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Stem Cells and Cancer Stem Cells

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1. Introduction

The different organs in human organism are constituted by tissues with mature and specialized cells and its specific stem cells. Stem cells represent only a minuscule fraction of cells that constitute each tissue but they are the only cells with self-renewal capacity. The organ-specific stem cells have specific properties that maintain tissue integrity and are defined mainly by their capacity to undergo self-renewal, as well as differentiation into mature cell types that comprise each organ (Shipitsin & Polyak, 2008).

The malignant neoplasias are believed to result from sequential mutations that can occur as a consequence of progressive genetic instability and/or environmental factors. An accordant observation in several investigations has been the association between deregulation of stem cells and carcinogenesis, because there are regulatory mechanisms of self-renewal in normal stem cells that also frequently regulate oncogenesis. In consequence, experimental and clinical evidences that have recently been accumulated support the hypothesis that cancer may arise from mutations in normal stem cell populations, and that these cells would be subject to ongoing genetic and epigenetic changes that could help to establish the disease.

The cancer stem cell (CSC) hypothesis states that normal stem cells may be the cells of cancer origin, and that a specific subset of cancer cells with stem cell characteristics can give rise to a hierarchy of proliferative and progressively differentiated bulk of tumoral cells, leading to tumor initiation, progression, and recurrence. In fact, there are several investigations that recently have identified specific CSC markers showing similar expression profiles than the normal stem cells of same organ. Moreover, CSCs can be prospectively isolated based on expression of a specific molecule or combination of molecules, and have the ability to give rise to new tumors when xenografted in immunodeficient mice.

Additional confirmations that stem cells can play a role in carcinogenesis are the homologies found between normal adult stem cells and cancer cells. Besides self-renewal capacity, these characteristics include the production of differentiated cells, activation of antiapoptotic pathways, induction of angiogenesis, resistance to apoptosis and drugs (due to active telomerase expression and elevated membrane transporter activity), and the ability to migrate and propagate (Wicha et al., 2006). Notwithstanding, different from normal adult stem cells that remain constant in number, CSCs can increase as the tumors grow, and originate the progeny that can be both locally invasive and/or colonize distant sites. Therefore, the consolidation of CSCs knowledge into our current view of multistep cancer development has important implications for defining the target population for therapeutical

approach and for understanding specific events required for realization of malignant potential, and the advances in CSC knowledge can help to build further evidences for potential targeting pathways in treatment of several cancer types.

2. Stem cells

Despite wide variety of cells that can be identified in adult tissues, all cells derive from a single egg cell after fertilisation of an ovule by a spermatozoid. Egg fertilisation results in creation of totipotent stem cells, which are the precursor cells of all tissues of embryo, yolk sac, amniotic sac, allantois, and embryonic portion of placenta – chorion and others placental membranes. Approximately four days after fertilisation, these totipotent stem cells undergo several mitotic divisions to form identical cells, and after this point, they tend to lose their high proliferative potential and begin to specialise by becoming pluripotent stem cells, which then can generate most of tissues necessary for embryo formation. Subsequently, the pluripotent stem cells begin to divide, and they mature into more specialised stem cells – the progenitor cells. These progenitor cells are called multipotent stem cells. They are committed to generate specific cell groups that have distinct functions, such as haematopoietic stem cells, which produce erythrocytes, white blood cells and platelets. Furthermore, multipotent stem cells become more specialised and give rise to precursor committed cells or unipotent stem cells, which are able to differentiate into only one cell lineage.

The unipotent stem cells' function is to act as cell reservoirs for different tissues. Certain unipotent cells, such as adult hepatocytes, may even have long-term repopulating functions. Finally, from unipotent stem cells originates the nullipotent cells that are terminally differentiated and have lost their self-renewal capabilities. Therefore, stem cells show diverse degrees of plasticity or differentiation potential and can be defined as units of biological organisation that are clonal precursors of more identical stem cells; in addition, they can produce a defined set of differentiated and specialised progeny (De Miguel et al., 2009; He et al., 2009; Slack, 2008) (Figure 1).

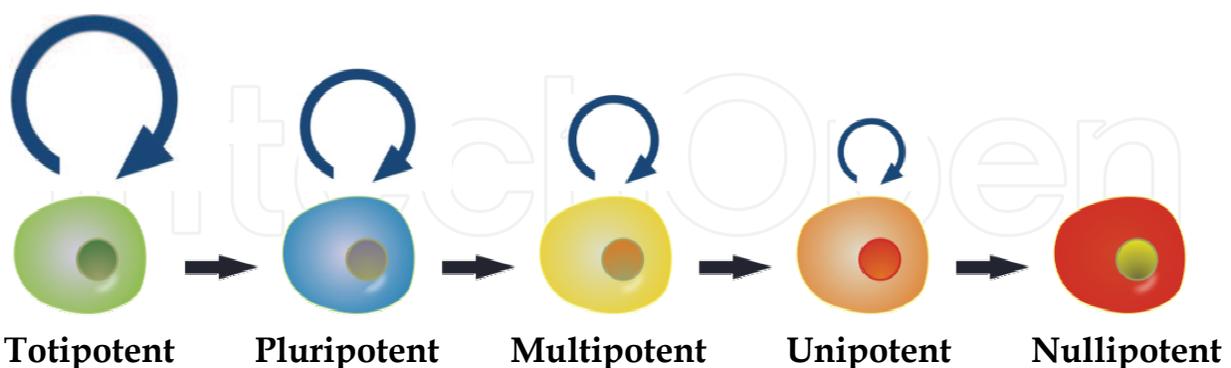


Fig. 1. Stem cells plasticity. Stem cells show diverse degrees of differentiation potential

The integrity of adult tissues is maintained by the continuous replacement of cells that regularly differentiate and die. Thus, in most adult tissues, there are pools of progenitor cells that are able to multiply and differentiate into specialised tissue of origin, while at the same time, they are able to maintain a reserve of undifferentiated cells. These adult progenitor cells are defined as adult tissue-specific stem cells or somatic stem cells.

The liver is probably the best example of a tissue with stem cells and differentiated cells because it has a remarkable regeneration capacity. Centuries ago, Greek mythology described liver regeneration through story of Prometheus, the mortal who stole the secret of fire from Zeus and introduced it to humans. Prometheus was then punished by having his liver plucked out by an eagle daily. His liver regenerated overnight, thus providing the eagle with eternal food and Prometheus with eternal torture. This phenomenon was later recognised in medicine, albeit at a slow rate, and it was probably first introduced into scientific literature in the 1800s in several German reports (Ankoma-Sey, 1999).

In modern times, the next significant scientific advance in elucidation of liver regeneration was introduced by Higgins & Anderson in 1931. They demonstrated experimentally that surgical removal of two-thirds of the rat liver (partial hepatectomy) was possible and that it resulted in regeneration of remaining lobes of liver by compensatory hyperplasia. The whole process lasted five to seven days (Higgins & Anderson, 1931).

During the 1960s, first genetic evidence of stem cells existence was detailed. The authors of these studies demonstrated that bone marrow contains a unique specific type of cell that could give rise to myeloerythroid colonies in spleen. In these experiments, genetically marked cells (random DNA breaks and translocations) were generated by sublethal irradiation of the donor bone marrow. These cells could self-renew and differentiate in spleens of conditioned transplanted host mice, indicating that the genetically marked stem cells were able to reconstitute and radioprotect mice after sublethal irradiation (Becker et al., 1963; Becker et al., 1965).

In summary, stem cells differ from other cells in the body because they have four major properties: a) they are undifferentiated and unspecialised; b) they are able to multiply for long periods while remaining undifferentiated (generally slowly cycling), such that a small number can create a large population of similar cells; c) they are capable of differentiating into specialised cells of a particular tissue (produce progeny in at least two lineages); and d) they can be serially transplanted. The combination of these properties is often referred to as “stemness” (Mikkers & Frisen, 2005).

Stem cells can divide symmetrically or asymmetrically. A symmetrical division occurs when two daughter cells share the same stem cell features, and it occurs when their numbers (stem cell pool) need to be expanded, such as during embryonic development or after tissue injury. An asymmetrical division occurs when one of the progeny remains undifferentiated, thereby replenishing the pool of stem cells, while the other daughter cell can proliferate and differentiate into specialised cells to generate new tissue mass (Figure 2).

2.1 Pluripotent stem cells

During embryonic development, the embryo originates from a single fertilised egg, also called a zygote, and it divides into extraembryonic (trophoblasts) and embryonic components (Gardner, 1983). The embryonic component is located inside the embryo. It refers to the inner cell mass of blastocysts, and is the originator of all tissues of embryo, foetus and adult organism (Brook & Gardner, 1997; Evans & Kaufman, 1981). The inner cell mass is also the source of embryonic stem (ES) cells and has the ability to give rise to all three embryonic germ layers: ectoderm (epidermal tissues and nervous system), endoderm (interior stomach lining, gastrointestinal tract, lungs), and mesoderm (muscle, bone, blood, urogenital) (Li & Xie, 2005; Thomson et al., 1998).

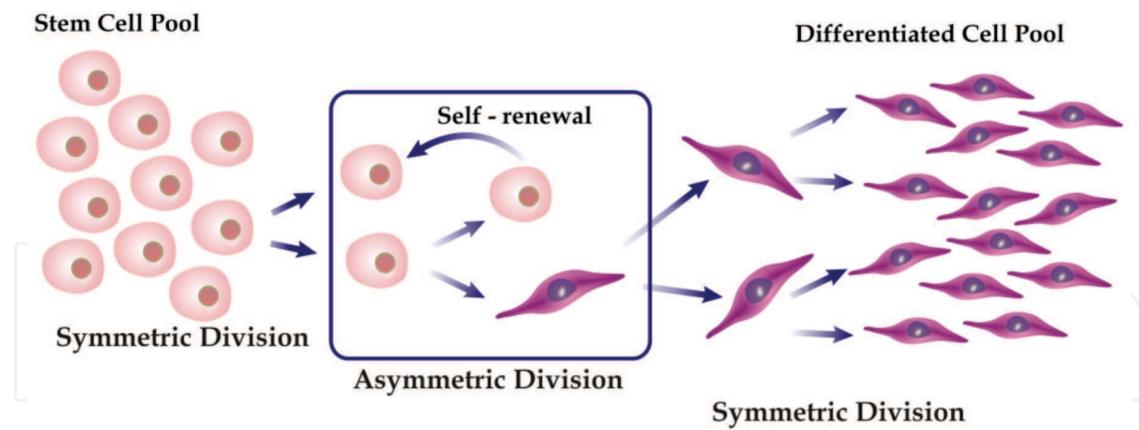


Fig. 2. Self-renewal is the fundamental characteristic of stem cells. Stem cell can be induced to undergo symmetric division when necessary and stem cells also are able to divide asymmetrically, originating one undifferentiated cell, which restores the stem cell pool, and another cell committed to differentiation

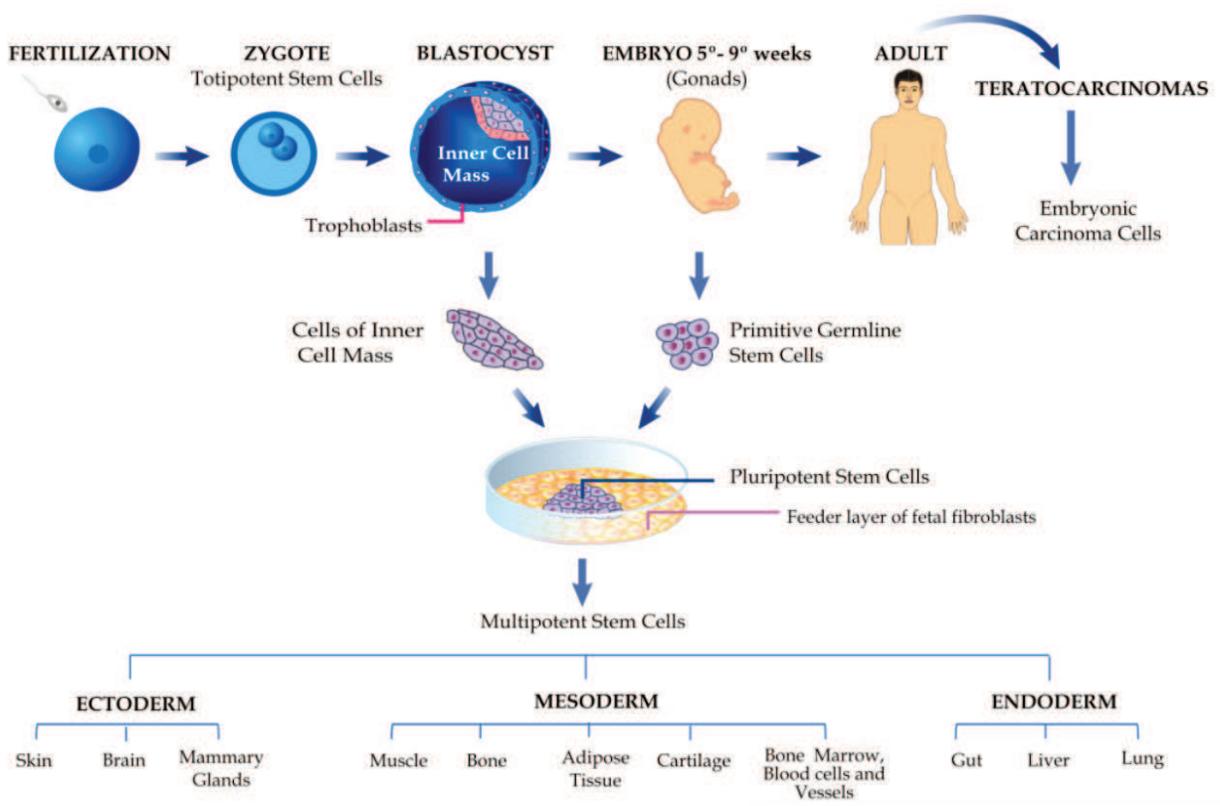


Fig. 3. The totipotent zygote is formed after fertilisation of an ovule by a spermatozoid and undergoes several mitotic divisions to form blastocyst, which is divided into extraembryonic (trophoblasts) and embryonic components (inner cell mass), from which all tissues of adult organism originate. Pluripotent stem cells can be isolated from inner cell mass or gonadal buds of embryo using a feeder layer of foetal fibroblasts, and these cells can be differentiated into cells of every lineage in human body. Stem cells restricted to one lineage (ectoderm, mesoderm or endoderm) are called multipotent stem cells

As development proceeds, the need for organogenesis arises, and over five-nine weeks post-fertilisation, the embryo proper forms germline stem cells for reproduction and somatic stem cells for organogenesis. Germline stem cells derive from gonadal buds of the embryo and are an alternative source of ES cells (Liu et al., 2004). The ES cells of the inner cell mass or the gonads are considered to be pluripotent due their ability to differentiate into cells of every lineage in the body (Anderson et al., 2001). Moreover, ES cells can undergo cell divisions without differentiation through symmetrical divisions (Fuchs & Segre, 2000) (Figure 3).

Interestingly, the term “ES cells” was introduced to distinguish pluripotent embryonic stem cells from teratocarcinoma-derived pluripotent embryonic carcinoma (EC) cells (Martin, 1981). Teratocarcinomas are malignant, multidifferentiated tumours containing a significant population of undifferentiated cells. These tumours were first described in 1970 when researchers reported that early mouse embryos grafted into adult mice produced teratocarcinomas (Solter et al., 1970; Stevens, 1970). In humans, teratocarcinomas are formed from a malignant form of primordial germ cells and usually occur in ovaries and testes. The EC cells proliferate extensively *in vitro* and remained undifferentiated even at high densities, and unlike ES cells and germline stem cells, they contain chromosomal alterations. Another characteristic of EC cells is that they have a more limited differentiation potential than ES cells *in vitro* and *in vivo* (Andrews, 1998).

The first isolated ES cells were obtained from mouse blastocysts in 1981 (Evans & Kaufman, 1981; Martin, 1981). After 17 years, James Thomson's team described the first human ES cells that were isolated (using a similar protocol as for mice) from fresh or frozen embryos obtained through *in vitro* fertilisation for reproductive purposes, which were donated by parents (Thomson et al., 1998). In same year, Shambloott & colleagues isolated pluripotent cells from human embryonic and foetal gonads. Since then, it has been possible to obtain several immortal ES cell lines from mice and humans using feeder layers of mouse foetal fibroblasts in presence of leukaemia inhibitory factor (LIF). These immortal cell lines present the same *in vivo* properties *in vitro* and grow indefinitely in laboratories under specific conditions. However, differences between mouse ES cells and human ES cells have been found, and subsequently, several lines of human ES cells has been described and added to a record that can be found on homepage <http://stemcells.nih.gov/research/registry>.

The criteria used to define cell lines as ES cells are the following: a) must be derived from pre-implantation embryos, b) must have prolonged proliferation in undifferentiated state, and c) must be able to differentiate into cells of the three germ layers, even after prolonged culture. In this manner, some investigators observed that ES cell lines subcutaneously injected into SCID mice could give rise to distinct tissues, such as neural epithelium (ectoderm); cartilage, bone and smooth/striated muscle (mesoderm); and gut (endoderm) tissues (Pera et al., 2000; International Stem Cell Initiative, 2007).

Human ES cells can grow as colonies, and they express certain undifferentiated stem cell markers, such as transcription factors Oct-4 (octamer-binding protein 4), Sox-2 and Nanog, as well as cell surface proteins SSEA (Stage Specific Embryonic Antigen)-3, SSEA-4, TRA (Tumour Rejection Antigen)-1-60, TRA-1-80 and alkaline phosphatase (Miguel et al., 2010). These cells have normal and stable karyotypes during continuous passaging and can be kept in their undifferentiated state for multiple cell divisions when cultured under specific conditions *in vitro* (Shambloott et al., 1998; Shambloott et al., 2001; Amit et al., 2000). On the other hand, when grown in conditioned media, ES cell lines can be induced to differentiate in tissue-specific manners or into several other tissues (embryoid bodies), which simulates

the development of a pre-implanted embryo. Moreover, human ES cell lines have been used to generate cells of different lineages, including neurons, cardiomyocytes, blood progenitors, hepatocytes, retinal precursors and β -cells of pancreatic islets (Cowan et al., 2004).

These remarkable characteristics of human ES cells have generated great interest among researchers around the world, and studies of ES cell lines have been conducted to elucidate the molecular mechanisms involved in totipotency and pluripotency of stem cells, as well as to develop methodologies of ES cell differentiation into different tissues. Future manipulation of these pathways involved in cell potency may serve as the basis for modification of adult tissue-specific stem cells into less differentiated cells, thereby increasing their ability to differentiate and proliferate. Furthermore, pluripotent stem cell lines could allow for the testing of new medications in several cell types, thereby aiding the advancement of drug development process. As a result, only drugs that are both safe and have beneficial effects in various tests on these cell lines will be forwarded to animal experimentation and human trials.

The most fascinating development in history of ES biology is the generation of ES-like cells, called "induced pluripotent stem (iPS) cells", that do not involve destruction of human embryos. The destruction of embryos has caused huge religious and ethical problems and significant public unease. Several countries (e.g., Austria, Germany, Italy, and Brazil) have introduced legislation prohibiting human embryo research. However, in 2006, Takahashi and Yamanaka demonstrated that retroviral-mediated overexpression of a set of only four pluripotent genes, Oct4, Sox2, c-Myc and Klf4 (Kruppel-like factor 4), was sufficient to reprogram murine fibroblasts to ES-like cells. The first iPS cells generated were from mice, but within months, the same group described the generation of human iPS cells (Takahashi et al., 2007; Yu et al., 2007).

2.2 Adult tissue-specific stem cells

Adult tissue-specific stem cells are indispensable components of tissue homeostasis because they support ongoing tissue regeneration by replacing cells that are lost due to natural cell death (apoptosis) or injury (Spradling et al., 2001). These cells are undifferentiated but are found in adult differentiated tissues, and most of them have self-renewal capacity throughout the entire lifetime of an organism; in addition, they can give rise to other adult tissue-specific stem cells and precursor cells that can produce mature differentiated cells by asymmetric division (Weissman et al., 2001).

Adult tissue-specific stem cells represent a small percentage of total cellularity. Previous studies have reported many kinds of adult tissue-specific stem cells, and their experimental assays have revealed different characteristics of stem cell behaviour. Adult bone marrow, for example, contains at least three distinct types of adult multipotent stem cells: haematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs). The HSCs are quite rare, with a frequency about of 1 in 10,000 of all bone marrow cells, and the selection of human HSCs is based on combined expression of CD34 and aldehyde dehydrogenase (ALDH) (Mirabelli et al., 2008). The MSCs are also scarce in human bone marrow aspirates (1-20 in 10,000), and they decrease in quantity with age. Human MSCs express a wide range of markers, such as CD105, CD73, CD90, CD29, HLA class-I, CD44, CD49e, CD34, CD31, CD14, CD19, and HLA class II. *In vitro*, MSCs adhere to plastic surfaces and can differentiate into bone, cartilage or fat. Finally, bone marrow-derived EPCs are a unique population of blood mononuclear cells that have a role

in postnatal neovascularisation during wound healing and tumour development. The identification of human EPCs relies on expression of VEGFR2, c-kit, or CD34.

In small intestine, there is estimated to be around 10 stem cells near the bottom of the crypt out of a total crypt population of less than 300 cells. Small intestinal stem cells are multipotent and can generate Paneth cells, mucin-producing goblet cells, columnar enterocytes and enteroendocrine cells (all four lineages) (Sancho et al., 2004). In skeletal muscle, satellite stem cells are unipotent and the major source of myogenic cells for growth and repair, and they comprise around 5% of adult muscle nuclei present within muscle fibres. The stem cell markers of these cells include M-cadherin and transcription factor Pax-7 (Goldring et al., 2002).

Kidney stem cells compose 0.8% of all cortical cells and have been isolated from the cortical interstitium. They have been shown to express Pax-2, CD133, and classical mesenchymal markers such as CD73, CD29, and CD44. *In vitro*, these cells have been shown to differentiate into epithelial and endothelial cells (Gupta & Rosenberg, 2008). In mammary glands, stem cells are bipotent, generating luminal and myoepithelial cells, and they can be identified from terminal ductal lobulo-alveolar units by expression of CD44, CK19, or epithelial surface antigen-positive (ESA) or by negative expression of common acute lymphoblastic leukaemia antigen (CALLA-) (Clarke et al., 2003). In the skin, epithelial stem cells are multipotent and give rise to epidermal progenitors for tissue repair and also hair matrix progenitors, which generate the hair shaft. These stem cells can be identified by CD34 and $\alpha 6$ integrin expression (Blanpain et al., 2004).

In summary, there are two criteria to define functional adult tissue-specific stem cells: self-renewal capacity and multipotentiality, which is most important when investigating new adult tissue-specific stem cell populations. However, there are controversies regarding the identity and functional potency of stem cells in some organs, such as in lung and pancreas. Kim & colleagues (2005) identified bronchoalveolar stem cells, but in 2009, Rawlins & colleagues revealed that these stem cells do not contribute to alveoli lineages during normal homeostasis and regeneration. Similar controversies have been debated in endocrine pancreas, which is composed of islets of Langerhans formed by α , β , δ and PP cells. Although embryonic pancreatic duct stem cells have the plasticity to give rise to endocrine and exocrine lineages, adult pancreatic duct stem cells generate acinar cells (exocrine pancreas) but not insulin-producing β -cells (Solar et al., 2009). Therefore, the distinction between adult tissue-specific stem cell characteristics, as well as their true potential, remains unclear. Thus, these facts should lead to future investigations aiming to clarify whether there are other common features among adult tissue-specific stem cells and to define the true roles of these cells that possess a wide *in vivo* differentiation potential.

2.3 Stem cell niches

Stem cells reside in a special microenvironment termed a "niche," which varies in nature and location depending on tissue type. The concept of niche was first proposed by Schofield (1978) to describe how bone marrow-derived haematopoietic stem cells, while in proliferative state, had increased proliferative potential when compared to haematopoietic cells that reside in spleen (spleen colony-forming cells, CFU-S). Historically, the term niche is typically used to identify the location of stem cells. Currently, the definition of niche is broader and includes the cellular components of microenvironment surrounding the stem cells, in addition to the signals that are emitted by these stromal support cells *in vivo*. Furthermore, the stem cell niche can be defined as a group of cells in a specific tissue whose

aim is the maintenance of adult tissue-specific stem cell pool (Spradling et al., 2001). Therefore, the niche provides a mechanism to precisely balance the production of stem cells and progenitor cells to maintain tissue homeostasis.

Although there are specific niches for each stem cell type and these special microenvironments appear to be structurally and functionally diverse, it is possible to find common features among them. The pioneering system used to study HSCs is the bone marrow, and currently, the HSC niche is conceptually divided into three parts: osteoblastic zone, vascular zone, and zone neighbouring haematopoietic stem cells. Another example is the neural stem cell niche, which supports neurogenesis in the adult brain and can be found in both subventricular zone (SVZ) and subgranular zone (SGZ) of hippocampal region. In these zones, the endothelial cells that form blood vessels and the specialised basal lamina are essential cellular components of neural stem cell niche (Doetsch, 2003).

The niche functions as a physical anchor for stem cells by generating factors that control stem cell proliferation and fate. Calvi & colleagues (2003) demonstrated in bone marrow that osteoblastic cells located in lining of endosteal surface express N-cadherin and physically attach HSCs, thereby acting as a regulatory component with capacity to control the HSCs number. In general, other mediating adhesion molecules can anchor cells to extracellular matrixes; for example, integrins, certain types of collagen (I-V), cadherins, and β -catenin play an important role in stem cell/microenvironment interaction (Simmons, 1997). With regard to the brain, it is known that endothelial cells can attach to astrocytes, which have stem cell features and give rise to neuroblasts in the SVZ and SGZ, thereby producing signals that control the stem cell population (Doetsch, 2003).

Inside the niche, stem cells are often in the quiescent state in terms of cell cycle. This quiescent state is vital for ensuring maintenance of tissues throughout life and prevents premature extinction of the stem cell pool caused by numerous conditions of stress experienced by cells. Niches for quiescent stem cells are located in hypoxic tissue regions that are poor in vasculature. For example, in the bone marrow, quiescent HSCs are maintained in osteoblastic niche (hypoxic niche), while the HSCs and haematopoietic progenitors in highly proliferative state are found in vascular niche (oxygenic niche) (Yin & Li, 2006; Jang & Sharkis, 2007). In response to injury, a microenvironmental change in tissue might actively signal to the niche to mobilise quiescent cells, which would induce the proliferation and transition of stem cells to the vascular niche area. Furthermore, after irradiation treatment, surviving HSCs must enter the proliferative stage to produce progenitor cells that will give rise to differentiated cells. Nonetheless, HSCs tend to exit of the cell cycle once the haematopoietic cells have been compensated (Suda et al., 2005).

Signalling pathways and molecular mechanisms can control stem cell fate decisions through a delicate balance between regulatory factors. To ensure appropriate control of cellular behaviour, the intrinsic stem cell factors must be subjected to microenvironmental regulation or extrinsic factors provided by niche. Therefore, both intrinsic and extrinsic factors are required to maintain stem cell properties and to direct stem cell self-renewal and differentiation. Several signalling molecules have been shown to be involved in maintenance of stem cell niche. For example, the Wingless-related protein (Wnt) signalling pathway is important for stem cell self-renewal, but expression of Wnt pathway inhibitors, such as axin, leads to inhibition of stem cell proliferation (Nusse, 2008). Studies using gene targeting have demonstrated that the bone morphogenetic protein (BMP) signalling pathway has an important role in the suppression of Wnt signalling pathway, thereby providing balanced control between stem cell activation and self-renewal. Homeobox genes induced by Wnt

activity, such as HoxB4 and Notch, can also participate in the process of stem cell expansion. The Notch pathway is important for maintaining stem cells in an undifferentiated state. Signals mediated by transformation growth factor beta (TGF- β) and family members, including BMPs, Nodal and activins, have been implicated in the maintenance and differentiation of various types of adult tissue-specific stem cells (Watabe et al., 2008). In short, there are several growth factors that operate at different stages in the stem cell lineages, indicating the need for strict control over cell division within the stem cell niche.

For maintenance of an adequate number of stem cells within their niches and meeting the demand of differentiated cells within the surrounding tissue, it is essential that there be a strict regulation of balance between symmetric and asymmetric stem cell divisions. The niche contributes to orientation of asymmetric division, with the aim of controlling the flow and direction of committed progeny. As a result, one daughter cell is destined to become a stem cell, stays in stem cell niche, retains its self-renewal properties and receives inhibitory differentiation factors. In contrast, the other daughter cell leaves the niche to become committed to proliferate and differentiate along a determinate lineage (progeny cell), and it can receive differentiation signals that can overcome this state to eventually become a functionally mature cell.

In general, both embryonic and adult stem cells must have the capacity to grow and differentiate in response to signals emitted by their specific niche. To sustain these functions throughout the organism's life span, there are essential mechanisms that control adult tissue-specific stem cells and the nature of a possible tumour transformation (Iwasaki & Suda, 2009).

3. Stem cells and cancer

3.1 Brief historical review of stem cells and cancer

The resemblance between stem cells and cancer cells was observed a long time ago. The first study concerning hypothesis of cancer origin from a rare population of normal cells with stem cell properties was proposed almost 150 years ago (Durante, 1874; Wicha et al., 2006). At that time, Cohnheim (1875) also proposed the hypothesis that stem cells could be misplaced during embryonic development and become the source of tumours that would be formed later in life.

This subject was revived over 40 years ago when several investigators confirmed the CSC hypothesis by demonstrating that a single tumour cell could generate heterogeneous progeny and give rise to a new tumour through studies performed in tumours derived from ascites fluid in rats and teratocarcinomas and leukaemias in mice (Bruce & Van Der Gaag, 1963; Kleinsmith & Pierce, 1964; Makino, 1956). In this vein, Park et al. (1971) observed certain myeloma tumour stem cells in mice using a primary cell culture assay, and Hamburger and Salmon (1977) corroborated the hypothesis that some cancers contain a small subpopulation of cells that are similar to normal stem cells. They observed in primary bioassays that the expansive growth of malignant lesions suggests the presence of a CSC population with stem cell properties, including indefinite proliferation.

In animal models, the ability of a small population of cells to originate a new malignant neoplasia was demonstrated in a classic experiment utilising transplantation of cells from human acute myeloid leukaemia (AML) that expressed certain cell surface markers associated with normal haematopoietic stem cells (Lapidot et al., 1994). The authors showed that these transplanted cells could initiate leukaemia in non-obese diabetic/severe combined

immunodeficient (NOD/SCID) mice, while other isolated cells could not. Since then, this assay has become the standard method for determining whether cell populations isolated from solid tumours are CSCs.

Based on ability of diverse purified populations to form leukaemia in NOD/SCID mice, various studies embarked on a search for stem-like cells in leukaemias. Bonnet and Dick (1997) recognised in AML that the injection of a small subset of leukaemic cells with a primitive haematopoietic progenitor phenotype (CD34⁺CD38⁻) resulted in leukaemias that could be serially transplanted into secondary recipients, and they also observed their ability to perpetually self-renew. Since then, putative CSCs have been isolated from many other tumour types, including brain, breast, colon, pancreas, prostate, lung, and head and neck cancer (Collins et al., 2005; Dalerba et al., 2007; Kim et al., 2005; Li et al., 2007; Prince et al., 2007).

3.2 Cancer stem cell hypothesis

The research fields of cancer and stem cell biology share common features regarding cellular proliferation properties. In humans, normal adult multipotent stem cells are usually self-renewing. This self-renewal ability allows stem cells to produce at least one progeny cell with a similar developmental capacity, and available current lines of evidence indicate that this cell population, through initial genetic or epigenetic alterations, can become the cells responsible for the development of several tumours through a progressive establishment of a CSC population.

It is widely accepted that genetic instability drives malignant transformation. The stem cell origin of cancer hypothesis considers that stem cells or other differentiated cells that have acquired self-renewal ability tend to accumulate genetic alterations and evade the strict control of their microenvironment, thereby giving rise to tumoural evolution (Shipitsin & Polyak, 2008). Thus, the CSC model suggests that tumour progression, metastasis and recurrence after therapy can be driven by a rare subgroup of tumoural cells that have the capacity to self-renew, while the bulk of the tumour does not have this capacity. Therefore, the deregulation of this self-renewal process leading to stem cell expansion may be a key event in carcinogenesis, and while self-renewal can drive tumorigenesis, the differentiation process may contribute to tumour phenotypic heterogeneity (Kakarala & Wicha, 2008; Shay & Wright, 2010).

Normal adult stem cells have relatively long telomeres compared to more differentiated somatic cells, they are usually quiescent or proliferate more slowly than their differentiated progeny, and they have increased longevity; for this reason, they are exposed to more damaging agents than more differentiated cells over time. Thus, they accumulate mutations that are then transmitted to the rapidly proliferating progeny (Dontu et al., 2003). Mutations in the DNA of normal adult stem cells appear to be the initiating events in several types of malignant tumours, and some of the strongest evidence supporting this hypothesis is that a specific group of cells can be prospectively isolated based on their peculiar features; later, these cells can be serially transplanted into immunodeficient mice (Alison et al., 2010).

If normal adult stem cells are the founding cells of several cancer types, then CSCs probably inherit many of their characteristics. The CSCs are a population of cells that are more tumorigenic than the bulk tumour population and can be defined mainly through the expression of unique properties, such as specific detoxification enzyme systems, molecular surface markers, and embryonic signalling pathways (Alison et al., 2010). The main hallmarks of CSCs are their properties of self-renewal, their ability to generate tumours from

very few cells, their slow cell division rate, their ability to give rise to phenotypically diverse progeny, and their selective resistance to radio- and chemotherapy (Reya et al., 2001).

The self-renewal and differentiation characteristics of CSCs lead to the production of all cell types in a tumour, thereby generating wide heterogeneity (Campbell & Polyak, 2007). The differentiated cells constitute the bulk of the tumour but are not usually tumourigenic due to their lack of self-renewal capacity and limited proliferation potential (Ginestier et al., 2007). However, it has been shown that the switch to carcinogenesis can occur in either the stem cells or their differentiated progeny, which sometime acquire the ability to self-renew (Dontu et al., 2003). In several tissue systems, it has been proposed that certain committed progenitor cells might become CSCs through a dedifferentiation process, which would occur by acquisition of stem cell properties (Cobaleda et al., 2007).

Further evidence indicates that stem cells can play a role in carcinogenesis. A previous study showed that there are similarities seen between normal stem cells and cancer cells. In addition to self-renewal capacity, these characteristics include activation of anti-apoptotic genes, production of more differentiated cells, induction of angiogenesis, resistance to conventional radio- and chemotherapy (e.g., due to active telomerase expression, high ALDH expression, elevated membrane transporter activity), and ability to migrate and disseminate in metastasis (Wicha et al., 2006).

Conversely, there are some important differences between these two types of cells, which also corroborates the CSC hypothesis. While normal stem cells are chromosomally stable and contain a normal diploid genome, cancer cells have a significant number of chromosomal rearrangements and are almost always characterised by aneuploidy. Moreover, cancer cells may lack cell cycle checkpoint activity that allows them to completely growth arrest. More importantly, a major difference that has been found between normal adult tissue stem cells and cancer cells is that stable telomere length is maintained in malignant cells (Shay & Wright, 2010).

Notwithstanding the evidence that has been found, the extensive characterisation of murine CSC models has not yet resulted in the identification of their human counterparts for all tumour types. More than one CSC type with a different phenotype per tumour type could be likely, which makes the search for a definitive cancer stem cell hypothesis even more difficult.

3.3 Isolation and purification of CSCs

Although the concept that cancers arise from stem cells was first proposed more than 150 years ago, it is only recently that advances in stem cell biology have allowed for more direct testing and validation of the CSC hypothesis. It is well settled that CSCs share some properties expressed by normal stem cells. Current methods for determining whether cell populations isolated from solid tumours are CSCs consist of purification of these cells from tumour samples based on the properties of normal stem cells, such as expression of specific cell surface markers of stemness (Al-Hajj et al., 2003), their ability to form spheres in culture (Dontu et al., 2003), membrane efflux activity through drug-efflux pumps (Goodell, 2002), and enzymatic activity detection of cytoprotective enzymes as aldehyde dehydrogenase 1 (ALDH1) (Nagano et al., 2007). Additionally, purified cells are then tested for the capacity to originate tumours when injected into immunodeficient mice.

The tumour initiation aspect of CSCs refers to the ability of these cells (at a reduced number) to originate malignant tumours in immunocompromised mice. The expression of some specific cell surface markers has been investigated to facilitate the identification and

purification of CSCs, and there are currently several stem cell markers that are shared by CSCs in multiple human tumour types, but this issue is best addressed in the next section of this chapter.

Hoechst 33342 membrane efflux activity is a discriminating characteristic of quiescent stem cells that is lost when these cells enter in cycle, and this activity allows the identification through flow cytometric analysis of a small stem-like cell population designated as a side population (SP). In fact, it has been hypothesised that the main characteristic of the SP is a universal stem cell phenotype (Zhou et al., 2001). Although heterogeneous, SP cells are observed in primitive retinal and cardiac cells (Bhattacharya et al., 2003; Hierlihy et al., 2002); in epidermal, neural, mammary, and haematopoietic stem cells; and also in certain embryonic stem cells (Zhou et al., 2001).

The SP cells are also associated with resistance to toxins and drugs, and this characteristic is a result of increased expression of membrane transporter proteins (ABC drug transporters), such as P-glycoproteins or BCRPs (breast cancer resistance proteins). In addition to acting as functional regulators of stem cells, they contribute to the defence against damaging agents through the elimination of xenobiotic toxins (Zhou et al., 2001). Thus, tumours might have a population of drug-resistant pluripotent cells that can survive chemo- and radiotherapy and subsequently repopulate the tumour (Charafe-Jauffret et al., 2008).

The *ALDH* gene superfamily encodes a family of NAD(P)⁺-dependent metabolic enzymes that are involved in detoxifying a wide variety of aldehydes to their corresponding weak carboxylic acids. ALDH activity, as a CSC specific marker, was discovered recently after great investigation, especially of haematological and breast malignancies (Charafe-Jauffret et al., 2009; Ginestier et al., 2007), although it has also been implicated as a CSC marker in several others tumour types (Ma et al., 2008). In human breast cancer cell lines, high ALDH activity has been used successfully to select CSCs (Charafe-Jauffret et al., 2009).

In vivo tumourigenic xenotransplantation assays performed in immunodeficient mice (NOD/SCID) are currently the gold standard for successful CSC isolation and purification. These mice have a lack of major elements of the immune system, and therefore, they do not reject human cells. Because a large amount of human tumour cells must be xenotransplanted into immunodeficient mice to originate tumours, it was initially thought that CSCs were infrequent in tumours. However, this might be because the human cells in this assay are in a foreign microenvironment, as transplantation of mouse tumour cells into other mice indicates that CSCs can be quite common in some determined cancers. This *in vivo* assay is frequently supplemented by a clonogenicity assay that assesses the ability of the cells to form spheres and determines the frequency of which these isolated cells can form colonies (neurospheres, mammospheres, or colonospheres) when they are plated at a low density under non-adherent conditions in semi-liquid medium. This technique is based on the unique property of stem cells to survive and grow in serum-free suspensions, while differentiated cells undergo anoikis and die under these conditions. The resulting spheres of cells can be then serially passaged for experiments, originating secondary and tertiary spheres with a cellular composition resembling that of primary spheres and proving their self-renewal capacity (Alison et al., 2011).

Therefore, the standard procedures for the isolation of CSCs have been similar in several investigations. Among the most used *in vivo* models is tumour cell fractionation according to cell-surface markers with stem cell characteristics, which is followed by a clonogenicity assay to verify the sphere formation capacity and their implantation into NOD-SCID mice to assess xenograft growth and cellular composition (Shipitsin & Polyak, 2008).

3.4 CSCs markers in human tumours

CSCs have been prospectively isolated on the basis of the expression of specific surface molecular markers, and recent interest in CSCs arose from experiments suggesting that cells with stem-like properties can be sorted from solid tumours based on the expression of these markers. However, there is still no apparent consensus regarding the more reliable markers associated with the identification of CSC phenotype in some particular solid tumours, such as in gastrointestinal carcinomas (Alison et al., 2010). In haematological malignancies, the consensus is that the CD34⁺CD38⁻ phenotype can identify most of the CSCs, and the accumulated evidence found in other tumour types indicates that markers such as cytoprotective enzymes, cell-adhesion molecules, and drug-efflux pumps can be associated with a CSC phenotype. The main surface markers currently associated with stem cells and CSCs include CD133, CD44, and CD24 (Al-Hajj et al., 2003; Hermann et al., 2007; O'Brien et al., 2007).

The CD133 cell surface marker, also called prominin 1 (PROM1), was discovered as a marker of normal haematopoietic stem cells and was later used to purify putative CSCs in several tumour types. In brain tumours, Singh et al. (2004) found that CD133⁺ cells could successfully grow under non-attachment conditions with neurosphere-like formations, whereas CD133⁻ cells could not. According to other studies, CD133 also has been shown to play a role in migration and asymmetrical stem cell division (Beckmann et al., 2007).

The CD44 marker is a transmembrane glycoprotein cell surface receptor for hyaluronic acid that is frequently expressed as several isoforms, and it is involved in cell adhesion, migration, and metastasis (Shipitsin et al., 2008). It has been used to identify putative CSCs in breast tumours (Shipitsin et al., 2008), as well as in other tumour types, such as prostate (Collins et al., 2005), pancreatic (Li et al., 2007), and head and neck carcinomas (Prince et al., 2007). Shipitsin et al. (2007) found that CD44⁺ tumoural mammary cells were associated with more invasive, proliferative, and angiogenic tumour status, thereby predicting more aggressive tumoural cell behaviour. Furthermore, there was a correlation between CD44⁺ tumoural cells and decreased patient survival (Shipitsin et al., 2007).

CD24 is a mucin-like adhesion molecule expressed by neutrophils, pre B lymphocytes and a large variety of solid tumours. Functionally, CD24 enhances the metastatic potential of malignant cells because it has been identified as a ligand of P-selectin, an adhesion receptor on activated endothelial cells and platelets. It also enables cancer cells to bind to platelets, and these tumour-platelet thrombi protect cells in the bloodstream and in turn facilitate tumour invasion through interactions with endothelia. Lim & Oh (2005) investigated the role of CD24 in various human epithelial neoplasias and demonstrated that intracytoplasmic CD24 expression was found to be highly associated with adenocarcinomas of the colon, stomach, gallbladder, and ovaries. Positive or negative CD24 expression also has been used in combination with other markers to identify putative CSCs in tumours, and some studies have defined the phenotype of pancreatic CSCs as CD24⁺/CD44⁺ (Li et al., 2007). However, in breast and prostate cancer, putative CSCs were found with a CD24⁻/CD44⁺ phenotype (Al-Hajj et al., 2003; Hurt et al., 2008).

These investigations suggest that diverse stem cell markers can be expressed by CSCs in different tumours, and each tumour may express a phenotypic pattern with a specific CSC marker combination. The significance of these observations in most human cancers remains to be determined. Table 1 shows the most prevalent and specific CSC phenotypes according to stem cell markers in tumours from different organs.

STEM CELL MARKER	TUMOR
CD34/CD38	Acute myeloid leukemia
CD44/CD24-/ALDH	Breast carcinoma
Side Population (SP)	Bladder carcinoma
CD133, CD44, EpCAM, ALDH	Colorectal carcinoma
CD133, Side Population (SP)	Endometrial carcinoma
CD133	Ewing's sarcoma
CD44	Gastric carcinoma
CD44, Side Population (SP), ALDH	Head and neck carcinoma
CD133, CD44, ALDH	Hepatocellular carcinoma
CD133, Side Population (SP), ALDH	Lung carcinomas (non-small cell and small cell)
CD133	Medulloblastoma, Glioma
CD133, CD44, CD24, ALDH, EpCAM	Pancreatic carcinoma
CD133, CD44, ALDH	Prostate carcinoma

Table 1. Cancer stem cell phenotypes according to stem cell markers expression in tumors of different organs.

3.5 The CSC niche

Tumours in general have a hierarchical organisation that can be dynamically regulated by the surrounding microenvironment. In adults, the niche prevents tumorigenesis through strict control of stem cell behaviour and maintenance of the balance between self-renewal and differentiation, as well as between quiescence and proliferation. Accordingly, intrinsic mutations that regulate self-renewal, including those in the Wnt, Notch and Hedgehog pathways, can lead to stem cells escaping from niche control. These mutations can initiate dysregulation of CSCs and result in tumorigenesis. Thus, a specialised microenvironment, consisting of cells, matrix proteins and growth factors, is thought to physically restrain stem cells and enable them to maintain their stemness by providing the required factors.

The CSC hypothesis suggests that CSCs reside in a supportive niche with a poor vascular supply and frequently hypoxic conditions, which would result in poor drug perfusion and therefore contribute to an ineffective chemotherapy response (Deonarain et al., 2009). Furthermore, in addition to normal adult stem cells, CSCs appear to be regulated through molecular stimuli that are supplied from the microenvironment by neighbouring connective tissue cells, mainly the fibroblast-like (mesenchymal) and endothelial cells (Alison & Islam, 2009). There is increasing evidence that disruption of epithelial homeostasis, whereby tumour cells acquire a mesenchymal phenotype, is necessary for cancer development. In colorectal cancer, for example, the promotion of Wnt signalling in CSCs requires co-stimulation by hepatocyte growth factor (HGF) secreted by stromal fibroblasts (Vermeulen et al., 2010).

It has been established that the microenvironment adjacent to blood vessels can serve as the main CSC niche that controls some aspects of CSC behaviour, and this microenvironment is also associated with the highest tumour proliferation rates (Alison & Islam, 2009). In brain

tumours, CSCs identified through expression of CD133 and nestin were observed to be concentrated in a niche close to capillaries (Calabrese et al., 2007).

According to Charafe-Jauffret et al. (2008), genetic and epigenetic mechanisms in the progenitor cells, in addition to environmental influences in the niche where these cells grow, may contribute to the cellular heterogeneity found in the malignant neoplasms. Recently, it has been suggested that the microenvironment adjacent to tumours can regulate asymmetric versus symmetric divisions (Alison & Islam, 2009).

3.6 The embryonic self-renewal pathways

Different mutations associated with cancer occur in pathways that govern stem cell maintenance, suggesting that dysregulation of normal mechanisms of stem cell functionality may also be involved in carcinogenesis. Thus, the signalling pathways that regulate normal stem cell development and proliferation can be identical to those that promote carcinogenesis, possibly through initiation of CSC proliferation (Reya et al., 2001).

The CSCs generally have or can re-acquire the self-renewal mechanisms needed for their maintenance, development and expansion. In this manner, the embryonic signalling pathways, such as Wnt, Notch, Hedgehog (Hh), Bmi-1, PTEN, and p53, are fundamental for normal stem cell development and organogenesis, and these same pathways are also involved in driving CSC activity (Takebe & Ivy, 2010).

The Wnt pathway is clearly important for the preservation and self-renewal of stem cells. Wnt signalling is known to regulate cell fate decisions and influence morphology, proliferation, differentiation, apoptosis, migration, and stem cell self-renewal (Turashvili et al., 2006). Moreover, Wnt proteins can assist in maintaining stem cells in an undifferentiated state within their niche, and defects in the Wnt pathway have been observed in breast and colon cancer carcinogenesis (Olsen et al., 2004).

In the same manner, the Hh pathway is associated with the maintenance of stem cells in several malignant neoplasms, including myeloid leukaemia (Zhao et al., 2009), multiple myeloma (Peacock et al., 2007), and colorectal cancer (Varnat et al., 2009). The Hh pathway is one of the main pathways that control stem cell fate, self-renewal, and maintenance. In human gliomas, Hh signalling represents a new therapeutic target through its essential control of the behaviour of glioma CSCs (Clement et al., 2007). Through the use of both *in vitro* culture systems and NOD/SCID mice, Liu et al. (2006) found that the Hh pathway, together with the polycomb protein Bmi-1, play important functions in regulating self-renewal of both normal and malignant human mammary stem cells. Furthermore, in agreement with Byrd & Grabel (2004), Hh signalling can target endothelial stem cells directly or stimulate blood vessel support cells to produce vascular growth factors.

Recently, the Notch pathway has attracted increased consideration because several Notch receptors and ligands are frequently overexpressed in tumours, as has been observed in breast and cervical cancers (Nickoloff et al., 2003). In a study performed in human breast cancer, the high expression of Notch intracellular domain in ductal carcinoma in situ (DCIS) has been shown to correlate with reduced disease-free survival time at five years after surgery (Farnie & Clarke, 2007). In experimental gliomas, Notch signalling activation appears to be dependent on nitric oxide (NO) released by endothelial cells of the perivascular niche, which is important for stem-like character promotion and CSC maintenance (Charles et al., 2010).

Oncogenic or tumour suppressor genes, such as *HER-2*, *PTEN* and *p53*, have also been implicated in the regulation of CSC self-renewal. These genes are usually impaired in CSCs,

leading to uncontrolled self-renewal, which in turn can generate resistant tumours in relation to current therapeutic approaches.

3.7 Targets for therapy

Cancer progression can be viewed as an evolutionary process that generates multiple novel clones, each with a specific identity. If CSCs are the origins of tumours, then these are the cells that must be specifically eliminated for effective therapy.

Currently, it is well known that several cancers are peculiarly resistant to conventional radiotherapy and chemotherapeutic drugs that typically kill the majority of cancer cells. These clinical responses may reflect the targeting of the bulk of non-stem cell population. On the other hand, there are several specific key intracellular signalling pathways implicated in CSC self-renewal and proliferation processes that appear to be promising therapeutic targets, and a wide and diverse range of advances to eliminate the CSCs in malignant neoplasms are becoming evident; however, although several seem promising, a major difficulty will be specifically targeting these cells to avoid undesirable toxicity *in vivo* (Oliveira et al., 2010).

An ideal therapeutic strategy might be to sensitise CSCs to chemo- and radiotherapy by inhibiting their stemness properties and then by promoting direct cytotoxicity. Furthermore, as previously mentioned, the CSC population is driven by embryonic signalling pathways, and the targeting of these pathways could result in increased likelihood of a successful cure. In this vein, several drugs directed toward the inhibition of embryonic signalling pathways are under development, and strategies based on targeting intracellular pathways active in CSCs, such as Wnt, Bmi-1, Hh, Twist, and Notch, have all been currently considered for therapeutic investigation.

Wnt signalling is a key pathway in cell development and has been shown to be upregulated in about 50% of cancers (Deonarain et al., 2009). Inhibition of the Wnt/ β -catenin signalling pathway has been shown to be effective at blocking epidermal squamous cell carcinoma development, and a new approach to antagonise Wnt signalling involves the stabilisation of axin, thereby maintaining the β -catenin destruction complex (Huang et al., 2009). The Bmi-1 molecule has been demonstrated to have a role in lung tumorigenesis and bronchioloalveolar stem cell expansion, and Hh signalling has been shown to be critical for normal lung development, lung injury repair, and lung carcinogenesis (Peacock & Watkins, 2008). Furthermore, another study has shown that the Hh pathway can maintain a tumour stem cell compartment in multiple myeloma (Peacock et al., 2007). The development of specific Hh inhibitors, such as cyclopamine, is currently underway for breast cancer, and clinical trials utilising these chemotherapeutical agents are in the planning stages (Liu et al., 2006; Kakarala & Wicha, 2008). Similarly, aberrant Notch signalling that has been observed in several human cancers, such as human T-cell acute lymphoblastic leukaemia, cervical cancer, and breast cancer, suggesting that inhibition of Notch may represent a potential effective therapeutic target (Nickoloff et al., 2003). Telomerase inhibition also could be another effective anti-cancer therapeutic approach that would target both the proliferating CSCs as well as the bulk of the cancer cells (Shay & Wright, 2010).

The inhibition of the Epithelial-mesenchymal transition (EMT) process through transcriptional pathways, such as Snail and Twist, can slow the generation of CSCs with metastatic capacity. There has been intense investigation with regard to further therapeutic strategies based on blocking molecules at the cell surface that are implicated in invasion, migration and metastasis, such as integrins, CXCR-4, and CD44. In basal-like breast cancer,

the inhibition of Wnt signalling was shown to block stem cell self-renewal and also to repress the expression of the CDH1 repressors Slug and Twist, which in turn, block metastasis dissemination. In ovarian tumours, it was observed that CD44⁺ cells expressing markers of pluripotent stem cells might have a selective advantage for dissemination through their adherence to the hyaluronic acid pericellular coat of adjacent mesothelial cells (Bourguignon et al., 2008).

As therapeutic resistance of CSCs can often be directly attributed to the activity of ALDH or ABC surface transporters, additional approaches based on targeting these molecules might sensitise CSCs to current standard adjuvant therapies. In addition, given that several types of cancer cells have a specific microRNA (miRNA) expression profile, the manipulation of mRNA expression levels through miRNAs is another promising strategy by which to target CSCs (Alison et al., 2010).

The stem cell status of the cells of certain cancers can be dynamically regulated by the tumour microenvironment. Like normal stem cells, CSCs depend on support from the vascular and stromal niche for survival. As the microenvironment adjacent to the blood vessels can serve as the local CSC niche, another interesting alternative that is being addressed is the targeting of the vasculature, and this strategy could destroy the niche as well as the tumour bulk (Calabrese et al., 2007).

Throughout cancer evolution, it is likely that the genetic instability initiated by several selection pressures, such as hypoxia, immune or nutritional status, may result in the selection of new phenotypically malignant clones with increased genetic and epigenetic alterations. These malignantly transformed cells can acquire a selective growth advantage over their normal cell neighbours through resistance to apoptosis or higher proliferation rates, and subsequently, a specific clone of cells will develop. Increasingly, additional tumour progression with mutations and clonal expansion may give rise to more abnormal clones. In this manner, the more advanced tumours exhibit a complex heterogeneous picture, whereas early tumours may be more homogeneous because they did not have appropriate time to develop this clonal diversity. The existence of these clones can then eventually compromise a targeted therapy against a specific CSC clone because some of the cells would tend to expand due to a mutation for selective growth or survival superiority (Alison et al., 2011). This is an important problem that must be addressed when designing therapies against CSCs.

The correct identification and targeting of signalling mechanisms that are specific for CSCs could provide an opportunity for selective targeting of these cells. In fact, there is currently a need for the development of highly specific therapies that target CSCs. Later, these therapies will need to be tested in the appropriate oncological patient population, along with the use of adequate pharmacodynamic markers. However, the use of combined targeting of different CSC pathways, together with the commonly used radio- and chemotherapy applications and other types of targeted therapies, remains to be further explored in cancer therapy.

3.8 The influence of CSCs on tumour prognosis

If CSCs are associated with carcinogenesis, it follows that their frequency in primary tumours correlates with the extent of tumour invasion and dissemination and consequently, with patient prognosis. Generally, it is believed that elevated stemness characteristics and a high proportion of CSCs in tumours are associated with a worse prognosis.

Tumoural recurrence, metastasis and survival might be determined by the behaviour of the more resistant CSC population. In most cases, patients with tumours expressing high levels

of molecules associated with CSCs have a poorer clinical outcome than patients with tumours that express low levels of these molecules. In breast cancer, a high prevalence of CSCs was associated with higher biological and molecular heterogeneity, as well as with less differentiated tumours (Pece et al., 2010).

In brain tumours, the ability of tumour cells to propagate neurospheres in culture and high CD133 expression on these cells are regarded as independent prognostic factors that are being considered by some studies as relevant parameters associated with a reduced time of disease-free survival and overall survival. In human pancreatic cancer, in which 60% of tumours are CD133⁺, the CD133⁺ CSCs that simultaneously displayed CXCR4 expression were directly involved in the occurrence of metastasis after orthotopic xenografting, and remarkably, these metastases could be blocked by a small molecule inhibitor of CXCR4 (AMD3100) (Hermann et al., 2007).

Elevated ALDH expression is also associated with a poor prognosis in several tumour types, including AML, prostate cancer, breast cancer, head and neck squamous cell carcinoma, and pancreatic cancer (Charafe-Jauffret et al., 2010; Ginestier et al., 2007; Rasheed et al., 2010). Similarly, high activity levels of the ABC transporters have also been reported to be a sign of poor prognosis in patients with AML (Guo et al., 2009).

Thus, fundamentally, patients with tumours expressing high levels of the molecules associated with CSCs tend to have a poorer clinical outcome than patients with tumours that express low levels of these markers.

4. The prognostic influence of cancer stem cell immunophenotypes in oral squamous cell carcinomas

We recently investigated the presence of CSC antigens by immunostaining to identify a putative CSC immunophenotype in oral squamous cell carcinoma (OSCC) and to determine its influence on prognosis (Oliveira et al., 2011).

The initial demonstration that the tumoural cells of head and neck carcinomas have a hierarchy of development and embody a subpopulation of cells with self-renewal and differentiation capacities was reported by Prince et al. (2007), who found CSCs in low percentages and were able to characterise them through CD44 immunoreexpression. However, to our knowledge, our study was the first to verify the association between prognostic factors in OSCC and conventional CSC immunophenotype markers.

The CD44 proteins are commonly found in epithelial tissues and were previously established as fundamental regulatory factors in squamous epithelium for processes such as cellular adhesion, cell-cell interaction, infiltration and metastatic dissemination (Bajorath, 2000). Our findings regarding CD44 immunoreexpression in OSCC showed that CD44⁺ tumour cells occurred at a frequency of 41.4% and were associated with basal cell morphology. Moreover, our results demonstrated that the overall survival curves presented significant differences between CD44⁺ or CD44⁻ immunophenotypes, as configured by an independent factor of poor prognosis in multivariate analysis (hazard ratio, 0.316 [95% confidence interval, 0.070–0.664]; $P = 0.033$).

These results were consistent with other prognostic studies, suggesting that alterations in adhesion molecules can act as either positive or negative regulators of progression and metastasis in OSCC, depending on stage when tumour is diagnosed (Wang et al., 2007; Wang et al., 2009). In agreement, Bankfalvi et al. (2002) also found that the high immunoreexpression of CD44 (specifically the CD44v9 alternative splice isoform) was significantly associated with a poorer clinical outcome in OSCC.

Nevertheless, the effect of CD44 immunoeexpression on OSCC prognosis still shows discordant results, and there are several candidate stem cell markers that need to be assessed. A trustworthy immunophenotypical marker that can be used to isolate the CSCs has yet not been definitively established in head and neck cancers, and the identification of reliable markers required to characterise CSCs in OSCC could certify the clinical effectiveness of future targeted therapies, possibly resulting in a more effective outcome for the patients.

5. Final considerations

There are still many aspects that remain to be discovered in the field of CSCs. Although there is much to be learned about the mechanisms that regulate normal stem cell function and how they can be used by malignant cells to propagate the disease, the careful identification of the main differences between normal adult stem cells and CSCs, as well as of their overlapping aspects, are important to discern how cancers progress and to transform the advances in CSC biology into effective therapies that could help patients in the near future. Therefore, the interaction between the expression of CSC markers and malignant behaviour need to be adequately understood as they relate to prognostic factors in several cancer types.

We believe that most human solid and haematological cancers contain a subpopulation of CSCs. Experimental and clinical evidence sustain the hypothesis that in humans, the process of tumorigenesis initiates in an adult normal stem cell, although other more committed cells, particularly in the haematopoietic system, might also be the founder cells of malignancy. Several therapeutic approaches have been shown to be promising by targeting CSCs in tumours, which is a great challenge given that these cells seem to be specifically resistant to currently available therapies.

6. References

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Over the last thirty years, the foremost inspiration for research on metastasis, cancer recurrence, and increased resistance to chemo- and radiotherapy has been the notion of cancer stem cells. The twenty-eight chapters assembled in *Cancer Stem Cells - The Cutting Edge* summarize the work of cancer researchers and oncologists at leading universities and hospitals around the world on every aspect of cancer stem cells, from theory and models to specific applications (glioma), from laboratory research on signal pathways to clinical trials of bio-therapies using a host of devices, from solutions to laboratory problems to speculation on cancer's stem cells' evolution. Cancer stem cells may or may not be a subset of slowly dividing cancer cells that both disseminate cancers and defy oncotoxic drugs and radiation directed at rapidly dividing bulk cancer cells, but research on cancer stem cells has paid dividends for cancer prevention, detection, targeted treatment, and improved prognosis.

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