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Review on Stem Cell Therapy and their Role in Cancer Treatment

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Abstraction

In the world, cancer remains a major cause of mortality. Despite great progresses have been made in understanding the molecular basis of cancer, the progress in cancer detection and treatment, mortality is still high and there is not a cure despite great improvements have been made in therapies. Stem cell based regenerative therapies have raised hopes for novel therapeutic approaches. Stem cells are cells that have the ability to perpetuate themselves through self renewal and to generate mature cells of a particular tissue through differentiation. A critical comparison of the attributes of several types of stem cells is presented with particular emphasis on properties that are critical for the application of these cells for therapeutic purposes. The importance of an autologous source of pluripotent stem cells is stressed. Like normal tissue stem cells, CSCs are capable of self-renewal, either by symmetric or asymmetric cell division and have the exclusive ability to reproduce malignant tumors indefinitely. Understanding of CSCs physiology and metabolism may be crucial for the development of novel effective therapies. Current chemotherapies cause severe side effects because which target all rapidly dividing cells. Although the treatments that target only CSCs which cause fewer side effects for patients due to the similarities between CSCs and normal tissue stem cells. The major obstacle to the development of effective cancer therapy is believed to be the absence of sufficient specificity. Since the discovery of the tumor-oriented homing capacity of mesenchymal stem cells (MSCs), the application of specific anticancer gene-engineered MSCs has held great potential for cancer therapies.

Keywords: *Cancer stem cell; Mesenchymal stem cell; Normal stem cell; Self renewal.*

Introduction

Cancer is one of the leading causes of mortality and morbidity throughout the world [1]. Over the past 50 years, many diseases that cause deaths have dramatically decreased but a cancer death has not been reduced [2]. Historically, much focus has been on the genetic and biochemical mechanisms that cause drug resistance. However, cancer is widely understood to be a heterogeneous disease and there is increasing awareness that intra tumoral heterogeneity contributes to therapy failure and disease progression [3].

Stem cell therapy holds the promise to treat degenerative diseases, cancer and repair of damaged tissues for which there are currently no or limited therapeutic options. Mesenchymal stem cells (MSCs) are the first type of stem cells to be utilized in clinical regenerative medicine. Current conventional cancer therapies such as surgery, chemotherapy and radiotherapy are symptomatic and passive in nature. The major limitation on the effectiveness of conventional therapies for cancer treatment is lack of tumor specificity [1]. In addition to the therapeutic specificity of anti-cancer agents, the development of drug resistance of tumor cells is another factor contributing to inefficient cancer therapy [4]. Stem cells (SCs) have the unique capacity to differentiate into one or more specialized cell types. Stem cell based regenerative therapies have raised hopes for novel therapeutic approaches in cancer [5]. Stem cells that used for regenerative medicine are embryonic stem cells (ESC), induced pluripotent stem cells (iPSC) and adult stem cells (adult SCs)[6].

Adult stem cells are now the basis of essentially all successful stem-cell based therapies. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which adult stem cells are found but current thinking suggests that these cells have greater importance as immune modulators with their regenerative properties being due to trophic paracrine effects rather than true regeneration [7]

Stem cells have three distinctive properties: self renewal, the capability to develop into multiple lineages and the potential to proliferate extensively. The combination of these three properties makes stem cells unique. The attribute of self-renewal is notable because its subversion is highly relevant to oncogenesis and malignancy [8][9]. Stem cells have the ability to build every tissue in the animal body. Therefore, Stem cells have great potential for future therapeutic uses in tissue regeneration and repair [10].

General methods for the identification and isolation of CSCs in malignancies include xenotransplantation assays which are the gold-standard for identification of CSCs; sorting based upon cell surface markers; efflux of Hoechst 33342 or Rhodamine dyes; the enzymatic activity of aldehyde dehydrogenase (ALDH) and colony and sphere forming assays requiring specific culture conditions[11]. Cancer stem cells reside in specialized microenvironments called niches, which have an important role in stem cell maintenance. The constituents of niche include fibroblasts, endothelial cells, perivascular cells, tissue macrophages, extracellular matrix and soluble factors excreted from cells or released from stroma [12].

Stromal cells within the niche may secrete some factors that regulate CSC self renewal properties [13]. Cancer stem cells prefer to reside in a hypoxic microenvironment to maintain their homeostasis rather than normal stem cells that prefer a glycolytic microenvironment. There is cross talk between CSCs and the niche, in a way that CSCs instruct the niche and are governed by the niche to proliferate, differentiate, invade and metastasize [12]. Cancer stem cells (CSCs) or tumor initiating stem-like cells (TICs) are a small subset of cancer cells which are capable of self-renewal and resist various chemotherapeutic drugs. This sub-population behaves like stem cells by undergoing either asymmetric or symmetric cell division by maintaining its population within the cancer [14]. CSCs and normal tissue stem cells possess self-renewal capacity; however, self-renewal is typically deregulated in CSCs because CSCs appear to be preferentially endowed with the capacity of self-renewal and responsible for long-term maintenance of tumor growth and progression. CSCs may also be primary cause of tumor metastasis [15] [3].

Current systemic cancer therapies frequently fail to eliminate advanced tumors, which may be due to their inability to effectively target CSC populations. Understanding CSCs physiology and metabolism may be crucial for the development of novel effective therapies [12]. Tumors irrespective of their origin are heterogenous cellular entities whose growth and progression greatly depend on reciprocal interactions between genetically altered (neoplastic) cells and their non-neoplastic microenvironment [16].

In general, all the cancer therapies including surgery, hormonal therapy, anti-angiogenesis therapy and immunotherapy are lack of efficacy in terms of long-term outcome because of their failure to target cancer stem cells and toxicity due to non-specific effects on normal cells [17]. Since the discovery of the tumor-oriented homing capacity of mesenchymal stem cells (MSCs), the application of specific anticancer gene-engineered MSCs has held great potential for cancer therapies. With the discovery of specific anticancer genes and the revelation of MSCs' capacity of tumor-directed migration and incorporation, a new research field has been inspired with the aim of achieving efficient therapy for cancer using engineered MSCs [1].

Therefore, the objectives of this manuscript are:

To explain stem cell therapy in cancer treatment compare to current cancer therapy like surgery, hormonal therapy, anti-angiogenesis therapy and immunotherapy.

To describe the important characteristics of stem cells in cancer treatment.

2. FUNDAMENTALS OF STEM CELLS

2.1. History of Stem Cells

During the 1940s and 1950s, various researchers conducted experiments that suggested that bone marrow contained cells that could reconstitute the blood and immune system. In 1961, two Canadian researchers finally proved the existence of what they called stem cells that are capable of regenerating tissues and even whole systems within a fully developed body. They used x-rays to kill a mouse's blood-forming cells and immune system cells and then injected bone marrow into the irradiated mouse. The injected bone marrow reconstituted the mouse's blood supply and immune system, proving that cells in the marrow are capable of producing all the different cell types in the blood. Eventually, these researchers showed that a single cell type what is known as hematopoietic stem cells. These experiments formed the basis of today's use of bone marrow transplants to treat leukemia and other kinds of blood disorders [18].

Adult stem cells (ASCs) are discovered in the mid 1950s. ASCs are found in low abundance in almost all adult tissues and in high abundance in the umbilical cord. ASCs are found in special regulatory niches as self-renewing progenitor cells that are able to produce one or more specialized cell types. ASCs are usually considered to be tissue specific, self-renewing populations of cells which can differentiate into cell types associated with the organ system in which reside [19].

Stem cells are cells capable of developing into other types of cells and tissues; for this reason they are often referred to as "pluripotential" cells. Historically, stem cells have been viewed within the context of the embryo because it is dramatic transitions of stem cells forming a range of tissues and organ systems [20]. Stem cells occur in many different somatic tissues and are important participants in their physiology. Populations of cells that derive from stem cells are organized in a hierarchical fashion, with the stem cell residing at the apex of the developmental pathway [9].

2.2. Concepts of Stem Cells

Stem cells are clonogenic cells capable of both self-renewal and multilineage differentiation [21] [19]. One of the most important issues in stem cell biology understands the mechanisms that regulate self-renewal. Self-renewal is crucial to stem cell function because it is required by many types of stem cells to persist for the lifetime of the animal. Moreover, whereas stem cells from different organs

may vary in their developmental potential, all stem cells are self-renew and regulate the relative balance between self-renewal and differentiation. Understanding the regulation of normal stem cell self-renewal is also fundamental to understanding the regulation of cancer cell proliferation because cancer can be considered to be a disease of unregulated self-renewal [22]. Self-renewal is the key biological process of cell division; a stem cell produces one (asymmetric division) or two (symmetric division) daughters that retain the capacity for self-renewal. Stem cell population is maintained or expanded for long-term clonal growth [3].

The capacity of prolonged self-renewal (proliferation) and multilineage differentiation (asymmetric replication) are characteristics of stem cells [19]. These characteristics are more pronounced in younger sources. By asymmetric replication, after every cell division, one cell retains its self-renewing capacity while the other transit-amplifying or TA cell enters a differentiation pathway and joins a mature non-dividing population. When an unspecialized stem cell differentiates, it assumes characteristics of a specific tissue. Stem cells are pluri, multi or unipotent. The zygote is the only mammalian cell capable of producing all cells and tissues of an organism and thus is considered totipotent. [23].

Pluripotent cells produce cells and tissues belonging to all three germ layers ectoderm, mesoderm and endoderm. Multipotent cells produce more than one cell lineage within a closely related family of cells. Unipotent cells only differentiate into a single cell phenotype [19]. Stem cells are quiescent or slowly cycling cells maintained in an undifferentiated state until their participation is required in normal functioning of the organism [24].

2.3. Sources and Classification of Stem Cells

Harvesting of stem cells may be divided into two domains, allogenic and autologous sources. Autologous stem cells are acquired from the host in which the cells are intended for use while allogenic cells are procured from an unrelated donor prior to transplantation. The use of allogenic stem cells may predispose an individual to various immunologic complications upon treatment, giving rise to the significant limitation of graft rejection with this method of treatment. Autologous treatments may be limited by their ability for propagation and cell yield. The immunological barriers such as graft versus host or required immunosuppression of the host are constant consideration in the therapeutic benefits and limitations of stem cell transplantation [25].

Stem cells for regenerative medicine include embryonic stem cells (ESC) which are derived from preimplantation embryos (morulae or the inner cell mass of the early blastocyst) and when grown under appropriate conditions can be induced to differentiate into cells of all three germ layers (ectoderm, mesoderm and endoderm). ESC can be readily grown as undifferentiated cells under defined conditions, providing an unlimited supply of pluripotent stem cells [26]. induced pluripotent stem cells (iPSC) established through reprogramming of somatic cells and various primary cell types including endothelial progenitor cells (EPC), mesenchymal stem cells (MCS), cardiac derived progenitor cells (CDP) and cardiac stem cells (CSC) collectively termed adult stem cells (adult SCs) [6].

There are several classes of stem cells including embryonic (ESC), adult stem cells and induced stem cells. Each class of stem cells has its own benefits, limitations and challenges in bioprocess development. All of them share as common features the ability to proliferate indefinitely (unlimited self-renewal capacity) and vary in their differentiation potential [27]. With the increasing diversity of stem cell sources emerging for donor cells in transplantation therapy, many laboratory to clinic translational factors such as source of cells, extraction, immunogenicity, capacity for proliferation and cell yield must be first considered [25].

Figure 1: Stem cell sources and characteristics (Adapted from [28])

2.3.1. Embryonic stem cells

Embryonic stem cells are totipotent which are capable of differentiation into every known daughter cell type [24]. ESC can be readily grown as undifferentiated cells under defined conditions, providing an unlimited supply of pluripotent stem cells [29]. Therefore, when established as a cell line, that could be

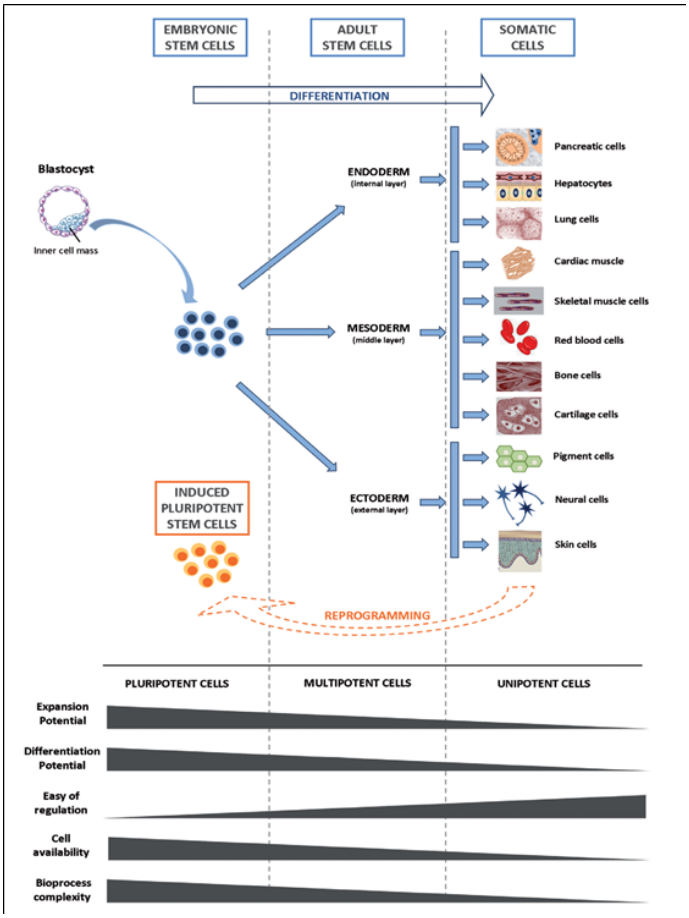

Figure 1: Stem cell sources and characteristics (Adapted from Placzek et al., 2009).

marketable and easily available as a therapeutic cell source [21].

There is a strong worldwide ethical debate about the ethics of using ESCs for therapeutic purposes [30]. If a therapeutic modality develops using human ESCs, there is a potential for these ethical issues to prevent the spread of this modality to certain populations. Tumorigenicity of ESCs after transplantation is a very important issue that should be properly addressed before starting ESCs transplantation clinical trials. It has been shown that these cells have the potential to induce tumor formation after transplantation. This tumorigenicity is mostly associated with ESCs and iPSCs [31].

The last concern is the fact that these cells are allogeneic and express high levels of major histocompatibility complex-I proteins and thus may be rejected on transplantation. In view of these issues, ES cells cannot be considered as the first choice in a clinical trial experiment until 2006 [21].

2.3.2. Adult stem cells

Adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ; it can renew itself and differentiate through progenitor cells to give the major specialized cell types of the tissue or organ. Basically, these cells renew themselves and become specialized to yield all the mature cell types of the tissue from which originated. It is shown that adult stem cells can develop not only into the specialized phenotypes of their tissue of origin but also into cell types of another tissue derived either from the same embryonic germ layer or from a different one. This is called plasticity [32]. Like embryonic stem cells, adult stem cells can reproduce themselves, or self-renew but they do so to a more limited extent. Unlike embryonic stem cells, adult stem cells do not usually form cells outside their tissue type [18].

Adult stem cells (ASCs) are found in low abundance in almost all adult tissues and in high abundance in the umbilical cord [33]. ASCs are found in special regulatory niches as self-renewing progenitor cells that are able to produce one or more specialized cell types. ASCs are usually considered to be tissue specific, self-renewing populations of cells which can differentiate into cell types associated with the organ system in which they reside. ASCs include mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), epithelial and neural stem cells. HSCs and MSCs originate in the bone marrow and differentiate into endothelium, liver, bone, muscle, skin or others [19]. Adult stem cells are now the basis of essentially all successful stem cell based therapies. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue [34].

On the other hand, ASCs do not present immunogenic complications on implantation since ASCs can be isolated directly from the patient. ASC exist in specific niches in the different organs like bone marrow, peripheral blood, pancreas, lung, brain, liver contributing to the regeneration/repair of the tissue/organ where ASC reside [35]. Depending on the source, ASCs can be isolated with relative ease. However, ASCs presently have major limitations such as the difficulty in obtaining pure populations, expansion capacity and the restricted differentiation potential [27].

2.3.3 Induced pluripotent stem cells (iPSCs)

Induced pluripotent stem cells are artificially derived from non-pluripotent cells, typically adult somatic cells (mostly fibroblasts of murine origin) and most frequently by epigenetic reprogramming and also by nuclear transfer or cell division [36]. The creation of these iPSCs elicited an explosion of scientific curiosity and industrial interest. This is mainly because iPSCs are similar to ESCs and thereby could potentially replace ESCs for clinical applications, circumventing the ethical concerns regarding the use of embryos [27].

This reprogramming process is called retrodifferentiation and is achieved by transfection of certain stem cell-associated genes into nonpluripotent cells such as adult fibroblasts or mononuclear leucocytes. Transfection may be achieved through viral vectors including retroviruses, adenoviruses and non-viral vectors. Transfected genes include the master transcriptional regulators Oct-3/4 (Pouf51) and Sox2, although other genes and epigenetic factors can probably enhance the efficiency of induction. iPSCs are postulated to be functionally identical to natural pluripotent stem cells such as embryonic stem cells, with respect to the expression of certain stem cell genes and proteins, chromatin methylation patterns and other epigenetic properties, doubling time, embryoid body formation, teratoma formation, viable chimera formation and potency and differentiability but the full extent of their capabilities and limitations in comparison to natural pluripotent stem cells is still being assessed [34].

Figure 2: Methods of production of iPSCs (Adapted from [36]

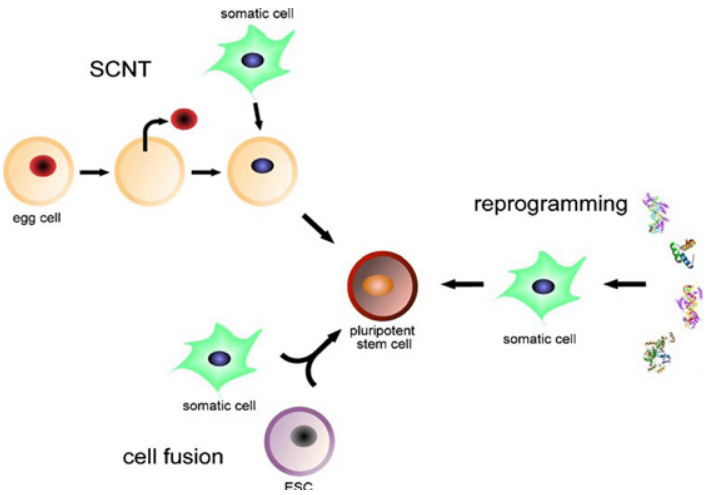

Figure 2: Methods of production of iPSCs (Adapted from Gonzalez et al., 2011).

The first methods to produce iPSCs required the use of retroviruses as the vectors to transfect cells to initiate the expression of the critical genes. This process is unacceptable as a prelude to the production of cells for therapy since retroviruses are known cancer-causing agents [37]. The problem is addressed through the use of a much safer adenovirus as the vector. However, the efficiency of the process, is dramatically attenuated with an adenovirus vector which certainly reduces tumorigenesis but does not eliminate this problem since the expression of endogenous oncogenes (proto-oncogenes) may potentially be triggered by the integration and harboring of exogenous sequences in the target cell genome. The problem of tumorigenesis is elegantly addressed by a technique that can remove oncogenes after the induction of pluripotency while removing or reducing the risk of tumor formation, greatly increases the complexity of the process. This advance is achieved by repeated treatment of the murine cells with four transcription factors, Oct4, Klf4, Sox2 and c-Myc channeled into the cells via poly-arginine anchors to induce pluripotency. Transcription factors or cell markers are the key mediators of cellular identity [38].

In addition to this, the production of iPSCs whether by a viral vector or protein induction and has been the subject of continuing concern on the basis that replacing oncogenes with the putative oncoproteins that are the product of the oncogenes may not eliminate the cancer risk on the same line of genetic-chemical flow of genetic information to phenotypic representation [34]. Two other strategies are developed to generate iPSCs with a reduced risk of tumor formation while viral integration is prevented. Firstly, induction of iPSCs has also been achieved without viral integration using adenoviral vectors or plasmids that encode the required reprogramming factors [37]. Secondly, chemicals and small molecules have been used successfully to generate iPSCs. These methods are based on the endogenous activation of reprogramming factors are reported for the reactivation of the Oct3/4 gene [39].

3. CANCER STEM CELL

According to the American Association of Cancer Research, CSCs are defined as cells within a tumor that possess the capacity of self-renewal and to cause the heterogeneous lineages of cancer cells that comprise the tumor. This promising concept originated in the 18th century when Rudolph Virchow postulated that cancer is originated in immature cells. More evidence for the existence of CSC is obtained in breast, brain, colon, prostate and lung tumors, where a small population of malignant cells with self-renewal capacity is observed [15].

Some researchers suggest that CSCs may originate from mutated normal stem cells upon aberrant alteration of the self-renewal pathways [22]. An alternative hypothesis is that CSCs originate from differentiated cells that have acquired stem-like features following multiple mutations. These features include the ability to self-renew and generate progenitors through asymmetric divisions to produce more committed progenitor cells or differentiated cells [40]. Cancer stem cells may arise from normal stem cells, progenitor cells or more differentiated cells through multiple mutations of genes as a result of their genomic instability [41].

A tumor is a complex ecosystem containing tumor cells, as well as various infiltrating endothelial, hematopoietic, stromal and other cell types that can influence the function of the tumor as a whole. These extraneous cell types can influence tumor cells directly and can create metabolic changes such as a hypoxic environment and nutrient fluctuations which contribute to heterogeneity in the function of malignant cells [3].

Specifically, only a minority of tumor cells have the capacity to regenerate the tumor and sustain its growth when injected into an immune-compromised mouse model. At least two models of cancer growth can explain tumor development. The first, termed the stochastic model, assumes that every cancerous cell has the capacity to extensively proliferate and regenerate a tumor. This is due to the assumption that all cancer cells have an equal, albeit low, probability of regenerating a tumor. In contrast, the cancer stem cell model assumes that only a very small subset of cells within the tumor population actually has the capacity to initiate and sustain tumor growth [42].

Cancer stem cells (CSCs) or tumor initiating stem-like cells (TICs) are a small subset of cancer cells which are capable of self-renewal and resist various chemotherapeutic drugs. This sub-population behaves like stem cells by undergoing either asymmetric or symmetric cell division thereby maintaining its population within the cancer [14].

Cancer stem cells have the capacity to maintain tumorigenesis because of their stem-like properties which include self-renewal and differentiation potential, characteristics also shared by normal stem cells. Based on this fact, one of the possible origins of cancer stem cells is obviously stem cells themselves. If this is true, CSCs use the existing machinery of stem cells to promote their own self renewal, promoting a longer lifespan compared with mature and differentiated cells, accumulating mutations and ultimately assuming a malignant phenotype [43]. Progenitor cells have also been described as a second possible origin for CSCs. However, it has been hypotheses that CSCs arising from normal stem cells are more aggressive than those from progenitor cells [17].

A third possibility is that CSCs may originate from mature and differentiated cells that enter a undifferentiation program becoming more stem-like, increasing resistance to stress [15]. The concept of cancer stem cells has now been used to explain why current cancer therapies are relatively ineffective [44].

3.1. Characteristics of Cancer Stem Cells

Cancer stem cells are cancer cells that possess characteristics associated with normal stem cells. At the molecular level, CSCs and normal stem cells share some common features including the capacity for self-renewal [22], the ability to differentiate, active telomerase expression, activation of anti apoptotic pathways, increased membrane transporter activity and the ability to migrate and metastasize [45].

Like normal tissue stem cells, CSCs are capable of self-renewal, either by symmetric or asymmetric cell division and have the exclusive ability to reproduce malignant tumors indefinitely. Most

cytotoxic therapies induce DNA damage or disrupt mitosis leading to cell death in dividing cancer cells. CSCs are protected against anti-neoplastic drugs through multiple defense mechanisms. These mechanisms can be divided into two groups: CSC-intrinsic and CSC-extrinsic. CSC-intrinsic mechanism can be due to more efficient DNA repair mechanisms, expression of drug pumps and altered cell cycle. CSC-extrinsic mechanisms refer to the effects of tumor microenvironment on CSCs [12]. In addition to these properties, CSCs display an anchorage-independent survival, active DNA-repair capacity and relative quiescence (slow cell cycling) [45].

3.2. Identification and Isolation of CSCs

The discovery of a universal marker for CSCs has not yet been made. General methods for the identification and isolation of CSCs in malignancies include xenotransplantation assays which are the gold-standard for identification of CSCs; sorting based upon cell surface markers; efflux of Hoechst 33342 or Rhodamine dyes; the enzymatic activity of aldehyde dehydrogenase (ALDH) and colony- and sphere-forming assays requiring specific culture conditions [11]. These CSC markers can be identified by staining cells with antibodies against them or by flow cytometry. CSC markers commonly used for various tissues are listed in Table 1 below.

Table 1: Markers used to Identify CSCs in Various Tissues

Tumor	CSC marker
Breast	CD44*+/CD24-/lin-/ALDH1+
Leukemia	CD34+/CD38-
Colon	CD133+/CD44+/ALDH1+
Head and Neck	CD44+
Brain	CD133+
Lung	CD133+
Prostate	CD133+/CD44+/α2β1high
Pancreas	CD133+/CD44+/CD24+/ESA+
Liver	CD90+

*CD44: hyaluronate receptor (p-glycoprotein 1); CD24: heat stable antigen; lin: linage markers; ALDH1: aldehyde dehydrogenase 1A1; CD34: hematopoietic progenitor cell antigen (GP105-120); CD38: cyclic ADP ribose hydrolase; CD133: prominin 1; α2β1: integrin α2β1; ESA: epidermal surface antigen; CD90: Thy-1 (Source: [12])

3.2.1. Cell surface markers

Experimentally, CSCs are currently identified by using cell-surface markers and their ability to reestablish a new tumor with identical heterogeneity by transplanting them into new immune deficient hosts in limiting dilutions. CSCs are isolated from primary tumors and cell lines by flow cytometry, fluorescence-activated cell sorting, or magnetic cell separation according to specific cell surface markers like CD133, CD44, CD24, CD34 and CD38 [11]. Biomarkers of the cancer stem cells not only the tools which are used to identify the cancer stem cells, but also might become the target of many drugs which were developed to cure cancer [17].

Although great progress has been made in understanding CSC surface molecules, it should be realized that these markers are not perfect for defining the tumor-initiating cell because some cells that do not belong to the CSC compartment may also express these markers. Furthermore, it has also been demonstrated that cell surface markers could be dynamically and reversibly expressed by tumorigenic cells [46]. Therefore, it is clearly insufficient to define a cancer stem cell based solely on surface markers [11].

3.2.2. Dye exclusion assays

This method is one of the most common methods employed to identify the side population (SP) based on its dye efflux properties in various types of cancer cells. SP cells constitute a subpopulation of cancer cells that can efficiently efflux the fluorescent DNA binding dye, Hoechst 33342 and ATP binding cassette transporter [14]. Although the use of Hoechst 33342 dye enables identification of Hoechst-negative CSC SPs, the possible toxicity of the dye may cause side effects during cell sorting [11]. Small differences in cell densities, dye concentrations and staining timings may affect the phenotype of the SP cells [14].

As the SP cells exhibit higher tumorigenicity than non-SP cells it is believed that this method is used to detect CSCs. As a control for sorting the CSCs, an ABC transporter inhibitor such as verapamil or reserpine is used in order to determine the SP gate. These DNA binding dyes inhibit the efflux of the Hoechst dye by SP cells thus serving as an essential control. By using the hoechst 33342 dye exclusion assay reports clearly show the presence of SP and NSP in various cancers such as brain, lung, prostate, and pancreatic [47].

3.2.3. Aldehyde dehydrogenase activity

This assay has been developed based on the increased aldehyde dehydrogenase (ALDH) activity in hematopoietic stem cells. ALDH is required for the oxidation of intracellular aldehydes thereby resulting in the oxidation of retinol to retinoic acid [14]. ALDHs belong to the oxido-reductase family, which oxidizes a wide range of endogenous and exogenous aldehydes to their corresponding carboxylic acid [11].

Aldehyde dehydrogenase activity is usually measured by using biodipy aminoacetaldehyde (BAAA), commonly known as Aldefuor [17]. BAAA passively diffuses into the living cells and gets converted into bodipy aminoacetate (BAA) by the intracellular ALDH. BAA is retained inside the cells until it is effluxed by ATP binding cassette transporters. To determine the background fluorescence an ALDH inhibitor, Diethylaminobenzaldehyde (DEAB) is used [14]. Increasing evidence has suggested that ALDH activity can be used either alone or in combination with cell surface markers to identify CSCs in hematologic malignancies and solid tumors including breast, colon, bladder, lung and skin. It has also been used as a prognostic indicator of metastases and poor survival [11]. However, ALDH does not appear to be a CSC marker in all tumor types [48].

3.3. Cancer Stem Cell Niche and Hypoxia

Cancer stem cells reside in specialized microenvironments called niches, which have an important role in stem cell maintenance. It has been shown that response of CSCs to antitumor drugs is different in vivo and in vitro, which may be due to the effect of the niche. The constituents of niche include fibroblasts, endothelial cells, perivascular cells, tissue macrophages, extracellular matrix and soluble factors excreted from cells or released from stroma [12]. Stromal cells within the niche may secrete some factors that regulate CSC self renewal properties [13].

Cancer stem cells prefer to reside in a hypoxic microenvironment to maintain their homeostasis rather than normal stem cells that prefer a glycolytic microenvironment. Hypoxia leads to an increase in the expression of Oct-4, Nanog and c-Myc which results in neurosphere formation, which is a stem cell property. Hypoxia Inducible Factors (HIF1α and HIF2α) play an essential role in cancer hypoxia and are shown to be associated with poor prognosis [11]. There is cross talk between CSCs and the niche, in a way that CSCs instruct the niche and they are governed by the niche to proliferate, differentiate, invade and metastasize. CSCs may generate niches as nascent domains or CSCs may use existing tissue stem cell niches [12].

4. ROLE OF STEM CELL THERAPY IN CANCER

Stem cell therapy holds the promise to treat degenerative diseases, cancer and repair of damaged tissues for which are currently no or limited therapeutic options. The potential of stem cell therapies has long been recognized and the creation of iPSC has boosted the stem cell field leading to increasing development and scientific knowledge [39]. For over 30 years, stem cells have been used in the replenishment of blood and immune systems damaged by the cancer cells [49].

Mesenchymal stem cells (MSCs) are the first type of stem cells to be utilized in clinical regenerative medicine. In addition to their capability of multipotent differentiation, MSCs show many other therapeutically advantageous features such as easy acquisition, fast ex vivo expansion, the feasibility of autologous transplantation and a powerful paracrine function. More recently, the specific tumor-oriented migration and incorporation of MSCs have been demonstrated in various pre-clinical models revealing the potential for MSCs to be used as ideal vectors for delivering anticancer agents [1].

4.1. Stem Cells as Delivery Vehicles in Gene Therapy

Stem cells can be classified as embryonic or adult depending on their tissue of origin. The role of adult stem cells is to sustain an established repertoire of mature cell types in essentially steady-state numbers over the lifetime of the organism. MSCs are a group of adult stem cells naturally found in the body [1]. Bone marrow and adipose tissue are the main sources of MSCs for cell therapy due to high expansion potential and reproducible isolation protocols [18]. The combination of cellular therapy and gene delivery is an attractive option for potentially protect the vector from immune surveillance and support targeted delivery of a gene or therapeutic proteins to the tumor sites [17].

Tumors can be characterized as “wounds that never heal”, serving as a continuous source of cytokines, chemokines and other inflammatory mediators. These signals are capable of recruiting respondent cell types including MSCs. The degree of inflammation in the tumor site plays an important role in the recruitment of MSCs. Irradiation resulted in apoptosis and increased release of inflammatory signals at the site of radiation like TNFα, PDGF, CLLS and CCR8. Radiotherapy is a traditional cancer therapy. Therefore, it could work in combination with MSC-based gene delivery to support improved targeting of MSCs to tumors [17].

Along with their tumor tropism, MSCs is integrating and persist in the tumor stoma. MSCs can efficiently produce biological products at the tumor sites and in a number of tumor models, MSCs expressing IFNβ have been shown to result in decreased tumor burden and increased animal survival [50]. A study has engineered MSCs to express TNF related apoptosis-inducing ligand (TRAIL) which causes apoptosis and death of cancer cells without harming normal cells by binding to specific TRAIL receptors and leading to activation of the extrinsic apoptosis pathway [51].

The recent concept of use of stem cells as delivery vehicles came from the fact that the tumors, similar to the wounds, send out chemo-attractants such as the vascular endothelial growth factor (VEGF) to recruit MSC to form the supporting stroma of the tumor and pericytes for angiogenesis. MSC transduced with an adenoviral expression vector carrying interferon-β gene has been demonstrated to increase the production of interferon-β at the local site [49]. Anticancer mechanisms of interferon are immunostimulatory and by inducing programmed cell death (apoptosis) [1].

4.2. Stem Cells in Immuno-Reconstitution

The stem cells have been used since many years in immuno-reconstitution following cancer development or following cancer treatments. The high dose chemotherapy has the adverse effects on the bone marrow causing myelosupression. It has been shown that chemotherapy can induce inhibitory factors such as Tumor Growth Factor (TGF)-β-Interferon(IFN-γ, IFN- α,) Tumor Necrosis

Factor(TNF)-α and Interleukin(IL)-4 with cytokines that causes myelosupression. The adequate number of the stem cells therapy is also reported crucial factors for speedy recovery [49].

5. CONCLUSION AND RECOMMENDATIONS

Stem cells and cancer cells share a lot of commonality. However, stem cells are proven and more primitive as compared to cancer and cancer stem cells. Under normal circumstances, stem cells maintain a homeostasis and replenish the adult cell pool while deregulation or imbalances of stem cells can give rise to cancer stem cells and eventually full blown cancer. The recent progress in both stem cell and anticancer gene studies has great potential for exploitation in new efficient cancer therapies. The combination of MSCs and specific anticancer genes can selectively act upon targeted tumor cells. There is a pressing clinical demand for more efficient remedies to replace existing symptomatic anticancer therapies. The extensive achievements of MSCs and anticancer agent studies have laid the foundation for the exploitation of MSC-based cancer therapies. Now the date, cancer therapy has entered in to an exciting new era with other therapies such as chemotherapy, radiotherapy and surgeries on one side while the stem cells on the other side. Stem cells therapies attract more attention within the new gene technologies due to their roles in immuno-reconstitution, potentially protect the vector from immune surveillance and support targeted delivery of a gene or therapeutic proteins to the tumor sites.

Therefore, based on above conclusion the following recommendations are forwarded:

Combined stem cell therapy with current therapy such as chemotherapy, radiotherapy and surgeries should be practiced in all types of cancer treatment in animals to support targeted delivery of a gene and proteins to the tumor sites.

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