Stem Cells and Cancer

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Abstract

Stem cells are undifferentiated cells that can differentiate into specialized cell and can divide to produce more stem cells. The concept of cancer stem cells has been discussed in the scientific literature since the 19th century. Circumstantial evidence suggests that most tumors are heterogeneous and contain a small population of cancer stem cells that exhibit distinctive self-renewal, proliferation and differentiation capabilities, which are believed to play a crucial role in tumor progression, drug resistance, recurrence and metastasis in multiple malignancies. Unlike normal adult stem cells that remain constant in number, cancer stem cells can increase in number as tumors grow, and give rise to progeny that can be both locally invasive and colonize distant sites-the two hallmarks of malignancy. Rapid advances in the cancer stem cell field have provided cause for optimism for the development of more reliable cancer therapies in the future. Strategies aimed at efficient targeting of cancer stem cells are becoming important for monitoring the progress of cancer therapy and for evaluating new therapeutic approaches.

Keywords: Cancer stem cell; Stem cell markers; Niche

Introduction

Stem cells are defined functionally as cells that have the capacity to self-renew as well as the ability to generate differentiated cells. More explicitly, stem cells can generate daughter cells identical to their mother (self-renewal) as well as produce progeny with more restricted potential (differentiated cells) [1]. By contrast, ordinary cells can only make copies of themselves when they divide and can only divide a limited number of times. Sometimes a stem cell may divide into two ordinary cells, producing no more stem cells. The ability of stem cells to produce new cells of specific types is of special interest to medical science [2].

Stem cells provide an opportunity to investigate the mechanisms that regulate embryonic development, cellular differentiation, and organ maintenance. Given their proliferation and differentiation capacities, stem cells have great potential for the development of novel cell-based therapies. In addition, recent studies suggest that dysregulation of stem cell properties may be the cause of certain types of cancer [3].

Characteristics of Stem Cells

Stem cell literature is replete with terms such as "immortal," "unlimited," "continuous," and "capable of extensive proliferation," all used to describe the cell's replicative capacity) [4]. Several properties besides self-renewal and differentiation potential are frequently ascribed to stem cells, including the ability to undergo asymmetric cell divisions, exhibit extensive self-renewal capacity, exist in a mitotically quiescent form, and clonally regenerate all the different cell types that constitute the tissue in which they exist [5].

Potency is the ability to differentiate to specialized cell types. Stem cells can be totipotent, pluripotent and multipotent. Totipotency is the ability to form all cell types of the conceptus, including the entire fetus and placenta. Such cells have unlimited capability; they can basically form the whole organism. Pluripotency is the ability to form several cell types of all three germ layers (ectoderm, mesoderm and endoderm) but not the whole organism. There are four classes of pluripotent stem cells. These are embryonic stem cells, embryonic germ cells, embryonic carcinoma cells and recently the discovery of a fourth class of pluripotent stem cell, the multipotent adult progenitor cell from bone marrow [6]. Multipotency is the ability of giving rise to a limited range of cells and tissues appropriate to their location, e.g. blood stem cells give rise to red blood cells, white blood cells and platelets, whereas skin stem cells give rise to the various types of skin cells. These can form a limited number of specialized cell types, and generally function locally to replace fully differentiated cells lost through depletion or damage [7]. Some recent reports suggest that adult stem cells, such as haemopoietic stem cells, neuronal stem cells and mesenchymal stem cells, could cross boundaries and differentiate into cells of a different tissue. This phenomenon of unprecedented adult stem cell plasticity has been termed 'transdifferentiation' and appears to defy canonical embryological rules of strict lineage commitment during embryonic development.

Another characteristic attributed to stem cells is the ability to regenerate clonally the entire adult tissue from which they derive, meaning all cell types that constitute that tissue. In practice, this is an extremely difficult criterion to satisfy. Even in the hematopoietic system, for example, certain classes of blood cells such as some kinds of T cells are only produced during fetal life and are maintained in the adult by proliferation of committed cells. The examples illustrate cases where stem cells regenerate only a subset of the differentiated cell types in each tissue.

The various types of stem cells not only have different potentials, but they also proliferate differently. Cells can divide symmetrically, whereby each daughter cell retains the properties of the parental cells, or asymmetrically, whereby one daughter cell retains the properties of the parental stem cell, whereas the other daughter cell begins the process of determination. The characteristic of embryonal stem cells is that they divide symmetrically. Each daughter cell remains a totipotent stem cell, resulting in a logarithmic expansion of cells during early embryonic growth. Then, as the germ layers of...
the early embryo form and the process of determination begins, the cells proliferate asymmetrically [8].

One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. However, unspecialized stem cells can give rise to specialized cells, including heart, muscle, blood or nerve cells. Stem cells can give rise to specialized cells. When unspecialized stem cells give rise to specialized cells, the process is called differentiation. While differentiating, the cell usually goes through several stages, becoming more specialized at each step [9].

Type of Stem Cells

Two basic types of stem cells occur in humans and animals: embryonic stem cells and adult stem cells. Other types of stem cells are related to embryonic or adult stem cells but have different properties. Stem cells can be divided into a long-term subset, capable of indefinite self-renewal, as well as a short-term subset that self-renews for a defined interval [10].

Embryonic stem cells

The complex architecture of our body originates from one fertilized egg, which proliferates and differentiates to give rise to various types of cells in multiple organs. Embryonic stem (ES) cells comprise the zygote, the descendants of the first two divisions and those from the inner cell mass of blastocyst. The zygote (fertilized oocyte) and the descendants of the first two divisions are totipotent, able to give rise to the embryo and to the placenta and supporting tissues [11]. ES cells can proliferate without differentiation since they are established. When the inner cells are, isolated and cultured on feeder cells, the cells can grow while retaining their potency to form all three layers: the endoderm, mesoderm, and ectoderm. Therefore, ES cells have been used in an in vitro model of early embryogenesis to investigate the detailed molecular mechanisms for developmental processes. ES cells can be generated from various species at different embryonic stages. Several groups have obtained ES cells from the mouse morula stage and epiblast of mouse embryos, termed epiblast stem cells.

Adult stem cells

Adult stem cells (ASC), also called somatic stem cells, are found in humans and animals after birth and remain active in the body throughout a lifetime. ASCs are organ-specific small subpopulations of quiescent slow-cycling-undifferentiated resident cells, with high proliferative and pluripotent potentiality and the ability to self-renew and to originate daughter cells, which finally differentiate into functionally mature cells, regenerating all the cell types of the tissue where they are located.

Cancer stem cells

The concept of CSCs has been discussed in the scientific literature since the 19th century. In 1874 Durante hypothesized that tumors derive from a rare cell population of stem cell characteristics. This concept was called the "embryonal rest theory". In the late 19th century this hypothesis was gradually replaced by dedifferentiation theory of carcinogenesis. It assumed that adult differentiated cells are the source of cancer stem cells after process of dedifferentiation, i.e. reversal of differentiation. In the mid-20th century, when stem cells were gaining more attention, the concept binding together tumors and stem cells became attractive again [12].

CSCs are cells that have the capacity of self-renewal, meaning they undergo asymmetric divisions to produce more CSCs and a variety of differentiated daughter cells forming the bulk of tumor [13]. The translational definition of CSCs and the gold standard for exhibiting 'stemness' in CSCs is the ability to regenerate primary tumor in immune compromised mice. This xenotransplantation demonstrates the capacity of specific cells (CSCs) to reproduce the variety of differentiated cells present in the original primary cancer. Different biomolecules are used as markers to detect and isolate of these self-renewal cells (CSCs) in various cancers [14]. CSCs may originate from tissue stem cells which have gained cancerous properties through genetic and epigenetic changes. Alternatively, they may arise from transformed progenitor cells that have acquired self-renewal capabilities [15].

A CSC population was first identified in acute myeloid leukemia where a subset of cancer cells showed serial transplantation ability. CSCs from solid tumors were more recently identified first from breast cancers and then from several others including the brain, colon, head and neck, pancreatic, melanoma, mesenchymal, hepatic, lung, prostate and ovarian cancers. However, not all cancers adhere to the hierarchical model and there has been considerable debate about the accuracy of the CSC hypothesis, particularly in studies using human solid tumors and potentially inaccurate xenograft transplantation assays [16].

Stem cell markers

Stem cell markers are genes and their protein products used by scientists to isolate and identify stem cells. In addition, stem cells can also be identified by functional assays which are considered gold standard for the identification and therapeutic purposes. Knowledge on the stem cell identification in respect to their therapeutic application is rather limited due to their extraordinary complexities, specificity, validity and lack of specific molecular markers. In addition, marker profiles of stem cell population often fluctuate depending on their site of origin, species. Despite the limited knowledge of marker functionalities, their unique expression pattern and timing provide us useful tool to identify and isolate stem cells [17].

In recent years, a wide range of cell surface markers and generic molecular markers have been reported to be indicative of undifferentiated ESCs, especially for human species. Proteins involved in several signal pathways are also known to have important functions in cell fate decision [18]. The expression of specific markers is linked to the maintenance of hESCs pluripotency and self-renewal. Such markers include the transcription factors Oct-4 and Nanog and various cell surface markers, such as the stage-specific embryonic glycolipid antigens (SSEA) 3 and 4, the keratan sulfate-related antigens TRA-1-60 and TRA-1-81, and alkaline phosphatase [19].

Some of the proteins are uniquely present or secrete in specific cell types. Therefore, cell surface proteins can act as cell markers. Membrane proteins are the most important marker type in recognizing ESC without breaking the cell membrane. Stage specific embryonic antigens (SSEA) were originally identified by three monoclonal antibodies recognizing defined carbohydrate epitopes associated with the lacto- and globo-series glycolipids SSEA-1, SSEA-3, and SSEA-4. These carbohydrate-associated molecules are involved in controlling cell surface interactions during development [20]. Cluster of differentiation (CD) markers are surface proteins that belong to several different classes, such as integrins, adhesion molecules, glycoproteins, and receptors. Antibodies recognizing CD markers are frequently used to identify and characterize various cell populations. The CD markers associated with pluripotent hESCs are CD9, CD24, and CD133. In addition, hESCs express markers such as CD29, CD90, and CD117. CD133 is also a hematopoietic stem cell marker.
Genes that function in the nucleus are always involved in important functions. Transcription factors are crucial for gene regulation. Unique genes appear and do functions in the nucleus means that the cell has responded to a certain condition. So, tracking these genes expression can be used as a marker for a specific cell situation. Two homeodomain transcription factors, Oct4 and Nanog, were the first proteins identified as essential for both early embryo development and pluripotency maintenance in ES cells. Emerging studies indicates that in addition to Oct4, Sox2 and Nanog, many other factors required for pluripotency have been identified, including Sall4, Dax1, Esrrb, Tbx3, Tc11, Rif1, Nac1 and Zfp281. These pluripotency factors regulate concomitantly to form a complicated transcriptional regulatory network in ES cells stem cells.

Octamer-binding Protein 4 (Oct4), a transcription factor also known as Oct-3, OCT-3/4, Oct3 or NF-A3, is encoded by the Pou5f1 gene (located on chromosome 6 in human and 17 in mouse) and belongs to the POU (Pit, Oct, Unc) family of DNA binding-proteins [21]. Sox2 belongs to the Sox gene families, which are HMG box transcription factors that interact functionally with POU domain proteins. The Sox family of genes is involved in maintaining pluripotency, like Oct3/4. However, it is associated with multipotent and unipotent stem cells, while Oct3/4 is exclusively expressed in pluripotent stem cells [22]. The Sox2 gene bears at least two regulatory regions that specifically function in pluripotent embryonic cells [23]. NANOG is a homeodomain transcription factor, which together with OCT4 and SOX2, constitute the pluripotency transcriptional core in embryonic stem cells [24]. The homeobox gene NANOG is expressed in mammalian embryonic stem cells where its product, a homeobox transcription factor, maintains the pluripotency of these cells [25,26].

CSC expresses a unique repertoire of surface biomarkers, which allows its isolation from non-tumorigenic cells in a reproducible manner [27]. The main markers used for isolation, identification and purification of CSCs include surface cell-adhesion molecules (e.g., CD133, CD24, hyaluronic acid (HA) receptor CD44, CD44, CD166, etc.), cytoprotective enzymes (such as aldehyde dehydrogenase, ALDH), transcription factors (e.g., OCT-4, Sox2-2), and drug-efflux pumps (e.g., ATP-binding cassette (ABC) drug transporters (ABC2, ABC5), multidrug resistance transporter1, MDR1), EpCAM, CXCR4, Nestin and LRCs [28-30]. Telomerase are also applied for identification of CSCs. In the following table, some of the known markers are indicated.

Accumulating evidence supports the notion that ESC self-renewal and pluripotency genes, including the transcription factor triad OCT-3/4 (i.e. OCT-4), SOX-2, and NANOG serve as neoplastic engines driving oncogenesis [31]. It has been shown that a transcriptional profile for maintaining the self-renewal state of CSCs is more akin to that of ESC than to that of adult stem cells. Moreover, key regulators (e.g., Oct4, Sox2 and Nanog) of ESC identity and their activation targets are more frequently overexpressed in CSCs in different types of cancers [32,33]. They are also more frequently overexpressed in poorly differentiated tumors than in well differentiated tumors [34]. Thus, it appears that the key regulators in ESC may also contribute to the pathogenesis of cancers by modulating the self-renewal and differentiation of CSCs. The mechanismic functions of OCT4, NANOG, and SOX2 in CSCs are a little different from their functions in ESCs. Although they both share the property of self-renewal, ESCs emphasize differentiation, whereas CSCs emphasize proliferation. OCT4, NANOG, and SOX2 together maintain the repression of lineage-specific differentiation in hESCs.

Expression of Oct4 is restricted to pluripotent stem cells, and expression is down regulated when differentiation is initiated during embryonic development. It is undetectable in adult normal tissue. However, some recent studies have shown that Oct4 is expressed in various tumor tissues. Sox2 is another important member of the transcriptional regulatory network that regulates pluripotency and self-renewal in ESCs. Sox2 has also been found to be expressed in various tumor tissues. Like Oct4 and Sox2, Nanog has been reported to be expressed in many tumor tissues, and Nanog knockdown inhibits tumor development [35]. Overexpression of Nanog predicts tumor progression and poor prognosis in colorectal and oral cancers, indicating that Nanog is a key factor regulating human tumor development. Transfection of healthy cells with Nanog could induce cell transformation.

Molecular mechanisms that regulate stem cell self-renewal in the early embryo may be re-activated during the dysregulated proliferation observed in tumorigenesis. OCT4 is reported to maintain the survival of CSCs partly by inhibiting apoptosis through the OCT4/TCL1/ AKT1 pathway. SOX2 participates in the SOX2/ORAIL/STIM1 pathway: Store-operated Ca2+ entry (SOCE) plays an important role in a variety of physiologic and pathophysiologic processes, including apoptosis. Reduced SOCE is one of the factors that contribute to the anti-apoptotic milieu of prostate cancer. The key components of SOCE are ORAIL1 and STIM1. NANOG is a direct target of the LIF-STAT3 pathway, and it also maintains self-renewal of CSCs through the IGF1R signaling pathway. NANOG overexpression enhances the expression of many CSC-associated molecules, such as CD133, ABCG2, ALDH1A1, and CD44.

**Stem cell niches**

Stem-cell populations are established in ‘niches’ specific anatomic locations that regulate how they participate in tissue generation, maintenance and repair. The niche saves stem cells from depletion, while protecting the host from over-exuberant stem-cell proliferation. It constitutes a basic unit of tissue physiology, integrating signals that mediate the balanced response of stem cells to the needs of organisms [36]. The stem cell niches encompassing a wide range of biochemical, biophysical, and biomechanical cues play a guidance role to modulate stem cell behaviors such as adhesion, proliferation, and differentiation [37].

Several factors, such as soluble/immobilized factors, chemical and physical signals, extracellular matrix (ECM) components, growth factors, cytokines, and the physiochemical nature of the environment, are important for the regulation of stem cell characteristics within the niche [38]. Stem cell fate could be determined by cell-cell interactions between stem cells, as well as interactions between stem cells and neighboring differentiated cells, interactions between stem cells and adhesion molecules, ECM components, the oxygen tension, growth factors, cytokines, and the physicochemical nature of the environment including the pH, ionic strength and metabolites, like ATP, are also important. The stem cells and niche may induce each other during development and reciprocally signal to maintain each other during adulthood) [39].

Germ line stem cells lie within the basal cell layer of the seminiferous tubules, epithelial stem cells reside within the bulge of hair follicles, neural stem cells reside within the lateral ventricle sub ventricular zone of the central nervous system, muscle stem cells reside among satellite cells under the basal lamina of myofibers, and HSCs reside within the bone marrow, close to endostereum and/or sinusoidal blood vessels. In each case these locations have been described as stem cell niches, and the factors that regulate the maintenance of these stem cells are beginning to be identified.
Like normal stem cells reside, CSCs require a similar microenvironment, termed CSC niche, which provides appropriate signals to regulate self-renewal and the normal homeostatic processes such as inflammation, epithelial-mesenchymal transition (EMT), hypoxia and angiogenesis.

Extrinsic signals that regulate stem cell behavior originate in the stem cell microenvironment. Although there is still relatively little detailed information on the composition and function of cancer stem cell microenvironments in different malignancies, tumor growth and metastasis are highly dependent on the tumor microenvironment [40]. The CSCs are sustained in undifferentiated state by the niche, which protects them from factors stimulating differentiation. The other way to sustain stemness by the niche is to limit the proliferation rate of stem cells. The elements forming the niche adhere to stem cells with adhesion molecules and control their function by signaling molecules, such as Shh (Sonic hedgehog), BMPs (bone morphogenic proteins) and Notch.

Given that cancer cells are characteristically less dependent upon survival factors and less restrained in their expansion than normal stem cells, they are unlikely to have an obligatory dependence on niches. Nonetheless, it is conceivable that supportive niches contribute to resistance to anticancer therapies by supplying growth factors that enhance the survival of tumorigenic cancer cells during treatment. The CSC niche itself is a part of the tumor microenvironment (TME). The TME makes up the stroma of the tumor, which occupies most the tumor mass, including the extracellular matrix (ECM), mesenchymal stem cells (MSCs), endothelial cells, immune cells, and, what is more, networks of cytokines and growth factors. ECM is major non-cellular component of niche. ECM constitution within the niche plays critical roles in pathways leading in maintenance of pluripotency or differentiation [41].

MSCs play an important role in orchestrating the tumor microenvironment through angiogenesis, modulation of both immune system and tumor stromal architecture. By secreting CXCL12, IL6, and IL8, MSCs promote cancer cell stemness through upregulating NF-B while CSCs secrete IL6 to attract more MSCs [42]. MSCs also produce the antagonist, Gremlin 1, to promote the undifferentiated NF- B while CSCs secrete IL6 to attract more MSCs [42]. MSCs also produce the antagonist, Gremlin 1, to promote the undifferentiated state of the niche, which protects them from factors stimulating differentiation. The other way to sustain stemness by the niche is to limit the proliferation rate of stem cells. The elements forming the niche adhere to stem cells with adhesion molecules and control their function by signaling molecules, such as Shh (Sonic hedgehog), BMPs (bone morphogenic proteins) and Notch.

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Signal for Self-Renewal and Differentiation of Stem Cells

Although there is still much to discover about the molecular mechanisms that govern stem cell-fate decisions and self-renewal, transcriptome profiling studies have highlighted several properties believed to be common to all stem cells at the molecular level. These essential attributes of ‘stemness’ are proposed to include: (i) active Janus kinase signal transducers and activators of transcription, TGFβ and Notch signaling; (ii) the capacity to sense growth factors and interaction with the extracellular matrix via integrins; (iii) engagement in the cell cycle, either arrested in G1 or cycling; (iv) a high resistance to stress with up regulated DNA repair, protein folding, ubiquitination and detoxification systems; (v) a remodeled chromatin, acted upon by DNA helicases, DNA methylases and histone deacetylases; and (vi) translation regulated by RNA helicases of the Vasa type. Considerable similarities have been found between cancer stem cells and normal stem cells on their dependence on certain signaling pathways. More specifically, the core stem cell signaling pathways, such as the Wnt, Notch and Hedgehog pathways, also critically regulate the self-renewal and survival of cancer stem cells.

The Wnt/β-Catenin pathway regulates stem cell pluripotency and cell fate decisions during development. It integrates signals from other pathways, including FGF, TGF-β, and BMP, within different cell types and tissues [47]. The Wnt ligand is a secreted glycoprotein that binds to Frizzled receptors, leading to the formation of a larger cell surface complex with LRP5/6 [48]. In the absence of Wnt signal, β-catenin, an integral E-cadherin cell-cell adhesion adaptor protein and transcriptional co-regulator, is targeted by coordinated phosphorylation by CK1 and the APC/Axin/GSK-3β-complex leading to its ubiquitination and proteasomal degradation [49]. Activation of the Wnt receptor complex triggers displacement of the multikinase kinase GS3K-3β from a regulatory APC/Axin/GSK-3β-complex. Stabilized β-catenin is translocated to the nucleus via Rac1 and other factors, where it binds to LEF/TCF transcription factors, displacing co-repressors and recruiting additional co-activators to Wnt target genes (Figure 2). Additionally, β-catenin cooperates with several other transcription factors to regulate specific targets [50,51].

Considering the importance of the Wnt pathway in stem cell biology, it is not surprising that aberrant Wnt signaling has been implicated in the tumorigenic potential of stem cells. A typical approach to prospectively identify putative cancer stem cells is via cell surface markers; however, these are also expressed on normal somatic stem cells. Many of these markers are in fact direct Wnt target genes (including LGR5/GPR49, CD44, CD24, CD133, ABC cassette genes, and EpCAM). The expression of these so called multidrug resistance genes has been shown to also be associated with cancer stem cells and partially responsible for poor therapeutic responses. Wnt/β-catenin signaling appears to play an important role in ABCB1/MDR-1 transcription [52].
Figure 1: The molecular and cellular basis of the cross talk between CSCs and their niches [43]. CSCs are metastatic cancer cells that can self-renew. Their plasticity and dormancy correlates with their therapeutic resistance. By secreting CXCL12, IL6, and IL8, MSCs promote cancer cell stemness through upregulating NF-κB while CSCs secrete IL6 to attract more MSCs. They also produce the antagonist, Gremlin 1, to promote the undifferentiated state. CSCs and CAFs produce CXCL12 to promote angiogenesis, and hypoxia causes both CSCs and endothelial cells to produce VEGF, which further induces angiogenesis. CAFs produce TNC and HGF to enhance WNT and NOTCH signalling for CSC maintenance.

Figure 2: The canonical Wnt signalling pathway [51]. In the absence of Wnt signalling (left panel), β-catenin is in a complex with axin, APC and GSK3-β, and gets phosphorylated and targeted for degradation. In the presence of Wnt signalling (right panel), β-catenin is uncoupled from the degradation complex and translocates to the nucleus, where it binds Lef/Tcf transcription factors, thus activating target genes.
It has long been known that misexpression of Wnt ligands induces mammary adenocarcinomas [53]. A role for the Wnt signaling pathway in glioblastoma stem cells has also recently been described. Wnt ligands are up-regulated in prostate cancer, and their expression often correlates with aggressiveness and metastasis. Many colorectal cancer is caused by mutations in key components of the Wnt signaling pathway. The adenomatous polyposis coli gene is a well-known tumor suppressor that plays a central role in the Wnt signaling pathway by targeting β-catenin for degradation. Germline loss-of-function mutations in the APC gene were originally identified to be associated with familial adenomatous polyposis (FAP), about 1% of which progress to CRC. Furthermore, 85% of cases of sporadic intestinal neoplasia have mutations in APC, while activating mutations in β-catenin were found in approximately 50% of CRC tumors lacking APC mutations [54].

Notch signaling is an evolutionarily conserved pathway in multicellular organisms that regulates cell-fate determination during development and maintains adult tissue homeostasis [55]. The Notch pathway mediates juxtacrine cellular signaling. Notch receptors are single-pass transmembrane proteins composed of functional extracellular (NECD), transmembrane, and intracellular (NICD) domains (Figure 3). Notch receptors are processed in the ER and Golgi within the signal-receiving cell through cleavage and glycosylation, generating heterodimer composed of NECD noncovalently attached to the TM-NICD inserted in the membrane (S1 cleavage) [56]. The processed receptor is then endosome-transported to the plasma membrane to enable ligand binding in a manner regulated by Deltex and inhibited by NUMB. In mammalian signal-sending cells, members of the Delta-like (DLL1, DLL3, DLL4) and the Jagged (JAG1, JAG2) families serve as ligands for Notch signaling receptors. Upon ligand binding, the NECD is cleaved away (S2 cleavage) from the TM-NICD domain by TACE (TNF-α ADAM metalloprotease converting enzyme). The NECD remains bound to the ligand and this complex undergoes endocytosis/recycling within the signal-sending cell. In the signal-receiving cell, γ-secretase releases the NICD from the TM (S3 cleavage), which allows for nuclear translocation where it associates with the CSL (CBF1/Su(H)/Lag-1) transcription factor complex, resulting in subsequent activation of the canonical Notch target genes: Myc, p21, and the HES-family members.

Notch signaling pathway plays a key role in stem cell self-renewal, cell proliferation, and differentiation. Consequently, it has important developmental functions, and its aberrant activation leads to many diseases and cancers [57]. Deregulated expression of Notch proteins, ligands, and targets, including overexpression and activation of Notch, has been described in a multitude of solid tumors, including cervical, head and neck, endometrial, renal, lung, pancreatic, ovarian, prostate, esophageal, oral, hepatocellular, and gastric carcinomas; osteosarcoma; mesothelioma; melanoma; gliomas; and medulloblastomas. Dysregulation of Notch signaling has been reported in some hematological malignancies other than T-cell acute lymphoblastic leukemia [58]. These include Hodgkin lymphomas, anaplastic large-cell non-Hodgkin lymphomas, some acute myeloid leukemias, B-cell chronic lymphoid leukemias, and multiple myeloma. In most cases, inappropriate activation of Notch signaling is oncogenic. In some cases, however, loss of function of Notch-1 has oncogenic effects. This has been demonstrated in the epidermis and, more recently, in a subset of head and neck squamous carcinomas. Notch signaling is essential to the orderly differentiation of squamous epithelia, and loss of Notch-1 causes loss of barrier in such epithelia. This in turn triggers an inflammatory response and cytokine cascade that may favor transformation.

The evolutionarily conserved Hedgehog (Hh) pathway is essential for normal embryonic development and plays critical roles in adult tissue maintenance, renewal and regeneration. Proper levels of Hh signaling require the regulated production, processing, secretion...
and trafficking of Hh ligands—mammals this includes Sonic (Shh), Indian (Ihh) and Desert (Dhh). Hh ligands are released from the cell surface through the combined actions of Dispatched and Scube2, and subsequently trafficked over multiple cells through interactions with the cell surface proteins LRP2 and the Glycophan family of heparan sulfate proteoglycans (GPC1-6) [59].

Hh proteins initiate signaling through binding to the canonical receptor Patched (PTCH1) and to the co-receptors GAS1, CDON and BOC. Hh binding to PTCH1 results in derepression of the GPCR-like protein Smoothened (SMO) that results in SMO accumulation in cilia and phosphorylation of its cytoplasmic tail. SMO mediates downstream signal transduction that includes dissociation of GLI proteins (the transcriptional effectors of the Hh pathway) from kinesin-family protein, Kif7, and the key intracellular Hh pathway regulator SUFU. In response to activation of Hh signaling, GLI proteins are differentially phosphorylated and processed into transcriptional activators that induce expression of Hh target genes [60].

Because hedgehog (Hh) signaling regulates progenitor cell fate in normal development and homeostasis, aberrant pathway activation might be involved in the maintenance of such a population in cancer [61]. Abnormal Hh signaling has been associated with diverse human malignancies including basal cell carcinoma, medulloblastoma, pancreatic and lung cancer. Interestingly, data suggest different mechanisms of action in the various tumor environments. Constitutive pathway activation through loss-of-function mutations epigenetic modifications or reduced expression of the negative regulators PTCH, HHIP, and SPUT3 or gain-of-function mutations and epigenetic changes in the positive regulator SMO have been observed in several solid malignancies. To date, no mutations have been identified in hematological malignancies; however, epigenetic modifications have been observed in a cohort of pediatric AML patients, correlating with disease status [62].

The major mechanisms by which the Hh pathway is aberrantly activated in cancer can be attributed to mutations of Hh pathway constituents (Type I: ligand-independent), excessive expression of Hh pathway ligands (Type II-IIIb: ligand-dependent) and the generation of a cancer stem cell (CSC) phenotype (Type IV). Type I: ligand-independent, tumor cell-intrinsic signaling tumors exhibit mutations in the Hh pathway components that promote cell-intrinsic growth and survival. Type II: ligand-dependent, autocrine stimulation is characterized by the response to the Hh ligand that is self-secreted. Type III: ligand-dependent, paracrine signaling is defined by the secretion of the Hh ligand from the tumor cells that acts on adjacent stroma, in turn creating a favorable microenvironment for tumor growth. In contrast, in Type IIIb ligand-dependent, reverse paracrine signaling, the Hh ligand is secreted by the adjacent stroma and acts on the tumor cells [63].

Cancer Stem Cells Role in Cancer

Cancer stem cells differ considerably from most cells of the tumor mass. It is assumed that the unlimited growth capacity of the tumor as well as the capability to develop metastases rest on the CSC population. Cancer stem cells divide relatively slowly and are essentially drug resistant, two properties which make them refractory to conventional chemotherapy. CSCs promote blood vessel formation; and they prompt cell motility. The acceptance of the CSC concept therefore demands re-evaluation and potentially re-direction of cancer therapies: instead of trying solely to reduce the tumor mass, the CSC subset should be specifically targeted.

Two distinct models, the clonal evolution model and the CSC model, have been proposed to explain tumor origin and tumor cell heterogeneity. According to the clonal evolution model, a normal cell becomes neoplastic due to an irreversible genetic change or a hereditary epigenetic change and gives rise to a clone of neoplastic cells. The clones accumulate further genetic changes and evolve into new clones; selective pressure favors one or more of these clones and ultimately leads to cancer and its inherent tumor cell heterogeneity. According to this idea, tumor initiation takes place once multiple mutations occur in a random single cell, providing it with a selective growth advantage over adjacent normal cells. As the tumor progresses, genetic instability and uncontrolled proliferation allow the production of cells with additional mutations and hence new characteristics. These cells may leave many offspring by chance, or the new mutations may provide a growth advantage over other tumor cells such as resistance to apoptosis. In either case, primarily the latter, new subpopulations of variant cells are born, and other subpopulations may contract, resulting in tumor heterogeneity. Through this process, which occurs throughout the lifetime of a tumor, any cancer cell can potentially become invasive and cause metastasis or become resistant to therapies and cause recurrence [64]. The CSC hypothesis posits that tumor cells are organized in a hierarchy and that only cells that reside at the apex of the hierarchy can regenerate the tumor when implanted into immunocompromised mice and in so doing recapitulate the heterogeneity of the original patient tumor. Furthermore, according to the CSC hypothesis only a small subset of cancer cells has the enriched ability to proliferate extensively and form tumors. The heterogeneity and hierarchy between all the cells within a tumor result from asymmetric division of CSCs. As result of aberrant signaling pathways, cancer stem cells acquire its unique ability to initiate carcinoma and promote recurrence after surgery.

Metastasis and systemic tumor dissemination from primary tumors are the most detrimental events that occur during cancer progression, accounts for over 90% of lethality in cancer patients. It has been hypothesized that a small subpopulation of cancer cells, namely metastasis-initiating cells (MICs), might exist, although these cells have not yet been prospectively identified. Multiple lines of recent evidence strongly suggest that MICs might exist within small subpopulations of CSCs inside of tumors. First, CSCs possess a high tumor-initiating capacity, which is an essential characteristic that enables the formation of new tumors (secondary and tertiary foci) beyond the point at which the original tumor formed. Second, CSCs express epithelial-mesenchymal transition (EMT) markers, which are associated with the ability of tumor cells to migrate into other tissues or organs. More specifically, some studies have suggested that potential MICs might be present within small CSC populations, for example, CD44+/CD24−/low breast cancer cells with stem cell-like properties have been proposed to exhibit enhanced tumorigenic and metastatic properties in tumor xenograft models.

Importantly, recent studies have established a crucial link between passage through EMT and the acquisition of molecular and functional properties of stem cells. Thus, in addition to bestowing migratory and invasive potential, induction of EMT in immortalized and transformed human mammary epithelial cells significantly enhanced their self-renewal and tumor-initiating capabilities and led to the expression of stem-cell markers, typically associated with breast CSCs [65].

Tumor-induced angiogenesis and lymphangiogenesis play an important role in promoting tumor growth and metastasis [66]. Various lymphatic growth factors and vascular growth factors participate in regulating tumor-induced angiogenesis and lymphangiogenesis.
Most of these factors are shown to have dual effects and interact with each other during angiogenesis and lymphangiogenesis making distinguishing difficult. CSCs show greater potential for angiogenesis and lymphangiogenesis than non-stem cell-like tumor cells [67]. A contemporary study has demonstrated that stem cell-like glioma cells (SCLGCs) enhance glioma angiogenesis through a mechanism that uses vascular endothelial growth factor (VEGF). In this study, SCLGCs was found to form more vascular tumors and expressed higher levels of VEGF in immunocompromised mice than non-SCLGCs [68]. In addition, cancer stem cells have also been found to be responsible for vasculogenic mimicry in human melanoma, whereby the process of de novo blood vessel formation is mimicked by the tumor to support tumor growth [69].

Evasion of apoptosis is a hallmark of most, if not all cancers, because defects in its regulators invariably accompany tumourigenesis and sustain malignant progression. Apoptotic signaling pathways, including extrinsic and intrinsic pathways, are also deregulated in CSCs [70]. cFLIP, a negative modulator of death receptor-induced apoptosis, are upregulated in CD133+ glioblastoma, breast cancer, and T-cell acute leukemia cells. Survivin is enriched in hematopoietic stem cells, neuronal precursor cells, CD34 (+)/38(-) AML stem cells and glioblastoma and astrocytoma CSCs. Other IAP proteins upregulated in CSCs include XIAP, c-IAP1, and Livin. Dysregulation of the intrinsic pathway in CSCs is mainly reflected in Bcl-2 family proteins and the DNA damage response. In most tumors, anti-apoptotic Bcl-2 family proteins are overexpressed in CSCs. For instance, CD133+ glioma CSCs express a high level of anti-apoptotic proteins Bcl-2 and Bcl-XL. Moreover, overexpression of OCT4, NANOG, and SOX2 in CSCs modulates signaling pathways to inhibit apoptosis.

Another way that cancer stem cells might contribute to the initiation and maintenance of tumor growth and disease progression is through evading and modulating the immune system. In general, immunocompromised human patients have been described to have a significantly higher risk of developing cancer [71]. CSCs possess similar features to normal stem cells in their ability of inducing immune modulation. Unfortunately, possession of these features by CSCs contributes to their escape from the immune system recognition and thus failure of the treatment and tumor relapse. Therefore, there is growing interest in understanding the mechanisms that regulate CSC immune modulatory properties to develop more effective therapy that can eradicate these cells. There are many signs that tumors in general show signs of immune tolerance [72].

The different mechanisms that are developed by tumor cells are a defect of expression of antigens on the tumor cell surface, attract CD4+ or CD8+ FOXP3+ regulatory T-cells, expression of B7-H1, lack of co-stimulatory molecules like CD80 and CD86 (positive co-stimulatory molecules that are required for optimal T-cell activation) and occasional lack of MHc class I molecules. Immune inhibitory molecules like B7-H1 is expressed on tumor cells (tumor infiltrating lymphocytes) and FOXP3+ regulatory T-cells are abundant in the tumor microenvironment in a group of breast cancer patients. Besides, CSCs produce immunosuppressive molecules such as TGF-β, PG E2 and adenosine, or of cytokines such as IL-6 and IL-10, the resistance to apoptosis, and/or the expression of Fas ligand (FasL), which leads to the death of tumor-infiltrating lymphocytes [73].

Moreover, tumor cells recruit macrophages called TAMs by secreting CSF-1, the chemokine ligand 2, 3, 4, 5, and 8 and VEGF. TAMs constitute the major inflammatory component of tumor microenvironment. Their functions within the tumor site are various and sometimes paradoxical. Indeed, according to the environmental stimuli, macrophages present two different phenotypes. Macrophages of the M1 phenotype kill pathogens and promote the activation of cytotoxic CD8+ T cells and the differentiation of naive CD4+ T cells into Th1 effector cells and Th17 cells. M2 macrophages stimulate CD4+ Th2 cells and regulatory T cell differentiation and can promote angiogenesis and tissue remodeling. Multiple studies have shown a correlation between many macrophages in the tumor microenvironment and a worse prognosis. TAMs, therefore, exercise different protumor functions associated with the M2 phenotype. During tumor initiation, TAMs create a favorable environment for tumor growth by secreting EGF, PDGE, TGF-β, IL-6, IL-1, and TNF-α and induce immunosuppression (via TGF-β, PGE2, and IL-10).

CSCs would be largely unaffected by standard therapies, because of their stem cell characteristics. Instead, they would survive and continue to divide, which would lead to the reappearance of the tumor with time. This could be compared with the effects of chemotherapy on other tissues, like the hair: the differentiated cells that form most of the hair are killed during treatment, but the stem cells responsible for hair growth survive. When the therapy is over, hair re-grows normally. CSCs have been found to exhibit several genetic and cellular adaptations that confer resistance to classical therapeutic approaches. These include relative dormancy/slow cell cycle kinetics, efficient DNA repair, high expression of multidrug-resistance-type membrane transporters, and resistance to apoptosis (Figure 4) [74].

Activation of different signaling pathways such as Notch, Wnt/β-catenin, TGF-β and Hedgehog has been reported in the attribution of therapy resistance of CSCs during or after treatment. Many studies have demonstrated that chemical intervention or downregulation of these signaling pathways increased the therapy sensitivity of CSCs in various cancers (Table 1). CD133+ glioblastoma stem cells increase the expression of the genes involved in Notch and Hedgehog pathways, making the glioblastoma insensitive to chemotherapy (temozolomide) [75]. Inhibition of components of these pathways with gamma-secretase inhibitors (Notch pathways) and cyclopamine (Hedgehog pathways) increased the sensitivity of CSCs to the treatment. Aberrant Wnt signaling pathways have been found to be involved in pathogenesis in different cancers and their resistance to chemoradiation therapies. Genetic inactivation or pharmacologic modulation of β-catenin (a target of Wnt pathway) remarkably increased the sensitivity of hematopoietic stem cells to a chemotherapy drug imatinib [76]. In addition, other pathways like PI3K/Akt/mTOR and JAK/STAT, also contribute to the therapy resistant properties of CSCs. Thus, CSCs can be regulated by the modulation of different genetic pathways and this in turn contributes to the therapeutic resistance of CSCs.

**Approaches in Targeting Cancer Stem Cells**

Current therapeutic strategies against cancer have severe limitations that frequently lead to treatment failure. A common cause of treatment failure in multiple malignancies is resistance to chemotherapy and radiotherapy. In addition, many strategies that are not sufficiently selective against CSCs can be toxic to healthy tissues, and patients usually face the risk of recurrence and metastasis because most therapies cannot eliminate CSCs. Accumulated evidence has established that CSC populations are more resistant to conventional cancer therapies than non-CSC populations. Therefore, the elimination of CSCs is crucial in treating malignant diseases. Currently, many different therapeutic approaches are being tested for prevention and treatment of cancer recurrence. These
CSCs and tumorigenicity. mAbs against the Wnt cascade have shown as inhibitors of Wnt signaling, which are involved in the regulation of cancer [77,78]. Several pharmaceuticals are under investigation such as inhibitors of Notch1 can significantly reduce the CD44+CD24− subpopulation and lower the incidence of brain metastases of breast cancer [79]. Fortunately, it seems that CSCs have their own specific enhanced signaling pathways such as Notch, BMI1 and Wnt. However, a series of signaling pathways are common between stem cells and CSCs which makes it difficult to target CSCs without affecting normal stem cells. Some therapeutic strategies can successfully kill CSCs, while others are still under preclinical and clinical evaluation.

CSCs may possibly be eradicated by targeting treatment against signaling pathways such as Notch, BMI1 and Wnt. However, a series of signaling pathways are common between stem cells and CSCs which makes it difficult to target CSCs without affecting normal stem cells. Fortunately, it seems that CSCs have their own specific enhanced signaling pathway. Targeting these pathways has been proposed to overcome drug resistance in several ongoing clinical trials. For instance, inhibition of Notch1 can significantly reduce the CD44+CD24− subpopulation and lower the incidence of brain metastases of breast cancer [77,78]. Several pharmaceuticals are under investigation such as inhibitors of Wnt signaling, which are involved in the regulation of CSCs and tumorigenicity. mAbs against the Wnt cascade have shown promise in the treatment of colorectal cancer. Several groups have exploited cycloamine, an SMO signaling element inhibitor, to block the Hedgehog cascade and inhibit the growth, invasion and metastasis of many malignancies both in vitro and in vivo [79,80].

There is solid evidence that OCT4, NANOG, and SOX2 can contribute to cancer treatment. Further work should focus on functional analysis to define the roles of these transcription factors in determining the CSC phenotypes, revealing the precise regulatory mechanisms and identifying new components of the transcriptional regulatory networks that may be relevant to tumor transformation, tumorigenesis, and metastasis. Tumors may be controlled by restricting the expression of OCT4, NANOG, and SOX2 or by disrupting the molecular pathways that are altered in CSCs.

The tumor microenvironment can create a niche to nurse and protect CSCs from drug-induced apoptosis. Considering the significant role of CSCs niches, apart from affecting CSCs directly, targeting CSCs niche factors may also prove to be a powerful modality for the treatment and prevention of tumor progression. Some attempts to target niches have already shown promising results. For example, investigations have indicated that tumor angiogenesis can be related to CSCs survival and drug resistance, and CSCs in the vascular niche establish an autocrine loop in which VEGF promotes CSCs activity by governing both microvasculature formation and intrinsic self-renewal pathways. Targeting VEGF with inhibitors or antibodies can lead to the normalization of tumor vasculature, disruption of the CSCs niche and inhibition of tumor growth. Targeting tumor hypoxia is another attempt to manipulate the niche of quiescent, drug-resistant cells. HIF-1α and HIF-2α, which promote cell cycle progression via c-Myc, represent a promising therapeutic target for glioma patients. Targeting cells within the tumor-associated stroma (e.g. myofibroblasts and tumor-associated macrophages) which are likely to play a prominent role in controlling CSCs homeostasis in many types of tumors also presents an alternative strategy.

Elevated expression of ABCG2 has been observed in several putative CSCs from retinoblastoma, lung, liver and pancreas cancer. In addition, ABCG2 and CD133, the widely identified CSC marker, are co-expressed in melanoma and pancreatic carcinoma cell lines. Recent report showed ABCG2+ cells could be purified from human hepatocellular carcinoma cell lines [81]. CSCs are protected against external toxic agents by the high expression of ABC transporter proteins. Recent report showed ABCG2+ cells could be purified from human hepatocellular carcinoma cell lines [81]. CSCs are protected against external toxic agents by the high expression of ABC transporter proteins. Recent report showed ABCG2+ cells could be purified from human hepatocellular carcinoma cell lines [81]. CSCs are protected against external toxic agents by the high expression of ABC transporter proteins. Recent report showed ABCG2+ cells could be purified from human hepatocellular carcinoma cell lines [81]. 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Inhibition of the ABCG2 transporter using low molecular weight inhibitors, including fumitremorgin C and
tryprostatin A, has been investigated as one of the effective strategies to sensitize and kill CSCs. Drug resistance of CSCs has also been associated with over-expression of other drug efflux transporters. For instance, P-glycoprotein (Pgp), also designated as ABCB1, possesses broad substrate specificity and is currently considered as the main negative factor in cancer therapy of leukaemias and solid tumors. Use of a monoclonal antibody (mAb) against ABCB5 was shown to sensitize melanoma cells to the anticancer drug doxorubicin, highlighting the role of efflux pumps in drug resistance.

**Future Perspective**

Despite the recent explosion of interest in CSCs, experimental studies have not been translated into improved survival outcomes for cancer patients. This presents a major question to the field: do cancer stem cells have meaningful relevance beyond the experimental systems in which they have been defined? Increasing evidence is pointing to CSCs having unique biologic properties (dormancy, drug/radiation resistance) that could permit them to survive therapies leading to eventual relapse. To date, CSCs have been studied in a relatively small number of patient samples and cancer types, so it is not known whether the CSC model is universal to all human cancers [82,83]. Major challenges in the CSC field still lie ahead. There is need to discover more specific markers and understand their physiological roles to better define the transition from pluripotency to various stages of tissue commitment and apply this knowledge to novel therapeutic targeting strategies.

The biologic relevance of CSCs in human cancer will be established by concentrating on the following research endeavors: improving the assay and purification of CSC and non-CSC subsets, carrying out detailed genomic or proteomic analysis on these subsets to identify CSC-specific signatures, and obtaining such signatures from a large number and wide range of tumors. Such an approach would make it possible to determine whether the "omics" of CSCs provide more predictive or prognostic relevance compared with analysis of the bulk tumor.

In the future, the field of CSC research will certainly be in the spotlight. Eliminating cancer cells with the potential for self-renewal and tumor propagation should be the target of cancer drug development. It is also important to discriminate CSCs from normal stem cells in cancer treatment, which will require the identification of drug targets unique to CSCs.

**References**


