a related family of cells, e.g. hematopoietic stem cells which differentiate into cells of the different blood cell lineages. Unipotent stem cells are restricted to production of one differentiated cell type only [5] (**Figure 1**).

The characterization and isolation of various stem cell populations, from embryonic to tissue-derived stem cells and induced Pluripotent Stem Cells (iPSCs), have led to a rapid growth in the field of stem cell research and its potentially clinical application in the field of regenerative medicine and tissue repair [5, 6, 7].

Stem cell therapy has been accepted as an emerging technology that could change the present approach toward curing many chronic disorders and degenerative conditions. Stem cell therapy can be applied for regenerative medicine which is another promising area of medical therapy for the coming years [8].

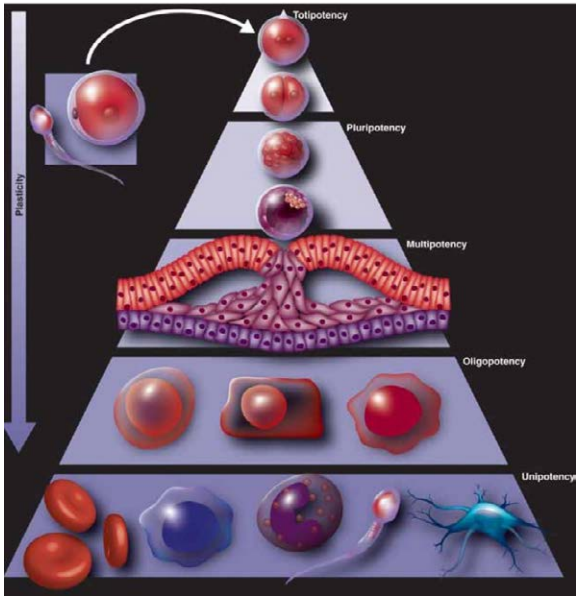

Figure 1: Totipotency, pluripotency, multipotency, oligopotency and unipotency [9].

Basic and clinical research accomplished during the last few years on embryonic, fetal, amniotic, umbilical cord blood, and adult stem cells has constituted a revolution in regenerative medicine and cancer therapies by providing the possibility of generating multiple therapeutically useful cell types. These new cells could be used for treating numerous genetic and degenerative disorders. Among them, age-related functional defects, hematopoietic and immune system disorders, heart

failures, chronic liver injuries, diabetes, Parkinson's and Alzheimer's diseases, arthritis, and muscular, skin, lung, eye, and digestive disorders as well as aggressive and recurrent cancers could be successfully treated by stem cell-based therapies [10, 11].

Embryonic Stem Cells

Embryonic stem cell characteristics include (i) derivation from the preimplantation or periimplantation embryo, (ii) prolonged undifferentiated proliferation, and (iii) stable developmental potential to form derivatives of all three embryonic germ layers even after prolonged culture [12, 13].

hESCs provide much promise in tissue engineering and regeneration since hESCs can act as an inexhaustible *in vitro* source of differentiated cell types. The potential use of hESCs in tissue engineering include, but are not limited to, organ substitutes, vascularization, and *ex vivo* cartilage/bone construction [14, 15].

Embryonic stem (ES) cells hold great promise for treating degenerative diseases, including diabetes, Parkinson's, Alzheimer's, neural degeneration, and cardiomyopathies. This research is controversial to some because producing ES cells requires destroying embryos, which generally means human embryos [16].

An important new source of embryonic stem cells is through use of a technique called somatic cell nuclear transfer, also referred to a therapeutic cloning [17, 18]. In this technique, nuclei from the cells of living patients with specific diseases are isolated and used for the generation of embryonic stem cells. This is achieved by placing one such nucleus into a donated unfertilized egg from which the genetic material has been removed and then stimulating the egg to divide to the stage when stem cells can be derived in culture. These cells can then be induced to develop in the laboratory into specialized cells such as nerve cells that are affected by the disease in question [18, 19].

SCNT has been proposed as an approach to generate patient-specific pluripotent stem cells for potential therapeutic applications. The embryonic stem cells derived from the SCNT embryo would be isogenic to the donor and thus free of immune rejection upon transplantation of the differentiated therapeutic cells back to the same donor. This concept is also known as 'therapeutic cloning' and has been demonstrated for proof of concept in mouse [20, 21].

The recent discovery that somatic mammalian cells can be epigenetically reprogrammed to a pluripotent state through the exogenous expression of the transcription factors OCT4, SOX2, KLF4, and c-MYC has yielded a new cell type for potential application in regenerative medicine, the induced Pluripotent Stem (iPS) Cell [22, 23].

Induced Pluripotent Stem (iPS) Cells share these salient characteristics of ES cells but are instead generated via reprogramming of somatic cells through the forced expression of key transcription factors [24,25]. The seminal achievement of induced pluripotency holds great promise for regenerative medicine. Patient-specific iPS cells could provide useful platforms for drug discovery and offer unprecedented insights into disease mechanisms and, in the long term, may be used for cell and tissue replacement therapies [25].

Mesenchymal Stem Cells and Their Clinical Significance

In vitro, MSC are characterized by plastic adherence, colony forming capacity and rapid proliferation. The immuno-phenotype of MSC, CD45−, CD34−, CD13+, CD44+, CD73+, CD90+, CD166+, CD80−, CD86−, HLA class Ilow, HLA class II−, distinguishes them from hematopoietic stem cells, which are CD34+, CD45+ and CD13−, and positions them close to fibroblasts. This phenotype also suggests MSC are low immunogenic [26].

At present no specific marker or combination of markers has been identified that specifically defines MSCs. Phenotypically, ex vivo expanded MSCs express a number of nonspecific markers, including CD105 (SH2 or endoglin), CD73 (SH3 or SH4), CD90, CD166, CD44, and CD29 [27, 28, 29]. MSCs are devoid of hematopoietic and endothelial markers, such as CD11b, CD14, CD31, and CD45 [27, 29].

One of the characteristic features of MSCs is their multi-differentiation potential under culture conditions, comprising lineage specific regulators. Although one is able to coax the differentiation of MSCs into a number of tissues *in vitro*, the resulting cell population/tissue often contains a mixture of cells and also does not mimic the targeted tissues entirely in their biochemical and biomechanical properties [30-33].

Via their immune-suppressive properties MSC may be able to prevent immune inflicted damage of tissues and organs and allow repair after injury. Immuno-suppression is, however, not the only aspect of the immunomodulatory capacity of MSC. Under immunological quiescent conditions, MSC promote T lymphocyte survival [34] and can stimulate the activation and proliferation of CD4+ T cells [35].

Preclinical models have demonstrated the ability of MSCs to regulate host immune-response and thus avoid recognition and subsequent rejection by the recipient [33, 36-38].

Besides BM, MSCs can also be isolated from adipose tissues [39, 40], fetal liver [41], cord blood and mobilized peripheral blood [42, 43], fetal lung [44], placenta [45], umbilical cord [46, 47], dental pulp [48], synovial membrane [49], periodontal ligament [50], endometrium [51], trabecular and compact bone [52, 53].

In contrast, the umbilical cord tissue or Wharton's jelly is an excellent source for isolating MSC [54-56]. The collection of Mesenchymal Stem Cells (MSCs) from UCB that is discarded at the time of birth is an easier, less expensive and non-invasive method than collecting MSCs from bone marrow aspirates [57, 58]. These MSCs attract special interest due to these specific advantages over embryonic and adult stem cell counterparts, since there are also no ethical issues associated with UCB. Another important characteristic of UCB-MSCs is that they are less immunogenic, and therefore do not elicit the proliferative response of allogeneic lymphocytes *in vitro* [58, 59]. UCB-MSCs expanded *in vitro* also retain low immunogenicity and an immunomodulatory effect from the UCB elicit a lower incidence of graft rejection and post-transplant infections compared with Moreover, cells derived other sources [58, 60].

HMSCs display a very high degree of plasticity and are found in virtually all organs,

however, the bone marrow contains the highest density [61, 62]. HMSCs serve as renewable source for mesenchymal [62, 63] and potentially epithelial cells and have pluripotent ability of differentiating into several cell lineages, including osteoblasts, chondrocytes, adipocytes, skeletal and cardiac myocytes, endothelial cells, and neurons *in vitro* upon appropriate stimulation, and *in vivo* after transplantation [62, 64].

Although the pathophysiologic functions of hMSCs are critically under investigation, the *in vitro* pluripotency of hMSC suggests a role in tissue regeneration, wound healing, or tissue repair after transplantation [62, 65]. These characteristics make hMSCs good vehicles for autologous transplantation with the genuine benefits for tissue regeneration or cell-based gene therapies [62, 66].

Mesenchymal stem cells are also multipotent cells which can support hematopoiesis, have immunomodulatory properties, may differentiate into osteocytes, chondrocytes and adipocytes, and specifically migrate to damage sites. The mesenchymal stem cell migration is mediated by growth factors, chemokines, adhesion molecules and toll-like receptors. Understanding the fundamental mechanisms underlying mesenchymal stem cell migration holds the promise of developing novel clinical strategies in regenerative medicine [67].

Because these cell populations can be readily isolated from patients for expansion and differentiation *in vitro* into at least three different lineages [27, 68, 69], MSCs are of great interest for clinical therapies. Indeed, protocols for injections of autologous MSCs are already in clinical trials not only for various musculoskeletal tissue replacement therapies including bone, cartilage, and intervertebral discs, but also to treat organ failure (cardiac, lung, liver, pancreas among others) and autoimmune diseases [69-72]. Moreover, MSCs are being developed as a critical cell source in tissue engineering, which involves the ex vivo creation of biological implants intended eventually to replace tissues or functional organs [69, 73].

Cells with properties of MSCs have also been isolated from tissues in several pathological conditions, sometimes with distinctive features. For instance, in the rheumatoid arthritic joint, MSC-like cells appear to express Bone Morphogenetic Protein (BMP) receptors [74, 75]. In the peripheral blood of acute burns patients, [75, 76], reported increase in circulating MSC-like cells compared with healthy donors, with greater numbers found among younger patients with more extensive burns. It is postulated that MSCs are mobilized into the bloodstream following acute burn signals which have not yet been elucidated. In other pathological conditions, such as obstructive apnoeas and bone sarcomas, studies provide evidence of possible mobilization of MSCs which increase in their circulating numbers compared to healthy individuals [75, 77, 78]; these reports are initial studies, often imprecise in the definition of MSC phenotype, and therefore they warrant further more accurate studies to understand the mechanisms underlying MSC mobilization *in vivo*, its biological significance and possible clinical impact in terms of recruitment to tissue and wound healing [75].

Briefly, MSCs can be used to support HSC engraftment, inhibit immune response after organ transplantation, reduce manifestations of graft versus host disease, treat various autoimmune conditions and cancer, and repair heart, liver, lung, kidney, and

CNS tissue [79-83]. They can be used as building blocks for artificially engineered tissues, including bone, cartilage, tendon, and muscle [84-88]. Furthermore, MSCs can be used as vehicles to deliver specific genes to target tissues, which represent one of the most promising therapeutic approaches using combined cell and gene therapy [89-92].

Tissue Engineering

Although the procedures for organ transplantation and reconstruction surgery improve the quality of life, and in some cases save life, there are problems associated with them. In most cases these procedures require either organ donation from a donor individual or tissue transplantation from a second surgical site in the individual being treated. The major problem with organ transplantation is that there exists a drastic shortage of donor organs. In 1996 alone, only 20,000 donor organs were available for 50,000 patients in need. In fact, patients are more likely to die while waiting for a human donor heart than in the first two years after transplantation [93, 94]. The problem with second site surgeries is that these procedures are associated with pain and morbidity. As a result of these problems, the science of tissue engineering has emerged with the goal of developing organs, tissues, and synthetic materials outside of the body ready for future transplant use [93-100].

Tissue engineering is a newly emerging field that combines the use of cells, scaffolds and biological factors for the purpose of tissue/organ repair/regeneration [101, 102]. The goal of tissue engineering is to surpass the limitations of conventional treatments based on organ transplantation and biomaterial implantation [96]. It has the potential to produce a supply of immunologically tolerant 'artificial' organ and tissue substitutes that can grow with the patient. This should lead to a permanent solution to the damaged organ or tissue without the need for supplementary therapies, thus making it a cost-effective treatment in the long term [103].

In basis, tissue engineering attempts to mimic the function of natural tissue. Therefore, to optimize the development of functional biological substitutes, the natural circumstances of the specific tissue have to be fundamentally understood. Biological tissues basically consist of cells, signaling systems and ExtraCellular Matrix (ECM) [96]. The cells are the core of the tissue, however, can not function in the absence of signaling systems and/or of the ECM. The signaling system consists of genes that secrete transcriptional products when differentially activated, and urges cues for tissue formation and differentiation [96]. The ECM is a meshwork like substance within the extracellular space and supports cell attachment and promotes cell proliferation [104, 105].

The concept of tissue engineering embodies the creation of a scaffold structure that has the appropriate physical, chemical, and mechanical properties to enable cell penetration and tissue formation in three dimensions [106, 107].

Ideally, scaffolds for tissue engineering should meet several design criteria: (1) the surface should permit cell adhesion, promote cell growth, and allow the retention of differentiated cell functions; (2) the scaffolds should be biocompatible, neither the polymer nor its degradation by-products should provoke inflammation or toxicity *in vivo*; (3) the scaffold should be biodegradable and eventually eliminated; (4) the porosity should be high enough to provide sufficient space for cell adhesion,

extracellular matrix regeneration, and minimal diffusional constraints during culture, and the pore structure should allow even spatial cell distribution throughout the scaffold to facilitate homogeneous tissue formation; (5) the material should be reproducibly processable into three-dimensional structure, and mechanically strong [108].

Stem Cell Therapy in Neurodegenerative Diseases

Neurodegenerative diseases, such as Parkinson's disease (PD), stroke, Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS), are characterized by neurodegenerative changes or apoptosis of neurons involved in networks, leading to permanent paralysis and loss of sensation below the site of the injury [109].

Stem cells from a variety of sources have shown effectiveness in improving motor function after neurodegenerative diseases in animal experiments and clinical trials. Cell therapies in neurodegenerative disease are intended to protect neuronal populations susceptible to disease and replace dysfunctional or dying neurons [110].

Neurons and glial cells have successfully been generated from stem cells such as Embryonic Stem Cells (ESCs), Mesenchymal Stem Cells (MSCs) and Neural Stem Cells (NSCs), and stem cell-based cell therapies for neurodegenerative diseases have been developed [111].

NSCs used for clinical applications should be safe, effective and accessible in large amount in GMP conditions. A variety of different sources for NSCs have been tested, including fetal and adult CNS-derived NSCs, neural progenitors derived from pluripotent cells, and a range of non-neural stem cells, such as Mesenchymal (MSCs) and Bone Marrow-Derived (BMDSCs) Stem Cells [112].

Stem Cell Therapy in Cardiac Diseases

The majority of cardiovascular disease is composed of cardiac diseases which can be broadly divided into either ischemic including coronary artery disease and myocardial infarction or non- ischemic heart disease including vascular heart disease and hereditary cardiomyopathy [113, 114].

Pluripotent stem cells provide an opportunity to generate patient-specific cardiac cells, but tumorgenicity and poor engraftment after transplantation currently limit their use for regenerative cell therapy and tissue engineering [115, 116].

Clinical trials show that bone marrow cell therapy improves myocardial perfusion and contractile performance in patients with acute myocardial infarction, heart failure, and chronic myocardial ischemia. Bone marrow cells are thought to have paracrine effects on neovascularization, inflammation, wound healing and possibly resident stem and progenitor cells [115].

Among the cell types under investigation, adult mesenchymal stem cells are widely studied, and in early stage, clinical studies show promise for repair and regeneration of cardiac tissues. The ability of mesenchymal stem cells to differentiate into mesoderm- and non-mesoderm-derived tissues, their immunomodulatory effects, their availability, and their key role in maintaining and replenishing endogenous stem cell niches have rendered them one of the most heavily investigated and

clinically tested type of stem cell [117].

With increasing evidence, endogenous Cardiac Stem Cells (CSCs) represent an attractive and promising cell candidate for cardiac repair and regeneration due to their autologous origin, cardiac-committed fate, and ability to develop into three major myocardial lineages [114, 118].

Stem Cell Therapy in Diabetes Mellitus

Both type 1 and type 2 diabetes are characterized by a marked deficit in beta-cell mass causing insufficient insulin secretion [119, 120]. Curative therapy for diabetes mellitus mainly implies replacement of functional insulin-producing pancreatic β cells, with pancreas or islet-cell transplants. However, shortage of donor organs spurs research into alternative means of generating β cells from islet expansion, encapsulated islet xenografts, human islet cell-lines, and stem cells. Stem-cell therapy here implies the replacement of diseased or lost cells from progeny of pluripotent or multipotent cells [43, 121].

Bone-marrow cells can differentiate *in vitro* under controlled conditions into insulin-expressing cells [122, 123]. Such cells, transplanted under the kidney capsule of diabetic rodents, correct glucose. Removal of the grafted kidney returned the animals to a diabetic state [124].

When MSCs are systemically administered they can selectively migrate and engraft in damaged tissue [125, 126] and differentiate into insulin-producing cells [126-128]. As immunomodulatory cells, MSCs can limit inflammation in damaged tissue [126, 129], produce a broad range of trophic factors protecting parenchymal cells from dying by apoptosis and promote the proliferation and differentiation of endogenous precursors [126, 130].

One approach to produce more β-cells has involved differentiating MSCs into functional β-cells. Hisanaga et al., have shown that a simple protocol can induce murine bone marrow-derived MSC differentiation into insulin-secreting cells; the differentiated cells contained insulin-secretory granules and secreted mature insulin after glucose stimulation. Such cells reduced blood glucose levels when they were transplanted into diabetic mice [126, 131].

Conclusion

Diseases such as neurodegenerative, cardiac disorders and diabetes mellitus can not be cured completely and are important problem in medicine. Currently effective treatment of these diseases can be provided with the innovative approach called regenerative medicine. Embryonic and mesenchymal stem cells provide a significant potential utility.

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Chapter 4

Tissue Engineering

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Abstract

In the world, thousands of people lose their lives due to organ failure depending upon various reasons. Because finding a new organ is a very difficult process. However, in the presence of the organ, the drugs used after transplantation can cause significant side effects for the patient. Tissue engineering has become an important research area to overcome these problems. In this chapter components of tissue engineering were discussed.

Introduction

All over the world there are many patients who are treated in hospitals due to organ failure and waiting for organ donation. It is a very long and painful process to find suitable donors for organ transplantation. A variety of medications are used to prevent rejection of the transplanted organ by the body. However, side effects of these drugs are inevitable and weaken the immune system. In recent years, some studies in the field of tissue engineering have given hope to overcome the difficulties of organ transplantation [1]. As a result of these studies, it may be possible to produce artificial organs soon by tissue engineering studies.

The goal of tissue engineering is to surpass the limitations of conventional treatments based on organ transplantation and biomaterial implantation [2] and mainly consists of activating regenerative abilities of the body that have come to a standstill and, if necessary, replacing damaged tissues with tissue implants [3].

Tissue Engineering is an interdisciplinary discipline addressed to create functional three-dimensional (3D) tissues combining scaffolds, cells and/or bioactive molecules [4, 5]. This field involves scientific areas such as cell biology, material science, chemistry, molecular biology, engineering and medicine [2, 5]. Thus, tissue engineering may provide therapeutic alternatives for organ or tissue defects that are acquired congenitally or produced by cancer, trauma, infection, or inflammation. Tissue-engineered products would provide a life-long therapy and may greatly reduce the hospitalization and health care costs associated with drug therapy, while simultaneously enhancing the patients' quality of life [6].

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The concept of tissue engineering has been applied clinically for a variety of disorders, for example artificial skin for burn patients [7, 8], tissue engineered trachea [9], cartilage for knee-replacement procedures [8, 10], injectable chondrocytes for the treatment of vesico-ureteric reflux [8, 11] and urinary incontinence [12, 13].

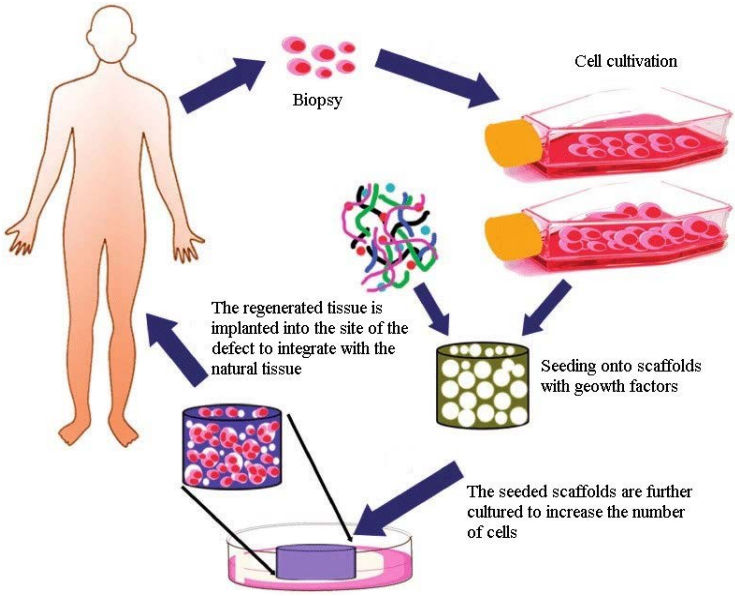

Figure 1: A typical tissue engineering cycle [14].

Components of Tissue Engineering

Cell Sources

Some of the most promising and frequent research in the field of regenerative medicine has focused on the use of stem cells. These cells, by definition, are undifferentiated cells with significant self renewal capabilities. Additionally, stem cells are able to proliferate and establish daughter cell lines for tissue generation [15, 16].

Stem cells are unspecialized cells that develop into the specialized cells that make up the different types of tissue in the human body [17]. Stem cells are divided into three groups according to their developmental potential: Totipotent, pluripotent and multipotent.

Totipotent stem cells have the ability of dividing and forming various differentiated cells including extra-embryonic tissues. In other words, these types of stem cells can create a whole human being. Pluripotent stem cells are derived from embryonic stem cells from the inner cell mass of the blastocyst. This type of stem cells can turn into three germ layers (ectoderm, endoderm and mesoderm) but not reveal the entire human being. Because they can't differentiate into extra-embryonic tissues. Multipotent stem cells are adult stem cells and they can differentiate into more than one cell type [18]. Hematopoietic and mesenchymal stem cells are multipotent stem cells [19].

Use of ESCs has been limited for tissue engineering because of the legal and ethical concerns regarding use of human ESCs [20]. These issues are less prevalent for adult stem cells, and as a result, adult stem cells from human sources in tissue engineering have been widely investigated [21, YOK]. For example, MSCs are used in tissue engineering because of their availability in various sources such as bone marrow [22], muscle [23], trabecular bone [24], dermis [25], adipose tissue [26], periosteum [27], blood [28], and synovial membrane [29]; and their ability to differentiate to multiple connective tissue cell types such as osteocytes [30], chondrocytes [31], adipocytes [32], and myocytes [33] and other cell types like hepatocytes [34] and neuron [35] in response to extracellular stimuli, including differentiation-inducing factors from protein and chemical origins.

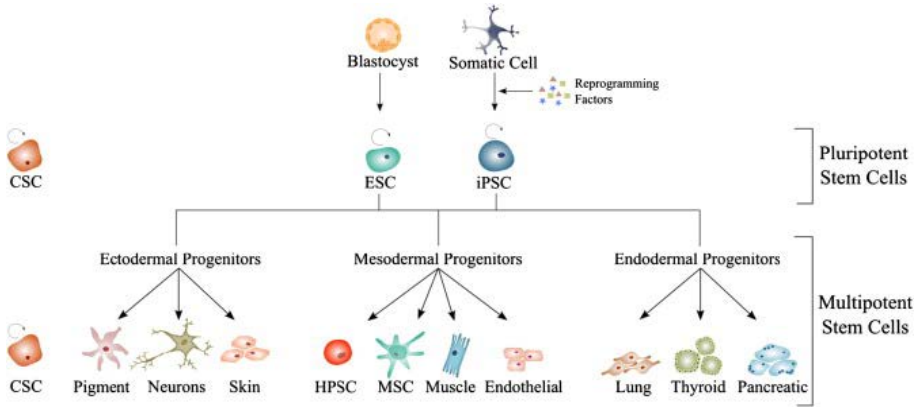

Figure 2: Differentiation potential of pluripotent stem cells [36].

Scaffolds

Cellular behavior is strongly influenced by biological and biochemical signals from the extracellular matrix. Therefore, the use of scaffolds as delivery systems for growth factors, adhesion peptides and cytokines is receiving considerable attention in the field [37-40].

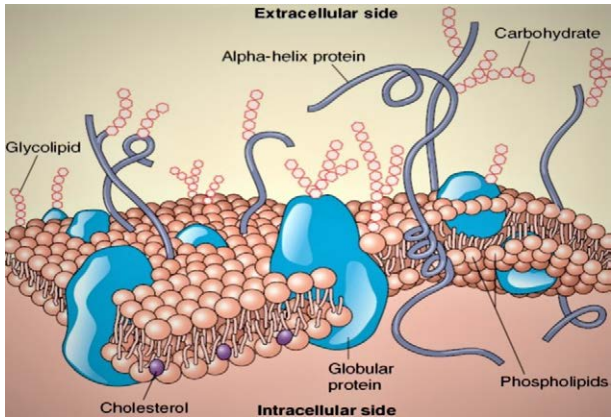

Figure 3: Extracellular matrix [41].

The appropriate scaffold for tissue engineering will be one that is created with biology in mind. The goal is for the new tissue grown in the scaffold to integrate with the host tissue. Ideally, the scaffold provides a temporary pathway for regeneration and will degrade either during or after healing, thereby obviating the need to remove the material later and eliminating possible side effects associated with leaving materials in the body. Of course, attention must be paid to ensure that degradation products are non-cytotoxic [42].

The scaffold should be i) biocompatible, meaning that it should not provoke any rejection, inflammation, immune responses or foreign body reactions, ii) provide a 3D template for the cells to attach and to guide their growth, iii) have a porous architecture with a high surface area for the maximum loading of cells, cell-surface interaction, tissue in growth, and transportation of nutrients and oxygen, iv) be degradable under physiological conditions and the degradation rate should match the rate of tissue regeneration to sustain tissue functionality, v) be mechanically strong to withstand *in vivo* biological forces, vi) support the cells in synthesizing tissue specific extracellular matrix components and growth factors required for healthy tissue growth and be sterilizable to avoid toxic contaminations without compromising any structural and mechanical properties [8].

Growth Factors

Tissue repair and regeneration is an important aspect of the process of wound healing, and is therefore key to the normal maintenance and survival of all organisms. Tissue remodeling spans the entire period, starting from injury and ending with repair [43]. The ability of damaged tissues to regenerate, and the extent of regeneration possible, determines the need for tissue engineering approaches, and hence the need for growth factors [44].

Growth factors are proteins and a subgroup of the polypeptides that are involved in transmitting signals which affect cellular activities Cell activities such as migration, proliferation, differentiation and protein expression are controlled by growth factors [44]. Growth factors synthesized by different cells act in different ways. They act on the same cell that produced it as autocrine stimulation, act on cells that are adjacent to the producer cell as paracrine stimulation and also enter the circulation to be transported to cells that are distant from the producer cell as endocrine stimulation [45, 46].

Growth factors play a central role in tissue engineering approaches by providing the right signals to cells, and thereby leading to accelerated tissue growth/ regeneration. However, growth factors that are provided exogenously tend to rapidly diffuse away from the site of tissue regeneration [48]. Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF), Insulin-like Growth Factor (IGF), Transforming Growth Factor (TGF), Nerve Growth Factor (NGF) and Fibroblast Growth Factor (FGF) are the examples of growth factors [49].

Signaling molecules used in tissue engineering can be added to the culture media as soluble factors or attached to the scaffold by covalent and non-covalent interactions. First of all, the direct delivery of these molecules in the media is frequently used to *in vitro* evaluate the effect of these cues. However, these biomolecules are rapidly degraded and deactivated by some cell-secreted enzymes, responsible for their short

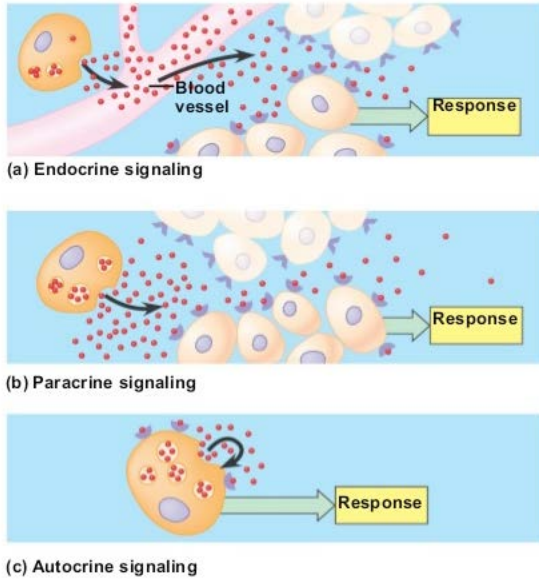

Figure 4: Signaling classification of growth factors [47].

biological half-live. For this reason, for clinical applications, bounding factors to the matrix helps to protect them from degradation [5, 39]. Consequently, the controlled release of different factors from scaffolds allows their constant renewal, having a great potential to direct tissue regeneration and formation. Several matrix systems, micro particles and encapsulated cells have been reported to locally deliver bioactive factors and to maintain effective concentrations for their use in the application areas, such as musculoskeletal, neural and hepatic tissue [5, 50-52].

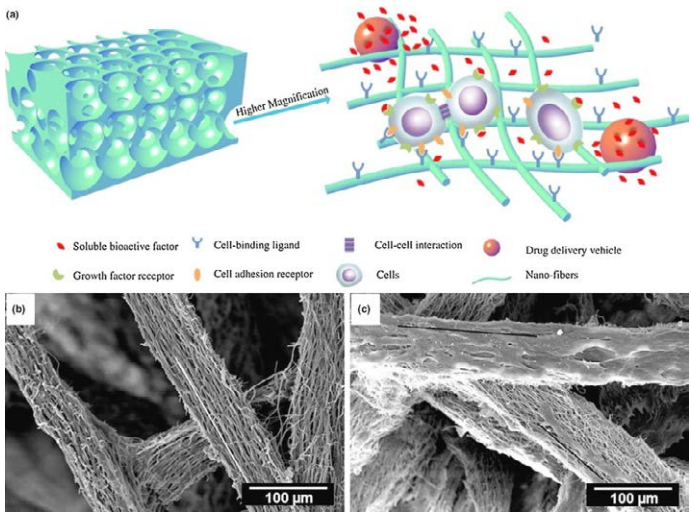

Figure 5: Growth factors and scaffold interactions [53, 54].

Importance of Mesenchymal Stem Cells in Tissue Engineering

Autologous tissue grafts often represent the current clinical "gold standard" for the reconstruction of defects resulting from trauma, chronic diseases, congenital anomalies, and tumor resection. However, autologous tissue grafting is based on the concept that a diseased or damaged tissue must be replaced by like tissue that is healthy. Thus, the key drawback of autologous tissue grafting is donor site trauma and morbidity [55].

MSC-based therapies can be autologous (from self) and thus eliminate the issues of immune-rejection and pathogen transmission or allogenic for potentially off-the-shelf availability. Autologous MSC-based therapies are also expected to be superior to other surgical approaches such as allogenic grafts, xenogenic grafts, or synthetic materials such as total joint replacement prosthesis [55].

A specific subtype of multipotent stem cells, mesenchymal stem cells (MSCs), are highly sought after in research due to their ease of isolation. The diverse *in vivo* distribution of MSCs includes bonemarrow, adipose, periosteum, synovial membrane, skeletal muscle, dermis, pericytes, blood, trabecular bone, human umbilical cord, lung, dental pulp and periodontal ligament [16, 56].

Although MSCs from different tissues show similar phenotypic characteristics, it is not clear if these are the same MSCs, and they clearly show different propensities in proliferation and differentiation [57]. In addition to tissue source, donor age and disease stage may directly affect MSC yield, rate of proliferation, and multipotency. There seems to be decreasing MSC number and proliferation rate as well as differentiation potentials with increasing age [58].

Because mesenchymal stem cells are culture-dish adherent, they can be expanded in culture while maintaining their multipotency [59]. The MSCs have been used in preclinical models for tissue engineering of bone, cartilage, muscle, marrow stroma, tendon, fat, and other connective tissues. These tissue-engineered materials show considerable promise for use in rebuilding damaged or diseased mesenchymal tissues [60].

Mesenchymal Stromal or Stem Cells (MSCs) are fibroblast-like shaped cells and appear ubiquitously in the human organism. They have the potential of multi-lineage differentiation and seem to exert numerous paracrine effects: by secreting different growth and signaling factors they seem to be able to modulate angiogenesis, (anti) inflammation or apoptosis [61].

MSCs are usually grown as a monolayer culture in medium typically containing 10% fetal calf serum at 37°C in a humid environment containing 5% CO_2. As for many other adult stem cells, MSCs are traditionally considered to only be capable of differentiating into cell types of their own original lineage, i.e., mesenchymal derivatives. We and many other groups have shown MSCs to be capable of forming osteoblasts, chondrocytes and adipocytes both *in vitro* [62, 63] and *in vivo* [64]. The ability of clonally expanded cells to form these three distinct cell types remains the only reliable functional criterion available to identify the genuine MSC and

distinguish it from preosteoblast, preadipocyte or prechondrocytic cells which each only give rise to one cell type [65].

MSC stem cells can be grown for many generations in the laboratory and still retain a stable morphology and normal chromosome complement. MSC, are contact inhibited, can be grown in culture for about 20 to 25 passages [66].

Clinical studies show high capacity of MSCs in improvement of allogeneic stem cell transplantation and in reducing chronic GVHD complications. In fact, these cells exert their anti- inflammatory and immunomodulatory effects through activation of T suppressor lymphocytes and secretion of a number of immunomodulatory agents. On the other hand, these cells identify the damaged area by their paracrine effects and implant there to accelerate the repair process of the damaged area by secreting a number of factors [67-69].

The differentiation tendencies of stem cells are closely linked to several factors including adhesive contexts, mechanical signals, and the physical responses of the cells [70, 71]. Recently, the mechanical properties of the ExtraCellular Matrix (ECM) of MSCs have become an area of interest because the elastic properties of the ECM significantly affect differentiation. Stem cells on a soft ECM or matrix are more likely to differentiate into neurons, whereas cells on a hard matrix are more likely to differentiate into osteoblasts [71, 72]. Differentiation is also closely linked to the intrinsic mechanical properties, including the elasticity and viscosity of individual MSCs. González-Cruz et al. [71, 73] reported that, among ADSCs from the same source that were treated under the same conditions, the stiffest cells tended to differentiate into osteoblasts while the “softest” cells tended to differentiate into adipocytes [71].

Conclusion

Currently organ failure is one of the biggest problem. For many patients, the only treatment is organ transplantation and patients are wait for the appropriate tissue or organ. In our rapidly changing and aging world, new and different organ-tissue regeneration technologies need to be developed to meet the increasing need for organ transplantation. Using regeneration mechanisms to produce the tissue and organ we need, or to warn damaged tissues for repair, is the main goal of regenerative medicine. For this purpose, new technologies are being developed for organ construction.

The organs cloned from human tissues will stop waiting for organ transplantation in liver and heart disorders in the future. Organs enlarged in the laboratory using the patient’s own stem cell will prevent the complications due to organ rejection. Evolving tissue engineering procedures and artificial organ technologies will revolutionize the delivery of tissue rejection and similar problems in classical organ transplantation.

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Chapter 5

Cancer Stem Cells

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Abstract

Cancer Stem Cells (CSCs), which are highly tumorigenic and low differentiated, are a very small part of the heterogeneous cell population consisting of cancer cell mass. Cancer stem cells are thought to play an important role in failure of the treatment. Cancer Stem Cells (CSCs) play a major role in the development of drug resistance and renewal of the disease. Therefore, treatments targeting cancer stem cells come forward. In this chapter we discuss the features of cancer stem cells and the importance of eradication of them.

Introduction

Malignant tumors are comprised of morphologically diverse cells and phenotypically heterogenous populations that possess high clonogenic and tumorigenic activity, and a varying degree of ability for self-renewal and differentiation into multiple cell types [1-5]. Cancer Stem Cells (CSCs) possess several characteristics including self-renewal, pluripotency and tumorigenicity and constitute a rare population in a tumor mass. Because conventional cancer therapies can not kill CSCs, these cells are responsible for tumor relapse and metastasis. Currently, with advances in the identification of CSCs, the importance of these cells is increasing in the field of cancer diagnosis and prognosis. In addition, clarifying the mechanisms responsible for the maintenance of CSCs properties led to the development of CSC-targeted therapies [6] (**Figure 1**).

While cancer stem cells are often phenotypically and functionally similar to normal stem cells from the same tissue, the cancer stem cell model does not imply that cancer stem cells must arise from nor- mal stem cells [7-9]. The cancer stem cell model describes the observation that cancer cells are heterogeneous and exist within a hierarchy of proliferative potentials, regardless of whether the cancer stem cells arise from the transformation of normal stem cells, downstream-restricted progenitors, or differentiated cells [10, 11]. In reality, many cancer stem cells are likely to arise from the transformation of normal stem cells as normal stem cells are

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the only mitotic cells that persist long enough in many tissues to accumulate the mutations necessary for transformation [10].

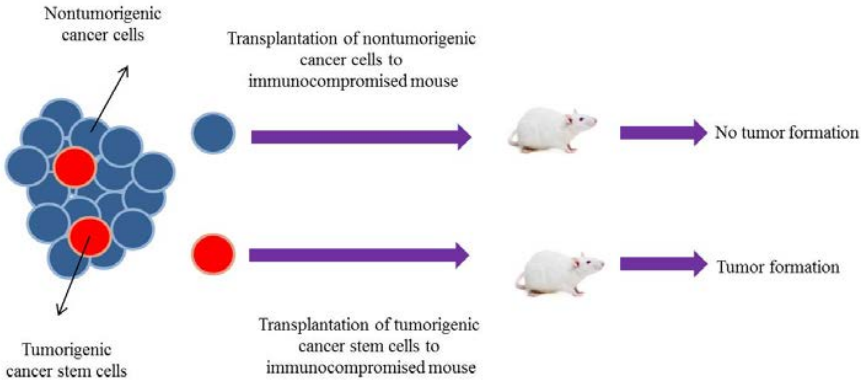

Figure 1: Heterogenity of tumor mass

The cancer stem-cell model provides one explanation for the phenotypic and functional heterogeneity among cancer cells in some tumor [10, 12-15]. The model posits that some cancers are organized into a hierarchy of subpopulations of tumorigenic cancer stem cells and their non-tumorigenic progeny. In these cases, cancer stem cells are thought to drive tumor growth and disease progression, perhaps through therapy resistance [16-18] and metastasis [19, 20].

Tumor heterogeneity indicates important implications for successful cancer therapies. Currently there are two models describing the heterogeneity in tumor, the stochastic and CSC models. The essential difference between them is that every cell or just a distinct subset tumor cells have the potential to behave like a CSC [12].

A CSC population was first identified in Acute Myeloid Leukemia (AML) where a subset of cancer cells showed serial transplantation ability [21]. CSCs from solid tumors were more recently identified first from breast cancers [22] and then from several others including the brain [23], colon [24-26], head and neck [27], pancreatic [28, 29], melanoma [30], mesenchymal [31], hepatic [32], lung [33], prostate [34] and ovarian [35] cancers.

Cancer Stem Cells in Solid Tumors

These cancer stem cells represent only 1% of the tumor and were the only cells in the tumor capable of transplanting the tumor into nude mice [36].

CD44 has a role in facilitation of cell to cell and cell-matrix interactions through its affinity for hyaluronic acid and is involved in cell-adhesion and the assembly of growth factors on the cell surface [37]. The lymphocyte homing receptor CD44 attracted considerable interest when it was described that CD44 splice variants (CD44v) suffice to confer the metastatic phenotype to locally growing tumor cells [38]. Meanwhile the importance of CD44v in tumor progression has been amply demonstrated in many types of cancer [39, 40]. More recently, CD44 has been described as a CSC marker not only for leukemia but also for colorectal, breast, prostate and pancreatic carcinoma [21, 28, 34, 41-43].

One of the most studied tumor stem cell-markers is cluster of differentiation 271 (CD271). CD271 (known as also nerve growth factor receptor, NGFR or p75NTR) is a neurotrophin receptor, which can bind all of the neurotrophins by similar affinity [44, 45]. It has contradictory actions; it functions to promote cell survival or induce cell death [45, 46]. Expression of CD271 has been found in several human neural crest-derived tissues and in some human cancers, including melanomas [45, 47]. Recently, CD271 has been used as an important cancer stem cell marker in melanoma [45, 48-50].

Aldehyde Dehydrogenase (ALDH) enzymes play a critical role in the metabolism of many molecules, and in the detoxification of external and internal substances, such as alcohol and toxins [51]. Different ALDH family members play diverse roles in detoxification pathways and retinoic acid bio-synthesis, as well as folate, amino acid, ethanol, and cyclophosphamide metabolism [52]. Stem cells from a variety of tissues express high levels of ALDH activity, which may be a characteristic of "stemness" [53, 54]. ALDH is found in every subcellular region such as cytosol, endoplasmic reticulum, mitochondria, and the nucleus, with some even found in more than one location [55]. Recent evidence suggests that enhanced Aldehyde Dehydrogenase (ALDH) activity is a hallmark of Cancer Stem Cells (CSC) measurable by the aldefluor assay [56].

The Epithelial Cell Adhesion Molecule (EpCAM) was considered a mere cell adhesion molecule and reliable surface-binding site for therapeutic antibodies. Recent findings can better explain the relevance of EpCAM's high-level expression on human cancers and cancer propagating cells, and its negative prognostic potential for survival of patients with certain cancers [57]. It has been reported that EpCAM is involved in the abrogation of E-cadherin-mediated cell-cell adhesion by disrupting the link between alpha-catenin and F-actin [58, 59]. In fact, modulation of cadherin-mediated cell-cell interactions by EpCAM points to a possible functional involvement of the molecule in tumor progression [60]. Besides, EpCAM has been shown to be involved in signal transduction and to support cell motility [61, 62]. Overexpression of EpCAM can also induce upregulation of the proto-oncogene c-myc and support cell proliferation via upregulated synthesis of cyclin A and E [63, 64] and regulates E-FABP (Epidermal Fatty Acid Binding Protein) expression [65].

CD133 is largely used as CSC marker in several tumors, including breast [66, 67], brain [23, 68], prostate [34], colon [24, 25, 69], liver [70, 71], ovarian [72, 73]. It was originally identified as a surface antigen expressed on hematopoietic stem cells [74]. CD133 was then used in the isolation of neural stem cells from human fetal brain. Despite the demonstration that cell sorting for CD133 expression can enrich for cells with tumorigenic potential in brain tumors [23, 75], the utility of CD133 in the isolation of brain tumor stem cells has been questioned in several studies [76-82].

Signaling Pathways

CSCs display many features of embryonic or tissue stem cells, and typically demonstrate persistent activation of one or more highly conserved signal transduction pathways involved in development and tissue homeostasis, including the Notch, Hedgehog (HH), and Wnt pathways [83]. The deregulation of these pathways,

resulting in stem cell expansion, may be a key event originating CSCs and, thereby, initiating carcinogenesis [84].

Notch signaling is an evolutionarily conserved pathway involved in cell fate control during development, stem cell self-renewal, and postnatal tissue differentiation. Roles for Notch in carcinogenesis, the biology of cancer stem cells, tumor angiogenesis, and Epithelial-to-Mesenchymal Transition (EMT) have been reported [85]. Notch pathways play a critical role in breast CSCs and, thus, may represent novel therapeutic targets to prevent recurrence of pre-invasive and invasive breast cancer [84].

Hh signaling contributes to tumor aggressiveness, affecting key tumorigenic processes such as proliferation, invasion and progression of cancer cells [86]. Hh signaling induces EMT and metastasis formation. Cells undergoing EMT under the influence of Hh signaling and become more motile and invasive as they acquire mesenchymal cell properties. This allows cells to escape from the primary tumor and circulate to distant sites. Once established at a distant site, Hh may be required for the clonogenic growth and self-renewal [87]. Emerging data from many human tumors including glioblastoma, breast cancer, pancreatic adenocarcinoma, multiple myeloma, and Chronic Myeloid Leukemia (CML) have suggested that Hh signaling regulates cancer stem cells [87-94, aynı]. Therefore, inhibitors targeting Hh signaling have drawn significant attention as novel, molecularly targeted drugs [86].

The Wnt signaling pathway is a key developmental pathway involved in a variety of biological processes including cell proliferation, survival and differentiation [95]. The Wnt/β-catenin signaling pathway is often aberrantly activated in CSCs, which are responsible for generation of metastasis and decreased survival of patients [96]. Therefore, targeting the Wnt/ β-catenin signaling pathway may potentially reduce the number of, or even eradicate, CSCs. To this end, a number of small-molecule inhibitors of Wnt signaling are being studied including existing drugs such as NonSteroidal Anti- Inflammatory Drugs (NSAID), new molecular-targeted agents, including many that are currently in the discovery, preclinical, or clinical testing stages [97].

CSC, EMT and Metastasis

The acquisition of a motile behavior early in metastasis depends on the Epithelial-Mesenchymal Transition (EMT), a process especially known in embryonic development, whereby epithelial cells switch to a mesenchymal progenitor-cell phenotype, facilitate detachment and reorganize the epithelial cell sheets during tumor invasion and metastasis [98]. The process of EMT involves a disassembly of cell-cell junctions [99], actin cytoskeleton reorganization [100] and increased cell motility [101, 102] and invasion [103], as characterized by down-regulation and relocation of E-cadherin and zonula occludens-1 (ZO-1) [104, 105, 106]as well as down-regulation and translocation of β-catenin from the cell membrane to nucleus, and up-regulation of mesenchymal molecular markers such as vimentin [100, 106, 107], fibronectin and N-cadherin [101, 108-110] (**Figure 2**).

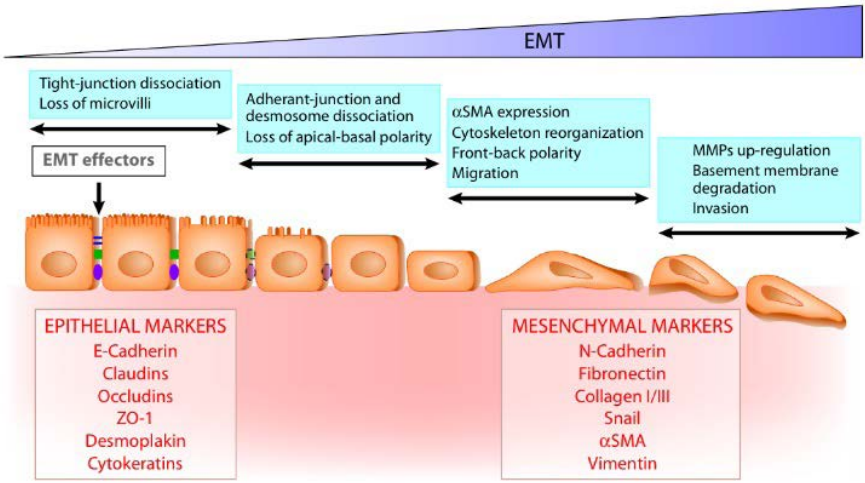

Figure 2: Key events during EMT. The diagram shows four key steps that are essential for the completion of the entire EMT course and the most commonly used epithelial and mesenchymal markers [111].

Recently, Cancer Stem Cells (CSCs) and Epithelial-Mesenchymal Transition (EMT)-type cells, which shares molecular characteristics with CSCs, have been believed to play critical roles in drug resistance and cancer metastasis as demonstrated in several human malignancies [106].

E-cadherin promoter is repressed directly or indirectly by specific developmental transcription factors such as Twist1, Snai1, Slug, ZEB1, ZEB2, FOXC2, KLF8 and E47, which disrupts the polarity of epithelial cells and maintains a mesenchymal phenotype [112-114].

Knockdown of E-cadherin by shRNA triggered EMT and resulted in acquisition of a mesenchymal phenotype and increased CSC activity in HMLER breast cancer cells [115].

Interestingly, the pro- metastatic CD26+ subpopulation within colon CSCs (see above) display reduced E-cadherin expression and express EMT markers such as N-cadherin [116].

Recent work has begun to address the importance of the tumor microenvironment in regulating the EMT during tumorigenesis and also found that the emergence of CSCs occurs in part as a result of EMT, for example, through cues from tumor microenvironment components. Both the EMT and CSCs play a critical role in tumor metastasis, therapeutic resistance and recurrence; however, each alone can not explain the sum of the cellular events in tumor progression and the significance of EMT in regulating the stemness of CSCs remains unknown until very recently [117].

Mechanisms of Therapeutic Resistance of Cancer Stem Cells

The most commonly used treatments strategies in clinically diagnosed different

types of cancers including leukemia and solid tumors such as skin, head and neck, brain, lung, kidney, bladder, prostate, breast, ovary, spleen, and the gastrointestinal system are surgical removal of the tumor tissue, hormonal therapy, radiation therapy and combination therapies. The classical treatment is effective in the initial stages of treatment, but in case of invasive or metastatic cancer is often resistant to treatment and progression of the fatal relapse is observed [118].

Conventional chemotherapy eradicates cycling differentiated cancer cells but is ineffective against quiescent CSCs. Under the influence of the stem cell niche, these cells not only thrive but also escape cell cycle and proliferation checkpoints by conventional therapies, leading to tumor recurrence and metastases [119]. There are several molecular mechanisms that may account for CSC therapy resistance. Many CSC are noncycling G0 cells, and would not be susceptible to cell cycle specific chemotherapy agents. ATP-binding cassette proteins (ABC transporter), known to efflux chemotherapy drugs, are often overexpressed in CSC [120].

Other mechanisms of CSC resistance to chemotherapy include quiescence, increased expression of antiapoptotic proteins, and multidrug resistance molecules. Several mechanisms appear to be involved in radiotherapy resistance including higher DNA repair capacity, lower reactive oxygen species (ROS) levels, activation of Wnt and Notch signaling pathways, induced autophagy, and the possible existence of a hypoxic CSC niche [121].

Targeting Cancer Stem Cells with Therapy

Tumor recurrence following treatment remains a major clinical challenge. Evidence from xenograft models and human trials indicates selective enrichment of Cancer-Initiating Cells (CICs) in tumors that survive therapy. Together with recent reports showing that CIC gene signatures influence patient survival, these studies predict that targeting self-renewal, the key 'stemness' property unique to CICs, may represent a new paradigm in cancer therapy [122]. Targeting the molecular signals that control self-renewal, survival, and proliferation of cancer stem cells is therefore considered a highly promising approach to tackle cancer at its very roots [9, 123, 124].

Tumor stem cells often exhibit the self-renewal capacity and asymmetric cell division characteristic of normal tissue stem cells. Often, in tumor stem cells these self-renewal pathways are dysregulated. However, it is important to note that such self-renewal pathways are not active in the bulk of more differentiated cells in the tumor. Thus, targeting these dysregulated pathways should allow opportunities to develop small-molecule therapeutics specific for tumor stem cells [106, 125].

In a new study in this issue of Nature Medicine, Kreso et al. [122] demonstrate that by targeting BMI-1, a gene that lies at the heart of stem cells' self-renewal machinery, they can effectively eliminate human colon cancer stem cells in mouse xenografts. They further show that a small-molecule BMI-1 inhibitor blocks tumor growth and metastasis in the absence of systemic toxicity, illustrating the feasibility of targeting self-renewal as a new strategy for treatment of colon cancer [122].

Promoting CSC differentiation is another approach to cancer therapy. Malignant cancer cells are generally derived from poorly differentiated cells, which make them

highly tumorigenic. As for CSCs, their selfrenewal and differentiation properties make them even more tumorigenic. Inducing differentiation into weakly tumorigenic cells could thus raise the potential for CSC eradication and thereby reduce the probability of recurrence after conventional therapy [126].

The presence of ABC transporters in the cells of the side population (cells with stem cell activity in the tumor mass) would facilitate the elimination of anticancer drugs such as mitoxanthrone, gemcitabine, doxorubicin or 5-fluorouracil [127]. Inhibition of ABC transporters can be a strategy for elimination of CSCs.

There are evidences indicating that inhibitors of DNA repair pathways together with certain DNA-damaging anticancer drugs increase the efficiency of the cancer treatment, due to the inhibition of the pathways that lead to the elimination of the toxic effects. There are many agents under study whose full analysis falls out of the scope of this chapter, since they are not specifically targeted at CSCs [128].

Manipulating the apoptotic machinery, including activation of pro-apoptotic pathways and inactivation of anti-apoptotic pathways to eradicate CSCs, displays great potentials [129].

It is known that stem cell niches tend to be hypoxic environments [130], and tumor masses also have a tendency to develop hypoxic regions due to the rapid cellular growth. Along these lines, the regulation of CSCs and niche ROS levels has been suggested as a potential way of therapy [131]. In this sense, enzymes that are involved in generation of reactive oxygen (such as superoxide dismutase) could be a possible target [128].

Conclusion

Because cancer stem cells are responsible for treatment resistance and cancer recurrence, interest in the study of these cells is increasing every day. Illuminating various features of these cells help the creation of new therapies and protocols. As a result, cancer stem cells are important in cancer cure and to increase the chance of and treatment.

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Stem Cells in Cell Therapy and Regenerative Medicine

The use stem cells in regenerative medicine are one of the most important issues of interest in the scientific field. Regeneration ability of stem cells, their differentiation characteristics offer a wide range of new projects and ideas. Valuable results in this regard provide important contributions to regenerative medicine. However, considering the presence of a cell group that affects the prognosis negatively, such as cancer stem cells, the issue becomes even more complicated. In this e-book, we aim to contribute to researchers working in the field of medicine, biology, molecular biology and genetics, pharmacy and veterinary medicine by referring stem cells, cancer stem cells and their importance in regenerative medicine.

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