

Cancer Stem Cells

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ABSTRACT: Cancer stem cells (CSC) are recently proposed to be the cancer initiating cells responsible for tumorigenesis and contribute to cancer resistance. Advances have been made in identifying and enriching CSC in leukemia and several solid tumors, including breast, brain and lung cancers. These studies suggest that, like normal stem cells, CSCs should be rare, quiescent, and capable of self-renewing and maintaining tumor growth and heterogeneity. Although the concept of CSC originates from that of normal stem cells, CSCs are not necessarily aberrant counterparts of normal stem cells. In fact, they may arise from stem cells or committed progenitors of corresponding tissues, and even cells from other tissues. At the molecular level, the alteration of stem cell self-renewal pathway(s) has been recognized as an essential step for CSC transformation. Better understanding of CSC will no doubt lead to a new era of both basic and clinical cancer research, re-classification of human tumors and development of novel therapeutic strategies specifically targeting CSC. (*Pediatr Res* 59: 59R–64R, 2006)

Stem cells are defined as undifferentiated cells that are capable of self-renewing and differentiating into a large number of diverse mature progeny. Amongst the various categories of stem cells, the embryonic stem (ES) cells are totipotent and able to differentiate into many cell types under appropriate conditions *in vitro* and contribute to all different tissues *in vivo* (1–3), making them a very promising foundation for stem cell-based therapeutics. Somatic stem cells from different organs, on the other hand, are pluripotent and responsible for tissue regeneration and repair. Adult stem cells have been identified in several organs, such as the hematopoietic system, brain, skin, mammary gland and lung, but it is not yet clear whether they are present in all other adult organs (4,5). The best-studied somatic stem cells are hematopoietic stem cells (HSC). With the aid of cell surface markers for positive identification, fluorescence-activated cell sorting (FACS) for prospective isolation, and *in vitro* and *in vivo* assays for functional testing; HSCs in mice and humans have been positively identified and successfully isolated by Weissman and colleagues (5,6). HSCs are known to be responsible for the generation of all cell types in the blood (Fig. 1, middle

panel), although their potential for giving rise to other tissues (or plasticity) is still controversial (4,5).

Dick et al. have recently revealed that, like the normal hematopoietic system, leukemia is organized as a hierarchy in which only a rare population retains a clonogenic capacity upon transplantation (7). Similarly, a solid tumor can be likened to an organ developed in an aberrant way, as it contains a heterogeneous mixture of cell types and abnormal tissue structures. More importantly, such an aberrant organ can be maintained and even formed at remote sites if no therapeutic intervention is performed. It is well established that tumor engraftment, although requiring a large number of cells, results in the formation of secondary tumors that recapitulate primary ones. The clonogenic and heterogenic nature of tumors suggests that a rare cell population in cancer, which acts like stem cells, is responsible for tumor growth and metastasis. These rare cells are named cancer stem cells (CSC) after normal stem cells, as both have similar abilities to self-renew and to give rise to heterogeneous differentiated cell types (8). Recent advances have begun to disclose the biologic identity and origin of CSC in several types of cancers and to elucidate the mechanisms underlying the transformation of normal cells into CSC. This review will highlight recent progress in the field, and discuss key issues of CSC research and their clinical implications.

THE IDENTITY AND ORIGIN OF CSC

To draw a true cellular and molecular picture of CSC, it is critical to identify CSC or purify the population to homogeneity. Recently, efforts have been made in isolating CSCs from human cancer samples as well as animal models as summarized in Table 1 (7,9–17). Although most of these studies are able to show cancers initiated by certain enriched populations for CSCs, homogeneity has not been reached. In fact, data revealing that CSCs can originate from either stem cells or progenitors (Table 1) raise the possibility that multiple CSC populations may be formed during cancer progression and even co-exist in advanced cancers.

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Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; APML, acute promyelocytic leukemia; CML, chronic myelogenous leukemia; CSC, cancer stem cells; HSC, hematopoietic stem cells; MPD, myeloproliferative disorder; NSC, neural stem cells

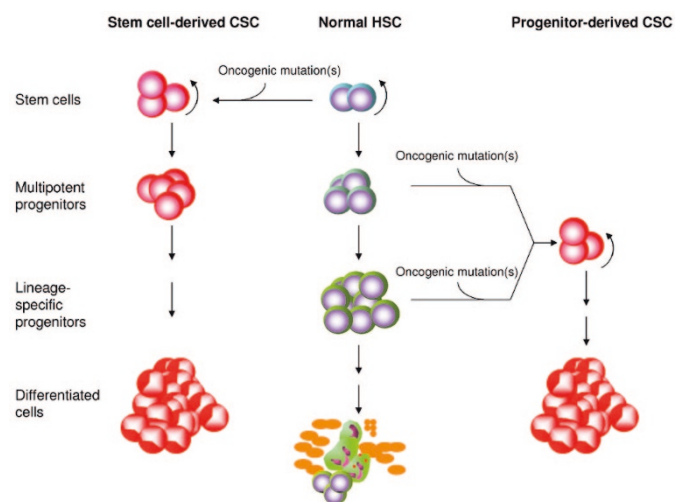


Figure 1. A schematic relationship between normal HSC differentiation and CSC and leukemia/lymphoma development. During normal hematopoietic development, long-term HSC maintain normal homeostasis by self-renewing and differentiating into functional mature cells of multiple lineages. Hematopoietic differentiation normally proceeds through the pathway of stem cells, committed multipotent progenitors, lineage-specific progenitors to mature cells (middle panel). Under rare conditions, an oncogenic event alters the tight regulation of stem cells or progenitors and transforms either of the two populations into CSC. The leukemic transformation also results in an aberrant hierarchical differentiation pathway. Stem cell-derived CSC may retain uncontrolled self-renewal capacity and be able to give rise to aberrant differentiating but immature leukemic cells due to maturation arrest (left panel). Conversely, progenitor-derived CSC may acquire self-renewal capacity but be subject to maturation arrest after transformation event(s) take place (right panel). In certain types of cancer, multiple oncogenic mutations may be required for full transformation.

The stem cell population is a logical candidate as a target for oncogenic transformation because of the inherent abilities of self-renewal and multilineage differentiation (Fig. 1, left panel). Using the hematopoietic system as an example, it has been known for some time that an oncogenic event can initiate at HSC level. It is well accepted that the t(9;22) chromosomal translocation (or the Philadelphia chromosome), which leads to the formation of the p210 BCR-ABL1 oncoprotein, is present in the HSCs of patients with chronic myelogenous leukemia (CML) (18,19). In a subset of acute lymphoblastic leukemia (ALL), this t(9;22) breakpoint was detected in the CD34⁺CD38⁻CD19⁻ HSCs (13). The transcripts of another leukemic fusion oncogene, *AML1-ETO*, were also detected in the CD34⁺Thy⁺CD38⁻Lin⁻ HSCs of patients with acute myelogenous leukemia (AML) in long-term remission (10). Consistent with the observations in human leukemia, only the murine *JunB*^{-/-} HSC population was capable of transplanting myeloproliferative disorder (MPD) to recipients (20). These lines of evidence underscore the stem cell origin of CSC. However, in most of the cases described above, additional mutations appear to be required for their malignant transformation.

In addition to stem cell origin, recent findings point out that CSC can also arise from committed progenitors that acquire self-renewal capacity (Fig. 1, right panel). Such progenitors are normally derived from self-renewable HSC but have no or

very limited self-renewal capacity. With progressive proliferation and differentiation, these progenitor cells are capable of producing terminally differentiated functional cells. The oncogenic fusion genes, such as *ETV6-RUNX1* or p190 *BCR-ABL*, could only be detected in CD34⁺CD38⁻CD19⁺ B progenitor population of some ALL patients (13). From these patients, the purified CD19⁺ B cells, but not the CD34⁺CD38⁻CD19⁻ HSC, exclusively reconstituted CD19⁺ leukemia in immunodeficient nonobese diabetic-severe combined immunodeficiency (NOD-SCID) mice (13), providing conclusive evidence of a B progenitor origin for CSC. In the case of acute promyelocytic leukemia (APML), the fusion gene *PML-RAR α* , resulting from the t(15;17) translocation, was detected only in CD34⁻CD38⁺ progenitors, but not in CD34⁺CD38⁻ HSC (9). The progenitor origin of CSC in APML is supported by the transgenic mouse model of *PML-RAR α* under the control of a human myeloid-specific promoter of *MRP8*, which is expressed in common myeloid progenitors (CMP) and granulocyte/monocyte progenitors (GMP). These *PML-RAR α* transgenic mice recapitulated human APML (21). In contrast, the transgenic mice expressing *PML-RAR α* in more differentiated CD11b⁺ myelomonocytic cells failed to develop leukemia (22). Results from these studies of hematopoietic malignancies suggest that CSC can also originate from lineage-committed and stage-specific progenitors (Fig. 1, right panel).

In addition to differentiation stage-dependent origin, a study of ependymoma, a CNS (CNS) tumor, adds regional difference to the origin of CSC. Taylor *et al.* reported that histologically identical ependymomas from the supratentorial, the posterior fossa and the spinal cord region might be initiated from altered radial glia cells (RGC, CD133⁺Nestin⁺RC2⁺BLBP⁺ progenitors). However, these tumors maintained different chromosomal abnormalities and only recapitulated the gene expression profiles of the progenitors from their respective anatomic sites (16).

Although CSCs are generally considered to be derived from mutated stem cells or progenitors of corresponding tissues or organs, some surprisingly originate from cells recruited from other tissues. This was shown in a mouse model of gastric cancer induced by chronic infection with *Helicobacter felis*, analogous to human *H. pylori* infection. In lethally irradiated mice transplanted with *LacZ*-positive bone marrow (BM) cells, these *LacZ*⁺ BM-derived cells could home to and repopulate the gastric mucosa and contribute to metaplasia, dysplasia and intraepithelial cancer after *H. felis* infection (23). *In vitro* culture further suggested that BM-derived mesenchymal stem cells (MSC) may be a candidate origin for CSC, as they, but not HSC, can acquire a gastric mucosa gene expression pattern when exposed to primary gastric cell cultures (23). However, it remains to be elucidated how the BM-derived cells are transformed to CSC.

MOLECULAR MECHANISMS UNDERLYING CSC FORMATION

Recent studies suggest that both cell intrinsic and environmental factors can control the normal stem cells and thus may

Table 1. A summary of putative cancer stem cells from cancer

Cancer	Species*	Definition	Cancer stem cells				Origins	Ref
			Fraction	Frequency† (cells)	Tumorigenic <i>in vivo</i> ‡	Replating‡		
AML	H	CD34 ⁺ CD38 ⁻	0.2–1%		Y		Myeloid progenitors	7
APML	H	CD34 ⁻ CD38 ⁺			Y		Myeloid progenitors	9
B-ALL (ETV6-RUNX1)	H	CD34 ⁺ CD38 ⁻ CD19 ⁺	1.1%		Y		B progenitors	13
B-ALL (p190 BCR-ABL1)	H	CD34 ⁺ CD38 ⁻ CD19 ⁺	1.1%		Y		B progenitors	13
B-ALL (p210 BCR-ABL1)	H	CD34 ⁺ CD38 ⁻ CD19 ⁺			Y		B progenitors	13
MPD in JunB ^{-/-} mice	M	Sca-1 ⁺ c-Kit ⁺ Thy1.1 ^{int} Lin ⁻		60	Y		Long term-HSC	20
CML blast crisis	H	CD34 ⁺ CD38 ⁺ IL3Rα ⁺ CD45RA ⁺				Y	Granulocyte/monocyte progenitors	25
Medulloblastomas	H	CD133 ⁺	6–21%	100	Y	Y	Stem cells/progenitors	15
Glioblastomas	H	CD133 ⁺	19–29%	100	Y	Y	Stem cells/progenitors	15
Ependymomas	H	CD133 ⁺ Nestin ⁺ RC2 ⁺ BLBP ⁺	0.001–1.5%	10,000	Y	Y	Radial glia cells	16
Breast cancer	H	ESA ⁺ CD44 ⁺ CD24 ^{-low} Lin ⁻	0.5–5%	200	Y		Stem cells/progenitors	12
Melanomas (metastatic)	H	CD20 ⁺ MCAM ⁺	20.0%			Y	?	14
Lung adenocarcinoma	M	SP-C ⁺ CCA ⁺					Bronchioalveolar stem cells	17

contribute to the formation of CSCs. As for cell intrinsic effects, studies of human hematopoietic malignancies in murine models indicate the existence of a hierarchy among oncogenic fusion proteins in their ability of endowing a self-renewal capacity to committed progenitors and blocking cell differentiation (24,25). *MOZ-TIF2* and *MLL-ENL* are AML-associated oncogenes that are capable of conferring a self-renewal capacity to myeloid progenitors and blocking cell differentiation. These oncoproteins alone are sufficient to transform committed progenitors to CSC (24,26). On the other hand, the oncoprotein p210 BCR-ABL1 in human CML, which provides HSC proliferation and survival advantage but not self-renewal activity, is not sufficient to trigger leukemic transformation on committed myeloid progenitors (24,27). This class of oncoproteins usually requires additional alteration(s), or a 2nd hit in the self-renewal pathways, *e.g.* Bmi-1 or Wnt/ β -catenin, for complete transformation. The differences in cellular transformation potency between these oncogenic fusion proteins remain to be further confirmed in human diseases. Since disease-specific chromosomal translocations are generally rare in epithelial tumors (28,29), multi-step mutational mechanisms are likely to be required for CSC formation in most of them.

Based upon the above observations, alteration of self-renewal pathways seems to be an important mechanism underlying CSC formation. It is known that the signaling pathways required for normal stem cell self-renewal are also involved in cancer development, such as Hox genes, Wnt, Sonic Hedgehog, and Notch signaling pathways (30,31). Recently, we have demonstrated that the PTEN tumor suppressor appears to negatively regulate the self-renewal of neural stem cells (NSC) (32) by modulating their G0-G1 cell cycle entry (33). The roles for these genes/pathways in normal and cancer stem cells have drawn an increased attention from both developmental and cancer biologists.

It is hypothesized that the self-renewal and differentiation of stem cells are maintained by asymmetric division, through

which a stem cell gives rise to two unequal daughter cells: one resembling the parental stem cell in the niche and the other proceeding toward differentiation. Any change in the control of asymmetric division may result in aberrant self-renewal activity of stem cells. Support for this hypothesis came from *Drosophila* studies. Aberrations in the stem cell asymmetric division, caused by mutations in polarity-controlling genes, *e.g.* *raps*, *mira*, *numb*, or *pros*, resulted in enhanced self-renewal activity and altered neuroblasts to form neuroblastoma-like tumors in adult hosts (34). It is suggested that the human tumor suppressor gene *LKB1*, which is reported to regulate polarity and is lost in the Peutz-Jeghers cancer syndrome, may contribute to tumorigenesis in mammalian systems via a similar mechanism (35).

Of those self-renewal regulators, the polycomb family transcriptional repressor Bmi-1 and Wnt/ β -catenin signaling pathway have recently been studied in the regulation of CSC self-renewal (25,36). Bmi-1 has been shown to be required for the self-renewal of adult HSC and NSC (37–39). Park *et al.* demonstrated that the number of HSC was normal in the fetal liver of *Bmi-1*^{-/-} mice but markedly reduced in postnatal BM. Moreover, *Bmi-1*^{-/-} fetal liver and bone marrow cells were only able to transiently reconstitute hematopoiesis (37). The studies of Bmi-1 in NSC further confirmed that NSC self-renewal is also dependent on Bmi-1, distinct from the Bmi-1-independent proliferation of restricted progenitors (39). Intriguingly, Bmi-1 plays an essential role in the self-renewal of CSC. Although introduction of *Hoxa9* and *Meis1* into *Bmi-1*^{-/-} fetal liver cells resulted in AML in host mice, leukemic cells from *Bmi-1*^{-/-}*Hoxa9*–*Meis1* recipients failed to reconstitute AML in any of secondary recipient mice (36). Besides Bmi-1, Wnt/ β -catenin signaling is reported to regulate *HoxB4* and *Notch1*, two critical regulators of HSC self-renewal activity (40). The ectopic expression of *Axin* or a frizzled ligand-binding domain, inhibitors of Wnt/ β -catenin signaling, led to inhibition of HSC growth *in vitro* and reduced reconstitution *in vivo*. This self-renewal role for Wnt/ β -

catenin signaling appears to be conserved in self-renewing CSC. For example, committed myeloid progenitors or GMP, putative CSC from CML patients in accelerated phase or blast crisis, showed increased nuclear β -catenin activity and self-renewal in a replating assay. Correspondingly, their replating capacity was reduced by the lentiviral introduction of *Axin* (25). PI3 K/AKT signaling is reported to positively regulate Wnt/ β -catenin signaling through AKT-mediated phosphorylation of GSK-3 β (41). In a murine MPD model carrying a conditional deletion of *Pten*, a potent negative regulator of PI3 K/AKT signaling, we also observed elevated cytosolic and nuclear β -catenin in leukemic blasts (W.G., J.L.L., and H.W., unpublished data).

Interestingly, these two self-renewal pathways appear to play important roles in the regulation of metastasis. Brabletz *et al.* observed that a high level of β -catenin was detected in mesenchyme-like colorectal tumor cells at the invasive fronts, implicating Wnt/ β -Catenin signaling in the epithelial-to-mesenchymal transition (EMT) or dissemination process of primary tumors (42). They proposed that low-level activation of β -catenin in the nucleus may be enough to confer self-renewal capacity, but its higher activation is required to trigger EMT, an essential step for metastasis (43). This phenomenon appears to hold true for the Bmi-1 pathway. Expression profiling on metastatic versus primary prostate tumors from human patients and the murine TRAMP transgenic model revealed substantial elevation of Bmi-1 and its associated molecular signature of 11 genes in metastatic cancer (44). Furthermore, this metastasis/stem cell signature was used to predict the risk of metastatic recurrence and poor clinical outcomes using samples from 1,153 cancer patients with 11 different types of cancer (44). Future studies are required to investigate the link between stem-ness and metastatic potential and the dual roles that Wnt/ β -catenin or Bmi-1 pathway may play.

As stem cell niches control normal stem cells, it is currently unknown how extrinsic or environmental factors control CSC formation. Studies on the development of *Drosophila* germ cells suggest that stem cell faith is determined by the instructive signals from their microenvironment—the “stem-cell niche” (45). In *Drosophila* germ cell niches, instructive signals are reported to be comprised of *dpp* and *Yb/Piwi/hh* in ovary or Unpaired (Jak-Stat signaling) in testis (45). In mammalian systems, signaling pathways conserved in niche-mediated stem cell control are reported to include TGF β /BMP, NOTCH, JAK-STAT, and context-dependent WNT (46,47). Furthermore, the size of a stem cell niche determines the number of stem cells in this niche. In *Drosophila*, the number of germline stem cells is positively correlated with the number of cap cells, a component of germline niches (48). Consistently, an increased number of murine osteoblasts, a component of HSC niches, leads to an increase in the number of HSCs (49,50). Although the role of the stem cell or CSC-specific niches in CSC formation remains unclear, some indirect evidence underscores their importance. For example, *dpp* overexpression induced by heat shock in *Drosophila* germlaria resulted in stem cell-like germline tumors (51).

CLINICAL IMPLICATIONS

The introduction of the CSC concept has provided exciting insights into the roots of carcinogenesis and sheds light on the future cure of cancer. The impact from current and future studies of CSC will revolutionize clinical practice with regards to both cancer diagnosis and therapy. Two of the implicated changes will be the re-classification of human tumors and development of novel therapeutic strategies targeting CSC.

The current diagnosis and classification of human cancer is mainly based on pathologic characterization of the entire tumor. However, we now understand that a rare CSC population(s) initiates and maintains cancer. As indicated by the above cases (13,16), although tumors are histologically identical, their CSC may be derived from different stem cell or progenitor populations, depending on where the transformation occurs. This difference at the level of CSC will likely be very important for diagnosis and therapy, especially for future therapies targeting CSC. Once CSC isolation approaches are established and verified, it will be critical to characterize human cancers based on both CSC and bulk tumor and establish a new system of cancer classification. However, since the current identification of CSC is fully dependent on *in vivo* reconstitution assays, a more practical and quicker approach to identify CSC will be essential for diagnosis. Both molecular signatures for altered self-renewal pathways, *e.g.* Bmi-1 (39) and β -catenin (25) in CSC, and *in vitro* replating capacity may serve as biomarkers for diagnosis based on CSC.

In most cases, current therapeutic strategies are developed to target the bulk of cancer and likely do not eradicate CSC completely, although they may have some effects on CSC, *e.g.* inhibition of CSC proliferation, and so reduce CSC number. For example, imatinib administration for CML can achieve complete remission, but the *BCR-ABL1* fusion gene is often detected in HSC of patients in remission, suggesting a potential risk of CML relapse. Thus, the complete eradication of CSC is likely the key to the cure of cancer. For clinical elimination of CSC, the cellular and molecular properties described below need to be taken into account.

The rarity of CSC will require therapeutic strategies different from conventional ones. Specific recognition of CSC from the tumor mass will be the first challenge. The identification of CSC-specific antigens may help develop specific targeting. Since the origins of CSC vary from cancer to cancer, the development of therapeutic strategies targeting different CSC population(s) will also be necessary. For stem cell-derived CSC which usually require additional mutations to generate malignancy, the use of inductive differentiation (*i.e.* transretinoic acid therapy for APL) or replacement with normal stem cells may be required, if it is hard to distinguish between normal stem cells and CSC. For progenitor-derived malignant CSC, eradication therapies targeting CSC and progenitors can be applied as long as a normal stem cell pool is still available for reconstitution in cancer patients (13).

Finally, multiple pathways/mechanisms will likely need to be targeted together for effective elimination. CSC may have or acquire stem cell properties that are more resistant to

therapies, such as survival advantage with increased anti-apoptotic activities and drug resistance due to increased levels of drug efflux pumps such as BCRP (breast cancer resistance protein) and MDR (multiple drug resistance) complexes. Future therapeutic strategies will need to integrate inhibition of these resistant mechanisms with CSC killing components. Moreover, combination therapies will help to prevent the generation of resistant CSC colonies due to mutations (52).

PROSPECTIVE

Despite recent progress in CSC research, our knowledge of these rare populations is still limited and many questions remain to be answered. Certain types of cancer are known to be multi-stage diseases, which generally progress into more malignant forms with the sequential accumulation of genetic and molecular alterations. For instance, hematological malignancies, such as CML, are often found to have two distinct phases: chronic phase and blast crisis (or leukemia). Similarly, some epithelial tumors, *e.g.* colon tumors, are thought to progress through at least five stages: pretumor patches/fields, hyperplasia, carcinoma *in situ*, invasive carcinoma and metastasis (30). One of the central questions in the CSC research is: how to link CSC to cancer progression in these tumors? Given sequential requirements of genetic and molecular alterations and distinct pathologic abnormalities associated with different stages of cancer progression, one may postulate that there could be multiple CSC populations, either intrinsically linked or generated independently, responsible for different stages of cancer progression.

To advance CSC research, we need to first understand the normal stem cells and critical pathways controlling stem cell properties. For this, identification of cell surface molecules for prospective stem cell isolation and biologically relevant stem cell assays are essential. In addition, technical improvement will expedite the studies of these rare and heterogeneous population(s). We should investigate the molecular mechanisms for the CSC formation and maintenance, especially their self-renewal regulation, which holds the key for the development of effective therapeutic strategies against CSC. Although stem cell niches have been shown to play an instructive and pivotal role in the regulation of stem cells, their implication in the CSC formation remains to be elucidated. Ultimately, with further improvements in our understanding of CSC, we will be able to develop better diagnostic and therapeutic methodologies, with which to classify, treat, and cure cancer.

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