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Stem Cells and Human Diseases

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Preface

The main objective of this book is to provide a comprehensive review on stem cells and their role in tissue regeneration, homeostasis and therapy. In addition, the role of cancer stem cells in cancer initiation, progression and drug resistance are discussed. The cell signaling pathways and microRNA regulating stem cell self-renewal, tissue homeostasis and drug resistance are also mentioned. An increased understanding of stem cell behavior and biology along with rapid advancement of high throughput screening has led to the discovery and development of novel drugs that control stem cell self-renewal and differentiation. In near future, these molecules will be very useful for treating and preventing several human diseases.

The authors represent a diverse group of experts who have endeavored to provide a historical perspective on the generation of stem cells and their roles in tissue regeneration, therapy and disease initiation and progression, to allow clinicians to assimilate these facts into their treatment algorithms. For this purpose we have considered both normal and malignant stem cells. While progress on the clinical front has been slower than desired, the use of stem cells for tissue regeneration and disease management has great potential in human health. Overall, these reviews will provide a new understanding of the influence of stem cells in tissue regeneration, disease regulation, therapy and drug resistance in several human diseases.

We greatly appreciate the exceptional contributions of the authors, each of which reflects their commitment to the field of stem cells and human diseases.

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Chapter 1
Cancer Stem Cells: Biology, Perspectives and Therapeutic Implications

Brahma N. Singh, Sharmila Shankar, and Rakesh K. Srivastava

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Abstract Cancer stem cells (CSCs) biology has come of age. The CSC theory is currently central to the field of cancer research, because it is not only a matter of academic interest but also crucial for the cancer therapy and prevention. Most cancers comprise of a heterogenous population of CSCs with marked differences in their proliferative potential as well as the ability to reconstitute the tumor upon transplantation. CSCs share a variety of biological properties with normal somatic stem cells in terms of self-renewal, the expression of specific cell surface markers and the utilization of common signaling pathways. Perhaps the most important and useful characteristic of CSCs is that of self-renewal. Through these properties,

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striking parallels can be found between CSCs and stem cells: tumors may often originate from the transformation of normal stem cells. This review will have significant ramifications for the biological basis and the therapeutic implications of the stem cell. In addition, dysregulation of CSC self-renewal is a likely requirement for the development of human cancers. Understanding the properties of, and exploring self-renewal, cell surface markers and signaling pathways specific to CSCs of different cancers, will lead to progress in therapy, intervention, and improvement of the prognosis of patients. In the near future, the evaluation of CSCs may be a routine part of practical diagnostic pathology.

Keywords Cancer stem cells • Stem cells • Notch • Sonic hedgehog • Wnt • Breasts cancer stem cells • Brain cancer stem cells • Prostate cancer stem cells • Pancreatic cancer stem cells • Progenitor cells

1.1 Introduction

Recent *in vitro* and *in vivo* research evidences have demonstrated that in hematologic and solid malignancies only a minority of cancer cells have the capacity to proliferate extensively and form new malignancies [3, 28, 86, 95]. These cancer stem cells (CSCs)/tumor-initiating cells (TICs) have been recognized and enriched on the basis of their expression of cell-surface markers (CSMs). Upon transplantation, TICs give rise to tumors comprising both new TICs as well as heterogeneous populations of non-tumorigenic cells reminiscent of the developmental hierarchy in the tissues from which the tumors arise. Most adult tissues are maintained over the lifetime of the host by somatic/or normal stem cells (SCs) that undergo expansion and differentiation to yield the functional elements of the organ [33]. Through self-renewal process, SCs are able to function over the lifespan of the host.

SCs are a class of undifferentiated cells that have the potential to perpetuate themselves through self-renewal and to generate mature cells of a specific tissue through differentiation. Commonly, SCs come from two main sources: embryos formed during the blastocyst phase of embryological development and adult tissue. Both types are generally characterized by their potency, or potential to differentiate into different cell types (such as skin, muscle, bone, etc.). In most tissues, SCs are rare. Although, it appears reasonable to propose that each tissue arises from a tissue-specific SC, the difficult identification, purification and isolation of these somatic SCs has been accomplished only in a few instances [12]. The genetic constraints on self-renewal restrict the expansion of SCs in normal tissues. Breakdowns in the guideline of self-renewal are likely a key event in the development of human malignancy as demonstrated by the fact that several cellular signaling pathways implicated in carcinogenesis. It also play a key role in normal SC self-renewal decisions. Thus, malignant tumors can be viewed as an abnormal organ in which a minority population of tumorigenic cancer cells have escaped the normal constraints on

self-renewal giving rise to abnormally differentiated cancer cells that have lost the potential to form tumors. This new model for cancer has important implications for the study and treatment of tumors [12]. Not only is ruling the source of cancer cells essential for successful treatments, but if current treatments of cancer do not properly finish enough CSCs, the tumor will reappear. Including the possibility that the treatment of for instance, chemotherapy will consent only chemotherapy-resistant CSCs, then the ensuing tumor will most likely also be resistant to chemotherapy. If the cancer tumor is detected early enough, enough of the tumor can be killed off and marginalized with customary treatment. But as the tumor size increases, it becomes more and more difficult to remove the tumor without conferring resistance and leaving enough behind for the tumor to regenerate.

Some treatments with chemotherapeutic agent such as paclitaxel in ovarian cancer (a cancer usually discovered in late stages), may actually induce chemoresistance (55–75% relapse <2 years) [21]. It potentially does this by destroying only the cancer cells susceptible to the drug by targeting those that are $CD44^+$ positive, and allowing the cells which are unaffected by paclitaxel ($CD44^+$ negative) to regrow, even after a reduction in over a third of the total tumor size. There are studies, though, which show how paclitaxel can be used in combination with other ligands to affect the $CD44^+$ positive cells [7]. While paclitaxel alone, as of late, does not cure the cancer, it is effective at extending the survival time of the patients [21].

The recent discoveries that these are bone marrow [15, 65, 75], and purified CSCs such as hematopoietic stem cell (HSCs) [57, 58], can give rise to non-haematopoietic tissues suggests that these cells may have greater differentiation potential than was assumed previously. Conclusive experiments are required to determine whether the cells from the bone marrow that are capable of giving rise to different non-haematopoietic lineages are indeed HSCs or another population. If further studies support the idea of CSCs plasticity, this will undoubtedly open new frontiers for understanding the developmental potential of CSCs, as well as expand their therapeutic application.

1.2 Self-renewal and Cancer

Self-renewal is a cell division in which one or both of the resulting daughter cells remain undifferentiated and retain the ability to give rise to another SC with the same capability to proliferate as the parental cell [23, 26]. Self-renewal is crucial to SC function, because it is required by many types of SCs to persist for the lifetime of the animal. Moreover, whereas SCs isolated from different organs may differ in their progressive potential, all the SCs must have to self-renew and regulate the relative balance between self-renewal and differentiation [24]. Propagation (unlike self-renewal), does not require either daughter cell to be a SC nor to retain the ability to give rise to a differentiated progeny [25]. The dedicated progenitor cell is destined to stop multiplying as with each cell division its potential to proliferate decreases.

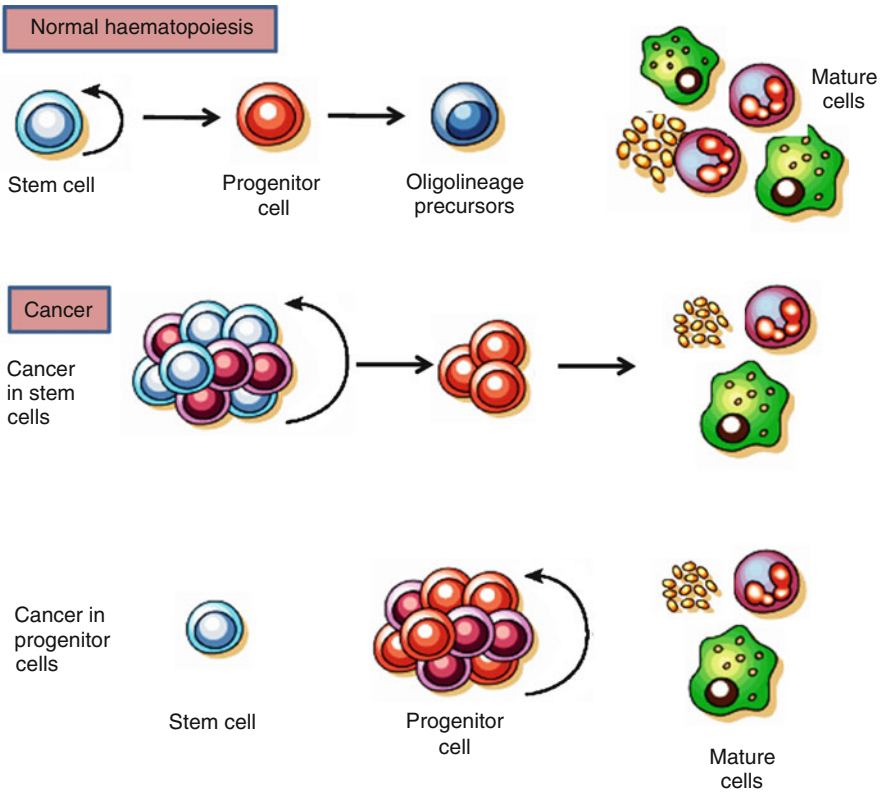

Fig. 1.1 Self-renewal during haematopoietic stem cell development and leukaemic transformation. Normal haematopoiesis, where signalling pathways that have been proposed to regulate selfrenewal are tightly regulated (*top*), during transformation of stem cells, the same mechanisms may be dysregulated to allow uncontrolled self-renewal (*middle*). Furthermore, if the transformation event occurs in progenitor cells, it must endow the progenitor cell with the self-renewal properties of a stem cell, because these progenitors would otherwise differentiate (*bottom*)

In the blood, both SCs and dedicated progenitor cells have an wide capacity to proliferate [19]. The normal SC self-renewal regulation is also fundamental to understanding the regulation of cancer cell proliferation, since cancer can be considered to be a disease of unregulated self-renewal. Although, up to 6–8 weeks devoted ancestor populations can maintain hematopoiesis [2, 8]. For example, a single HSC can restore the blood system for the life of the animal (Fig. 1.1). This incredible potential is a direct outcome of its capacity to self-renew.

Most tumors develop over a period of months to years and like normal tissues consist of heterogeneous populations of cells. The unregulated growth of tumors attributed to the serial acquisition of genetic events that resulted in the turning on genes promoting proliferation, silencing of genes involved in inhibiting proliferation, and avoiding of genes involved in programmed cell death. Another key event in tumorigenesis is the interruption of genes involved in the regulation of SC self-renewal.

Thus, some of the cancer cells within a tumor share with somatic SC the capacity to replicate without losing the potential to proliferate. Signaling pathways such as Bmi-1, Notch, Wnt and Sonic hedgehog (Shh) that have been identified to be involved in regulation of self-renewal in normal SC [6, 9, 94]. Recently, Reya et al. [81] demonstrated the requirement of normal HSC self-renewal decisions on Wnt-signaling through the canonical pathway. The capacity of purified Wnt3a to permit the *in vitro* expansion of transplantable HSCs has been observed [4, 49, 104].

The Polycomb and trithorax groups are parts of multimeric complexes that interact with chromatin leading to either a repressed or activated state of gene expression, respectively. Bmi-1, a member of the Polycomb group, targets the INK4a locus and overexpression of Bmi-1 consequences in inhibition of both p16 and p19Arf expression [47]. Post-natal mice deficient in the expression of Bmi-1 display failure of hematopoiesis and fetal liver and bone marrow SCs from Bmi-1 mice are able to contribute to recipient hematopoiesis only transiently indicating a primary defect in adult HSC self-renewal [61, 71, 99]. Bmi-1 also plays a key role in malignant hematopoiesis as HOXA9/MEIS 1 induced murine leukemia [61]. The importance of epigenetic events, such as modification of chromatin, in normal and malignant tissues is likely to remain a key focus of research on self-renewal property of SCs. Preliminary studies have examined the ability to reverse these epigenetic alterations through the transfer of nuclei from cells in a differentiated tissue into enucleated oocytes. Nuclei obtained from medulloblastoma tumor cells arising in Ptc1 heterozygous mice were transferred into enucleated oocytes [62]. Blastocysts derived from medulloblastoma were morphologically indistinguishable from those derived from control spleen cell nuclei without evidence of the uncontrolled proliferation. This study suggests that epigenetic reprogramming was responsible for the loss of the tumor cells' capacity to form tumors.

1.3 Cellular Origin of CSCs

The term 'CSC is an operational term defined as a cancer cell that has the potential to self-renew giving rise to another malignant SC as well as undergo differentiation to give rise to the phenotypically diverse non-tumorigenic. In foregoing years, the cell-of-origin for cancer SCs remains unclear: they may or may not be derived from their somatic cell counterpart. Recent evidence strongly favors a progenitor cell origin for many types of leukemic SCs in addition to the SC origin [64]. In solid tumors too, it is most likely that not only somatic SCs but also differentiating progenitor cells are capable of becoming CSCs [107].

The fact that multiple mutations are necessary for a cell to become cancerous [56] has implications for the cellular origin of cancer cells. As both progenitor cells and mature cells have a very restricted lifespan, it is unlikely that all of the mutations could occur during the life of these relatively short-lived cells. In addition, to maintain the disease, cancer cells must overcome the tight genomic constraints on both self-renewal as well as proliferation [66]. Because cancer SCs must possess the

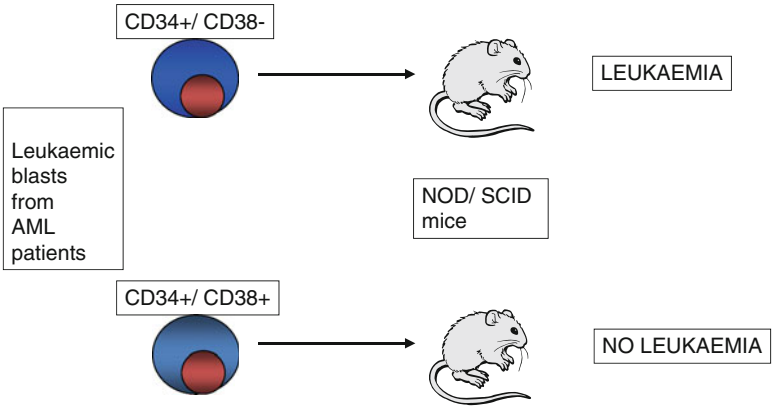

Fig. 1.2 Leukaemia stem cells exist in human acute myeloid leukaemia (AML). The cells capable of initiating human AML in NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mice have a $CD34^{+}CD38^{-}$ phenotype in most AML subtypes, and thus have a phenotype similar to normal HSCs

ability to self-renew, it follows that they are derived either from self-renewing normal SCs—which could be transformed by altering only proliferative pathways—or from progenitor cells that have acquired the potential to self-renew as a result of oncogenic mutations. In case of most cancers, the target cell of transforming mutations is unknown; nevertheless, there is considerable evidence that certain types of leukaemia arise from mutations that accumulate in HSCs. The cells capable of initiating human acute myeloid leukaemia (AML) in NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mice have a $CD34^{+}CD38^{-}$ phenotype in most AML subtypes, and thus have a phenotype similar to normal HSCs (Fig. 1.2) [14].

Feldman and Feldman [35] proposed a model of oncogene-induced plasticity for CSC origin by demonstrating reprogramming events triggered by a specific combination of oncogenes. [63] suggested that genomic instability is a driving force for transforming normal SCs to CSCs. In CSCs, a potential mechanism for cancer cell heterogeneity. A common phenotype for the LICs has been identified [10, 13, 14, 52]. Although the phenotype of the LIC is much related to that of the normal HSC, there are differences, including the differential expression of Thy1 and IL3 receptor a chain [13, 72]. These differences suggest that early mutations occurred in the HSCs and the final transforming events either alter the phenotype of the SCs or occur in early downstream progenitors.

A model of CML was reported recently in which the expression of the fusion product was targeted to myeloid/monocyitc progenitor cells using the hMRP-8 promoter. A subset of the hMRP8p210BCR/ABL mice develops a CML-like disease with elevated white cell counts and splenomegaly [48]. When crossed with hMRP-8bcl-2 mice, a proportion of the mutant mice developed a disease resembling AML. One explanation for this finding was that targeting the expression of the fusion protein to the committed progenitor instills in this population the capacity for

self-renewal. Additional studies examining the ability of purified hMRP8p210BCR/ABL progenitors to reconstitute the disease upon transplantation into primary as well as secondary recipients may be helpful to distinguish between these two possibilities. As with AML, the phenotype of breast cancer TICs may be similar to that of normal breast epithelial stem or progenitor cells because early multipotent epithelial cells have been reported to exhibit a similar phenotype to that seen in the tumorigenic breast cancer cells [1, 38, 91].

1.4 Identifying Characteristic Cell Surface Markers

Although functions have yet to be determined for many of these early surface markers, their unique expression pattern and timing provide a useful tool for scientists to initially identify and isolate SCs from the source. These are CD34 in several kinds of leukemia, CD44 in pancreas, prostate, breast, colorectal, head/neck cancers and some bone sarcomas, to detect and isolate CSCs from among the uncountable cancer cells and stromal cells occupying the entire tumor tissue. Representative cell surface markers for human hematologic and solid cancers reported to date are listed in Table 1.1.

The cell surface sialomucin CD34 has been a focus of interest ever since it was found expressed on a small fraction of human bone marrow cells [22]. The $CD34^+$ -enriched cell population from marrow or mobilized peripheral blood appears responsible for most of the hematopoietic activity [22]. CD34 has therefore been considered to be the most critical marker for HSCs. CD34 expression on primitive cells is down-regulated as they differentiate into mature cells [92]. It is also found on clonogenic progenitors, however, and some lineage-committed cells. In contrast to the high endothelial venules for which CD34 serves as a ligand for l-selectin, CD34 is not the ligand for l-selectin in hematopoietic stem/progenitor cells and ligands for hematopoietic CD34 remain to be identified [59]. Few lung CSC markers have been identified to date. Two recent reports suggest that CD133, together with the pan-epithelial marker EpCAM, can be used to isolate human lung CSCs.

CD44, originally described as a leukocyte-homing receptor, includes a family of glycoproteins encoded by a single gene, which vary in size due to alternative splicing CD44 has been used as a CSC marker for leukemia and for a variety of solid cancers as described above. CD133, a 120 kDa, glycosylated protein containing five transmembrane domains, identified initially by the AC133 monoclonal Ab, which recognizes a $CD34^+$ subset of human HSCs [108]. CD133 is a specific marker of CSCs in a wide spectrum of malignant tumors including brain tumors, colorectal, pancreatic, breast, prostate, ovarian cancers [55], and some lung cancers. CD133 was first reported as a novel marker for human hematopoietic stem and progenitor cells [108]. Recent studies have offered evidence that CD133 expression is not limited to primitive blood cells, but defines unique cell populations in non-hematopoietic tissues as well. CD133+ progenitor cells from peripheral blood can be induced to differentiate into endothelial cells in vitro [93]. In addition, human neural SCs can

Table 1.1 Specific cell surface markers for human CSCs

S. no.	Type of cancer	Cell surface markers	Reference
1.	Pancreatic	$CD133^+$, $CD44^+$, $CD24^+$, Lgr5	[22, 55]
2.	Prostatic	$CD44^+$, integrin	[55, 59]
3.	Breast	$CD44^+$, $CD24^{-/low}$	[55, 106]
4.	Ovarian	$CD44^+$, $MyD88^+$	[55]
5.	Colon	$CD133^+$, $CD44^+$, $CD166^+$, $E\text{-}CAM^{high}$, Lgr5	[55, 101]
6.	AML	$CD34^+$, $CD38^-$	[43]
7.	Myeloproliferative disorder	$CD117^+$	[55]
8.	Glioblastoma	$CD133^+$, Nestin, $CD15^+$	[50]
9.	Medulloblastoma	$CD133^+$	[55]
10.	Hepatocellular cancer	$CD133^+$	[55]
11.	Head and neck squamous cell carcinoma	$CD44^+$	[102]
12.	Metastatic melanoma	$CD20^+$	[43]
13.	Bone sarcomas	Stro-1^+, $CD105^+$, $CD44^+$	[55]
14.	Lung	$CD133^+$	[108]

be directly isolated by using an anti-CD133 Ab [102]. The aldehyde dehydrogenase (ALDH) superfamily denotes a divergently related group of enzymes that metabolize a wide variety of endogenous and exogenous aldehydes, At least 17 functional genes and 3 pseudogenes have been identified in human genomes [90]. ALDH also contributes to the oxidation of retinol to retinoic acid, a modulator of cell proliferation, which may also modulate SC proliferation [44]. Murine and human hematopoietic SCs [51], murine neural SCs [27], normal and malignant human mammary SCs [36], and normal and malignant human colorectal SCs [32] display ALDH activity and express this enzyme, strongly suggesting that strong ALDH activity and/or antigen expression can be used as a marker for SCs in a variety of cancers.

Breast CSCs have been isolated from human breast tumors or breast cancer-derived pleural effusions using flow cytometry to find subpopulations of cells with a specific pattern of cell surface markers [$CD44^+$, $CD24^{-/low}$, ESA^+ (epithelial specific antigen)] but lacking expression of specific lineage markers (Lin^-) [43]. These cells expressed (EMT) markers and had higher tumorigenic potential than bulk tumor cells after transplantation in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice [54]. It has also been shown that single cell suspensions of $CD44^+CD24^{-/low}Lin^-$ cells from human breast cancers were able to proliferate extensively and form clonal nonadherent mammospheres in a low attachment *in vitro* culture system [77]. These mammospheres were more tumorigenic than established breast cancer-derived cell lines including MCF-7 and B3R [77]. PROCR, identified using gene expression profiling of primary breast cancers [87], is also a known marker of hematopoietic, neural, and embryonic stem cells. An additional marker, CD133, was identified for breast cancer stem cells isolated from cell lines generated from $Brca1^{-exon11}/p53^{+/-}$ mouse mammary tumors [106] and is a known

marker of cancer stem cells in several organs including brain, blood, liver and prostate [45]. As in breast cancer, FACS analysis revealed heterogeneous surface marker expression for CD44, CD24, and ESA among pancreatic tumor cells.

Colon cancer cells such as $CD44^{+}CD166^{+}$ display a higher ability to form tumors in immunodeficient mice as compared to $CD44^{+}CD166^{-}$, $CD44^{-}CD166^{-}$ or $CD44^{-}CD166^{-}$ cell populations, making this an useful combination for the identification of colon CSCs [78]. Lgr5 is a Wnt target gene, exclusively expressed on colon CSCs and normal intestinal SCs and could thus also be a colon CSC marker [101]. On the basis of this hypothesis that the presence of tumor cells expressing SCs markers would affect the survival of glioblastoma patients, the expression of three SCs markers have been investigated: nestin, the prototypical marker for the identification of (NSCs); CD133 (cluster of differentiation 133), the most accredited marker for CSCs in various organs including the brain; and CD15, which is one of the most recently highlighted NSC markers and is also used to identify CSCs in central nervous system tumors [17]. Recent findings support the belief that cancer stem-like cells are responsible for tumor formation and ongoing growth. Differential expression was verified by Western blotting analysis of six interesting proteins, including the up-regulated Receptor-type tyrosine-protein phosphatase zeta, Tenascin-C, Chondroitin sulfate proteoglycan NG2, Podocalyxin-like protein 1 and CD90, and the down-regulated CD44. An improved understanding of these proteins may be important for earlier diagnosis and better therapeutic targeting of glioblastoma [40]. Neurons and glia cells also differentiate into abnormal cells with multiple differentiation markers and express many genes characteristic of NSCs and other stem cells, like the cell surface marker CD133, transcription factor SRY-related HMG-box gene 2 (Sox2) and neural RNA binding protein musashi 1 [41]. A population of cells in human brain tumors, medulloblastomas, astrocytomas, ependymomas and gangliogliomas that expresses the cell surface marker CD133 identified and elicit CSCs characteristics [88]; the $CD133^{+}$ isolated cells correspond to a small fraction of the entire brain tumor cell population, express the NSC marker nestin, exhibit increased self-renewal capacity, generate clonal tumor spheres in culture, and are capable of tumor initiation upon transplantation in to the brains of immune compromised mice. Sox2 is one of the key regulatory genes that maintain the pluripotency and self-renewal properties in embryonic SCs. Recently, Jia et al. [50] reported that Sox2 the potential to be a significant marker to evaluate the progression of prostate cancer and serve as a potentially useful target for prostate cancer therapy.

1.5 Pathways Regulating Stem Cell Self-renewal and Oncogenesis

It seems reasonable to propose that newly arising cancer cells appropriate the machinery for self-renewing cell division that is normally expressed in SCs. Evidence shows that many pathways that are naturally associated with cancer may also regulate normal SC development. For example, the prevention of apoptosis by

Table 1.2 CSC molecular signatures in different cancer types

Target type	Specific target	Cancer type	Use
Cell surface markers	$CD34^+/CD38$	AML	Identification has allowed for characterization of LSCs. Too broad to use as a target for chemotherapy but is very useful in identification for further characterization
	$CD33^+$	AML	Leukemia Gemtuzumab ozogamacin
	C-type lectin like molecule -1 (CLL-1)	AML	No clinical trials but efficacy seen *in vitro* and *in vivo* experimental studies
Signaling pathways	PI3K/Akt/mTOR	FDA approved therapy for renal cell carcinoma. Evidence that may be effective in other solid tumors	Temsirolimus, Everolimus FDA approved for renal cell Carcinoma
	Hedgehog	Evidence in basal cell carcinoma but has been identified as being up-regulated in many cancer types	Novel GDC-0449 and GANT-61
	HMG-CoA reductase	Increase ROS within cells leading to apoptosis, being investigated in many cancers including CML	Synergistic effect seen when imatinib and simvastatin in CML
	Wnt/β-catenin	AML, colon, pancreatic, breast, prostate, melanoma, glioblastoma etc	Celecoxib, Rofecoxib, Valdecoxib, AV-4126, ICG-001, Troglitazone and Rosiglitazone

enforced expression of the oncogene Bcl-2 results in increased numbers of HSCs *in vivo*, suggesting that cell death has a role in regulating the homeostasis of HSCs [29]. Other signaling pathways associated with oncogenesis, such as the Notch, AKT, Shh and Wnt signaling pathways, may also regulate SC self-renewal (Table 1.2) [94]. Notch activation in HSCs in SC culture using the ligand Jagged-1 have consistently increased the amount of primitive progenitor activity that can be observed *in vitro* and *in vivo* model systems, suggesting that Notch activation stimulates SC self-renewal, or at least the maintenance of multi-potentiality [53, 100]. Another pathway, Shh signaling has also been concerned in the regulation of self-renewal by the finding that populations highly enriched for human HSCs ($CD34^+Lin–CD38^–$) exhibit increased self-renewal in response to Shh stimulation *in vitro,* albeit in combination with other growth factors [9]. The involvement of both Notch and Shh in the self-renewal of HSCs is especially interesting in light of studies that implicate these pathways in the regulation of self-renewal of SCs from other tissues as well [80].

Our recent studies have been indicated that the aberrant reactivation of shh pathway is common event in pancreatic, prostate, brain, breast CSCs as downstream effectors of this pathway, Gli1, Gli2 and Gli3, induce genes that promote cellular proliferation and self-renewal. Therefore targeting Shh pathway could be a novel approach to prevent disease progression and metastatic spread. Small molecule inhibitors of Gli family proteins, such as GDC-0449, GANT-61, and other are used to block Shh signaling in human pancreatic, prostate, brain, breast CSCs that express Shh signaling components. Inhibition of the Shh signaling pathway by these inhibitory molecules induced significant cell death in CSCs. Gli1 and Gli2 expressions, promoter binding activity and Gli-luciferase reporter activity were also decreased. Increased level of Fas, DR4, DR5, caspase-3 and PARP cleavage were observed however expression of PDGFR-α and Bcl-2 was decreased following treatment of inhibitors. Silencing both Gli1 and Gli2 using shRNA abolished all the alterations produced by inhibitors. Collectively, these results highlight that these inhibitors induce apoptosis and cell death through inhibition of Gli family transcription factors (Gli1 and Gli2), and inhibition of SHH-signaling pathway, can be used for treatment of human pancreatic, prostate, brain, breast cancers.

One particularly interesting pathway that has also been shown to regulate both self-renewal and oncogenesis in different organs is the Wnt signalling pathway. Wnt proteins are intercellular signaling molecules [69] that regulate development in several organisms and contribute to cancer when dysregulated. The expression of Wnt proteins in the bone marrow suggests that they may influence SCs as well [16, 79]. Using highly purified mouse bone-marrow HSCs, overexpression of activated β-catenin (a downstream activator of the Wnt signalling pathway) in long-term cultures of HSCs expands the pool of transplantable HSCs determined by both phenotype and function (ability to reconstitute the haematopoietic system *in vivo*) was observed. Additionally, ectopic expression of Axin, an inhibitor of Wnt signaling, leads to inhibition of HSC proliferation, increased death *in vitro*, and reduced reconstitution *in vivo*. Soluble Wnt proteins from conditioned supernatants have also been shown to influence the proliferation of haematopoietic progenitors from mouse fetal liver and human bone marrow [6, 98]. Both *in vitro* and *in vivo* investigations into the PI3K/Akt/mTOR signaling pathways have also shown some potential for targeting CSCs. Integrin linked kinase (ILK) is also involved in phosphorylation of Akt and is upregulated in many malignancies including pancreatic cancer and AML blast cells [18, 67, 82, 85]. One of the hardest parts of targeting cancers is being able to target cells when they are quiescent. Interestingly, there is an over-expression of ILK during this phase which may play a part in the survival of cells or inhibition of apoptosis [67].

1.6 Stem Cells and Heterogeneity

Tumors are heterogeneous, but the mechanisms underlying this are unclear. Heterogeneity may result from mutations occurring early or late in a SC's maturation. For example, CML is believed to derive from an early progenitor SCs because

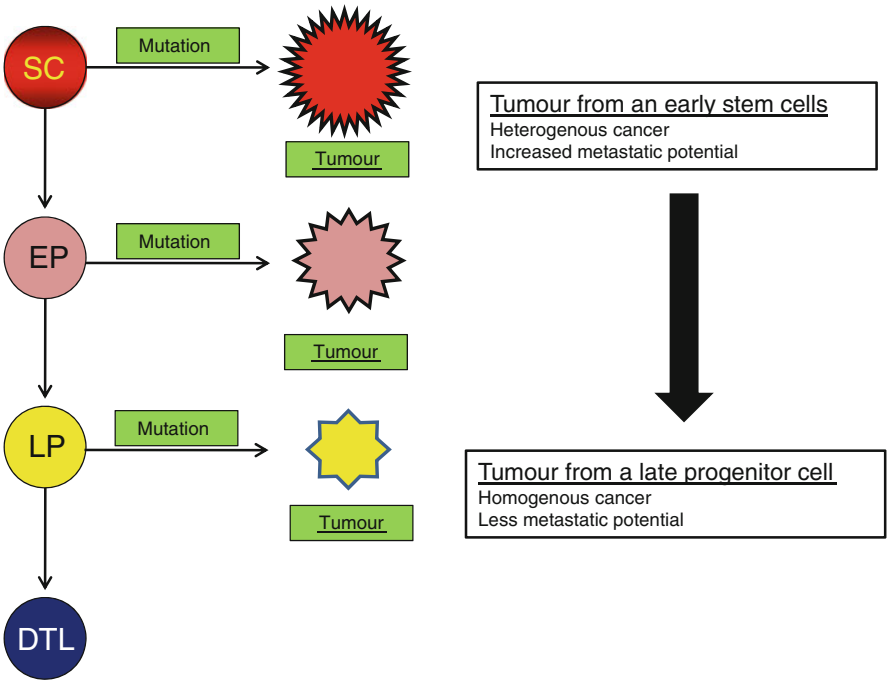

Fig. 1.3 In the stem cell model, only the stem cells or their progenitor cells have the ability to form tumours. Tumour characteristics vary depending on which cell undergoes the malignant transformation. *DTL* definitive tissue line, *EP* early progenitor, *LP* late progenitor, *SC* stem cell

its cytogenic marker (BCR-ABL) is present in several cell lineages, for example lymphoid, myeloid and platelet cells. Nevertheless, an abnormality in a late SC progenitor in the myeloid lineage at the promyelocytic stage results acute promyelocytic leukaemia [97]. Tumors derived from an early SC may develop a more heterogenous phenotype and have an increased metastatic potential. Mutations in late progenitor SCs may lead to tumors of a single cell type with reduced metastatic potential (Fig. 1.3).

As recently shown in an experiment, the mammary gland develops by differentiation from its mammary SC [84]. A diverse range of breast cancers may, hence, develop depending on where a mutation occurs in this pathway [30, 31]. Therefore, a SC model for estrogen receptor (ER) expression in breast cancer has been proposed, dividing breast cancer into three types, in an attempt to explain how ER-positive, ER-negative or heterogenous receptor status tumors can be created by mutations in the SC or progenitor cell populations. In early fetal life, SCs are ER negative, but presumably under the influence of oestrogen, progenitor cells that are both ER positive and ER negative can be identified at various times during growth, in particular during puberty and pregnancy [30].

Type 1 tumors develop from mutations in ER-negative stem/ progenitor cells, blocking differentiation and averting the development of ER-positive progenitors [30].

These tumors are poorly differentiated and seem to be more aggressive with a poorer prognosis. Less than 10% of these tumors are ER positive [30]. In the ER negative stem/progenitor cells Type 2 tumors are also derived from mutations. However, a variable percentage of the tumor will differentiate into ER-positive cells [31]. Antioestrogen therapy can produce a decrease in tumor size; however, the effect is short lived as ER-negative SCs are unaffected and tumor proliferation continues despite hormonal therapy [30]. This may explain why some ER-positive breast cancers continue to grow despite adjuvant hormonal therapy [30]. Type 3 tumors are well differentiated and result from mutations in ER-positive progenitor cells. Hormone replacement therapy use increases the risk of cancer formation. They respond best to antioestrogen therapy and have the best prognosis [30].

1.7 Therapeutic Implications

A practical importance of CSC heterogeneity is that strategies to induce apoptosis and cell death to treat cancer must address the unique survival mechanisms of the CSC within the cancer cell population. Most old-style cancer treatments have been developed and assayed based on their ability to kill most of the cancer cell population and result in tumor shrinkage. These treatments likely miss the CSCs, which have been shown in several cancer types to be quite resistant to standard chemotherapy and radiation. A prediction of the CSC model is that treatments that target the CSC will be required to result in an effective cure of cancer. As such, tumor shrinkage is not going to be a useful parameter to measure effectiveness of CSC therapies, and approaches to measure CSC burden will need to be devised.

The identification of CSCs has potential therapeutic implications [30]. As SCs are important for tissue growth and repair, they have developed highly efficient mechanisms for resistance to apoptosis [30]. Many have overexpression of anti-apoptotic genes such as Bcl-2 and may express drug-resistance transporter proteins such as MDR1 and ABC transporters [37, 70, 83]. These mechanisms permit normal SCs to become resistant to chemotherapy [3, 60, 88]. It has been proposed that CSCs also express these proteins at higher levels than the bulk population of tumor cells and may be more resistant to chemotherapeutic agents, allowing the reproliferation of tumors after chemotherapy [80]. Developing targeted therapies that are selectively toxic to CSCs while sparing normal SCs may lead to more effective treatment options for eradicating this crucial population of cells [70].

Although, there is clinical research based evidence that are indicating more clearly towards the origin of brain tumors from brain proliferative sections. The cell of origin for brain tumors is still unknown and these may vary from one tumor type to another or may be different in tumors occurring at different patient ages. One could claim that once the tumor exists, its cell of origin is not relevant, what is relevant is the CSCs and the directing therapy to this cell to effect a cure. The CSC hypothesis suggests that the CSC must be eliminated to cure cancer, but it is likely that different components of the tumor hierarchy will need to be targeted. This

hypothesis suggests that current therapies spare CSCs leading to tumor regrowth and clinical recurrence. One key factor for treatment may be the cell cycle status of the SCs, as most currently available treatments target cells that are promptly cycling. Although, normal SCs, and leukaemic SCs, have been shown to be quiescent, the cell cycle status of solid CSCs has not yet been well characterized. If the brain tumor SC is relatively quiescent, these cells will probably require distinct therapy from tumor progenitors that are rapidly proliferating.

Some studies suggest that Shh, Bim-1, Notch, Wnt/β-catenin signaling leads to increased tolerance of DNA damage, thus conferring radiation resistance of CSCs [20, 105]. Wnt/β-catenin signaling activities the DNA damage response, and one transcriptional target of β-catenin signaling is survivin, which is known to encourage cellular survival in CSCs in response to apoptotic stimuli such as ionizing radiation [5]. The complex nature of CSC survival mechanisms extends beyond Wnt/β-catenin signaling. Shh and Notch signaling has also been implicated in prostrate, pancreatic, brain, and breast cancer's response to radiation injury and targeting this pathway has shown effective antitumor response in preclinical trials [34, 76]. Alternatively, some studies suggest that the level of compaction of chromatin dictates accessibility to genomic DNA and subsequent mediation of DNA damage responses and that a looser configuration of chromatin in SCs leads to accelerated DNA repair following injury. Such has been shown in embryonic SCs that have lower levels of the chromatin structural protein histone-1. Embryonic SCs with lower levels of histone-1, which results in less chromatin compaction, had enhanced recovery from DNA damage in comparison to differentiated cells [68]. Pancreatic and prostrate CSCs likely share some of the signaling cascades involved in cellular responses to DNA damage present in other SC systems; however, the specific responses and mechanisms involved in the chemotherapy and radiation resistance of prostate and pancreatic CSCs remain to be elucidated.

There has been much interest in modern technique microarray analysis of tumors, the development of prognostic and predictive markers, allowing tumor subtyping and the possibility of developing specific tumor treatments [42, 103, 109]. The identification of SCs in several cancers such as AML, pancreatic, breast cancers and CNS tumors raises the possibility that decision-making on the basis of microarray analysis of the bulk tumor population may not be entirely appropriate because the gene expression profile of the CSC may be different to the rest of the tumors [80]. By comparing gene expression profiles of CSCs, the bulk tumor cell population, normal SCs and normal tissue, it may be possible to identify therapeutic targets that preferentially attack CSCs [70].

Understanding CS cell biology may lead to insights into the causes and treatment of tumor metastasis. The metastatic ability of a tumor cell may be related to properties of the SC of origin [30]. For example, the cytokine receptor CXCR4 is expressed on haematopoietic SCs and interacts with cytokines CXCL12/SCDF that are secreted by bone marrow stromal cells. This attracts haematopoietic SCs to the bone marrow [31]. The CXCR4 cytokine is also overexpressed on metastatic breast cancer cells. This may direct them to the bone marrow and may be one of several potential explanations for the increased incidence of bone metastases in breast cancer.

Existing remedies have been developed largely against the bulk population of tumor cells because they are often identified by their ability to shrink tumors. Because most cells with a cancer have limited proliferative potential, an ability to shrink a tumor mainly reflects an ability to kill these cells. It seems that normal SCs from various tissues tend to be more resistant to chemotherapeutic agents than mature cell types from the same tissues [39]. The reasons for this are not clear, but may relate to high levels of expression of anti-apoptotic proteins or ABC transporters such as the multidrug resistance gene [74, 96, 110]. If the same were true of CSCs, then one would predict that these cells would be more resistant to chemotherapeutic agents than tumor cells with limited proliferative potential. Even therapies that cause complete regression of tumors might spare enough CSCs to allow regrowth of the tumors. Therapies that are more specifically directed against CSCs might result in much more durable responses and even cures of metastatic tumors.

The defining characteristics of a circulating cancer stem cell (CTSC) are its capacity for self-renewal and for initiation of distant metastases; some of these cells are also resistant to traditional chemotherapy. The concept that circulating tumor SCs can be identified and targeted is attractive and has major diagnostic, prognostic, and therapeutic implications for patients with metastatic cancer. The development of metastasis is a late event in the linear progression model of metastasis, as opposed to the parallel progression model that suggests dissemination of circulating tumor cells can be an early event [89]. Some patients with Dukes' stage A CRC had detectable mRNA of CEA/CK/CD133 but failed to show any differences in overall survival and disease-free survival regardless of their CEA/CK/CD133 status [46]. These findings suggest that dissemination of CTSCs may indeed be an early event but also indicate that the prognostic significance of these cells in association with early disease may be negligible. Additional studies are needed to shed more light on the prognostic significance of CTSCs that are detected in patients with early-stage disease.

Genomics may provide a powerful means for identifying drug targets in cancer cells. Although targeting genetic mutations does not require isolation of the SCs, there are likely to be differences in gene expression between CSCs and tumor cells with limited proliferative potential. The application of microarray analysis to malignant tumors has shown that patterns of gene expression can be used to group tumors into different categories, often reflecting different mutations [11, 73]. As a result, tumor types that cannot be renowned pathologically, but that can be renowned on the basis of differences in gene-expression profile, can be examined for differences in treatment sensitivity. However, gene-expression profiling is often conducted on tumor samples that contain a mixture of normal cells, highly proliferative cancer cells, and cancer cells with limited proliferation potential. These findings in a composite profile that may obscure differences between tumors, because the highly proliferative cells that drive tumorigenesis often represent a minority of cancer cells. Gene-expression profiling of CSCs would allow the profile to reflect the biology of the cells that are actually driving tumorigenesis. Micro dissection of morphologically homogeneous collections of cancer cells is one way of generating profiles that reflect more homogeneous collections of cells [60]. The next boundary will be to

purify the CSCs from the whole tumor that keep unlimited proliferative potential and to perform gene-expression profiling on those cells. In addition to being a more efficient way of identifying new therapeutic and diagnostic targets, the profiling of CSCs might sharpen the differences in patterns observed between different tumors.

1.8 Conclusion

Self-renewal is the hallmark property of CSCs in normal and neoplastic tissues. Distinct signaling pathways control CSC self-renewal in different tissues. But perhaps within individual tissues, the same pathways are used consistently by both normal CSCs and cancer cells to regulate proliferation. For example, Wnt Shh, and Notch signaling pathways regulate the self-renewal of normal CSCs. Constitutive activation of these signaling pathways have been implicated in a number of epithelial cancers. The regulation and consequences of these pathways in normal and neoplastic cells need to be further elucidated. The discovery of CSCs in AML, pancreatic, prostrate, breast, brain cancers and some other tumors offers a new approach to understanding the biology of these conditions. Understanding the signaling pathways that are used by for normal SCs and neoplastic cells should facilitate the use of normal SCs for regenerative medicine and the identification of CSC targets for anticancer therapies. Further studies are needed to understand both normal and CSC development and whether CSCs are present in other tumor types. Ultimately, new prognostic and predictive markers, as well as targeted therapeutic strategies, may be developed to force tumors into permanent remission. There are many connections between SCs and cancer that are important to understand. Just as the signals that are known to control oncogenesis are providing clues about the control of self-renewal of normal SCs, studies of stem cell biology are lending insight into the origins of cancer and will ultimately yield new approaches to fight this disease.

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