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Concise Review: Cancer Stem Cells and Minimal Residual Disease

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ABSTRACT

Evidence gathered over the past two decades confirms earlier reports that suggested that hematologic malignancies exhibit a hierarchical differentiation structure similar to normal hematopoiesis. There is growing evidence that some solid tumors may also exhibit a differentiation program similar to the normal tissue of origin. Many excellent reviews on the topic of cancer stem cells (CSCs) document the recent explosion of information in the field, particularly highlighting the phenotypic and functional characteristics of these putative cells in vitro. Accordingly, here we only briefly discuss these concepts, and instead primarily examine the potential clinical relevance of CSCs, arguably the major unresolved issue in the field. Although it is generally accepted that CSCs are resistant to chemotherapy in vitro, only recently have data surfaced that suggest a role for these cells in disease relapse. Importantly, cancer cells with a stem cell phenotype have been found to be enriched in minimal residual disease of several malignancies. If the role of CSCs in relapse is confirmed, targeting these cells would hold substantial potential for improving the outcome of cancer patients. STEM CELLS *2012;30:89–93*

Disclosure of potential conflicts of interest is found at the end of this article.

HISTORICAL PERSPECTIVE

First formulated by Nordling in 1953 [1], the theory that cancer results from an accumulation of DNA mutations was further refined by Ashley [2], Knudson [3] and Nowell [4]. In this model of carcinogenesis, inherited mutations and/or environmental carcinogens lead to the development of premalignant clones. These cells further accumulate genetic hits until one cell reaches a critical genetic or epigenetic state that confers a growth and/or survival advantage over its normal counterparts. Over time, if it can evade the immune system, this abnormal cell would give rise to a malignant tumor. In the purest sense, the cell that suffered the "critical insult" is the primordial cancer-initiating cell and the tumor is its clonal expansion.

As postulated by Ashley, a cancer-initiating cell must survive long enough to accumulate three to seven genetic mutations necessary to generate cancer [2]. Moreover, it must already manifest proliferative capacity or, alternatively, develop it anew as a consequence of genetic mutation(s). Nowell [4] hypothesized that the inherent longevity and extensive proliferative capacity of a tissue stem cell make it an ideal candidate cancer-initiating cell. In contrast, most terminally differentiated cells are neither long-lived nor possess the ability to produce tumors with the limited number of divisions remaining in their differentiation program. Such cells could only acquire the multiple genetic mutations required for malignant tumor growth if such mutations occurred simultaneously or in rapid succession (e.g., as in the generation of induced pluripotent stem cells). However, longevity and extensive proliferative capacity are not traits restricted to classic normal tissue stem cells. To some degree, myeloid progenitors beyond the level of hematopoietic stem cells (HSCs) also retain these properties [5]. Moreover, within the lymphoid system, self-renewal capacity is preserved during differentiation through the memory lymphocyte stage to maintain life-long immunity [6].

The cancer stem cell (CSC) concept would explain why only a minority of cells from most hematologic malignancies and solid tumors are clonogenic in vitro and in vivo. In this CSC model, the cancer-initiating event, while conferring some advantages to the original cancer cell, does not completely alter its differentiation program; the malignant tumor would thus consist of a heterogeneous population of cells including the differentiated progeny of the original cell, mimicking to an extent the hierarchical structure of the normal tissue of origin. Since the primordial cancer-initiating cell or one of its progeny in this model possesses self-renewal capability and at least some differentiation potential—two of the defining features of normal stem cells—this cell naturally came to be called a CSC. Alternatively, it is also conceptually possible that the low clonogenicity of cancer is the result of all cells within a cancer retaining the capacity to proliferate but only at a low rate. Which of these two scenarios account for the low clonogenicity of most cancers has been debated for years. The first evidence supporting the CSC concept was published more than 40 years ago, when Fialkow et al. [7] demonstrated clonal hematopoiesis involving both the erythroid and myeloid lineages in patients with chronic myeloid leukemia (CML).

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Table 1. Phenotype of AML CSC

Phenotype	Xenograft model	Comments	References
CD34+CD38−	NOD/SCID		Lapidot et al. [13]
CD34+CD38+	NOD/SCID, NOD/SCID/β2m−/−, NOD/SCID/IL2Rγ −/−		Taussig et al. [14]
CD34+CD123+	NOD/SCID		Jordan et al. [15]
	NOD/SCID	Neutralizing antibodies reduces AML CSC	Jin et al. [16]
CD44+	NOD/SCID	Neutralizing antibodies reduces AML CSC	Jin et al. [17]
CD96+	Newborn Rag2−/−γc−/−		Hosen et al. [18]
CD34+CD38−CLL1+	NOD/SCID		van Rhenen et al. [19]
CD34−	NOD/SCID/β2m−/−, NOD/SCID/IL2Rγ−/−	Only NPM1+AML	Taussig et al. [20]
Lin−CD38−	NOD/SCID/IL2Rγ−/−	Lin−CD38− fraction had the highest AML CSC frequency but all populations showed some AML CSC activity	Sarry et al. [21]

Abbreviations: AML, acute myeloid leukemia; CD, cluster of differentiation; CLL1, C lectin like molecule 1; CSC, cancer stem cell; IL2Rγ−/−, interleukin 2 receptor gamma knock out; NOD/SCID: nonobese diabetes/severe combined immunodeficiency; NPM1, nucleophosmin; Rag2−/−, recombination activating gene 2 knock out; β2m−/−, beta-2 microglobulin knock out.

Identifying and Characterizing CSCs

Myeloid Malignancies

Probably not surprisingly, given that hematopoiesis is the best characterized somatic stem cell system, CSCs have been best characterized in hematologic malignancies. The stem cell origin of CML was confirmed nearly 20 years ago when several groups, using characteristics known to define normal HSCs, identified and isolated CML cells capable of expansion ex vivo [8–10]. Dick and colleagues extended these observations, showing that primitive HSCs purified from patients with CML would generate leukemia in vivo when injected into nonobese diabetes/severe combined immunodeficiency (NOD/SCID) mice [11]. Moreover, the expression patterns of CML stem cells closely resemble those of normal HSCs [12]. Thus, the accumulated evidence over the last 15 years suggests that CML stem cells share many properties with, and likely arise from, normal HSCs. Thus, there is now universal agreement that the cancer-initiating event in CML, the Philadelphia (Ph) chromosome, occurs in an early hematopoietic cell if not the HSC itself.

Acute myeloid leukemia (AML) was the first cancer in which malignant cells with the ability to recapitulate the disease in a NOD/SCID mouse were identified [13]. These AML stem cells not only reproduced the disease in NOD/SCID mice but also possessed self-renewal capacity and exhibited an HSC phenotype. However, the exact surface phenotype of AML stem cells continues to be a subject of debate, possibly because of the heterogeneity of AML. Nevertheless, most studies suggest that, like CML, most cases of AML arise from phenotypic HSCs. Thus, markers of HSCs, including CD34, absence of CD38 and lineage-specific markers, CD133, and expression of aldehyde dehydrogenase (ALDH) have been widely used to identify and isolate putative AML stem cells (Table 1).

Other Hematologic Malignancies

The first modern use of the term cancer or tumor stem cells was probably by Bergsagel and Valeriote [22], who found that only a minority mouse multiple myeloma cells were capable of clonogenic growth. Subsequent studies by Hamburger and Salmon [23] confirmed these findings with clinical myeloma specimens, revealing a cloning efficiency ranging from approximately 1:1,000 to 1:100,000 cells. Insufficient tools existed at the time to distinguish whether this low clonogenic potential was the result of proliferative capacity exclusively restricted to a small subset of cancer cells or by all cancer cells retaining the capacity to proliferate but only at a low rate. Work from our laboratory suggests that the cancer-initiating cells in myeloma are found within the memory B-lymphocyte population, with the $CD138^+$ plasma cells terminally differentiated progeny of these malignant myeloma B cells [24]. These malignant $CD138^{neg}$ myeloma B cells expressed CD19, CD20, and CD27, along with high levels of ALDH. Moreover, myeloma CSCs and the plasma cells that comprise the bulk of the tumor exhibited disparate drug sensitivities. The CSCs seem to be resistant to most clinically active agents (e.g., dexamethasone, lenalidomide, bortezomib), perhaps in part by co-opting normal stem cells' intrinsic defense mechanisms such as quiescence, efflux pumps, and detoxifying enzymes [24, 25].

Hodgkin and Reed-Sternberg (HRS) cells, the hallmark of classic Hodgkin lymphoma (HL), also belong to the B lymphoid lineage. However, they are unlike any normal cells of that lineage, and their limited proliferative potential belies the clinical aggressiveness of the disease. More than 20 years ago, Newcom et al. [26] identified a population of cells that phenotypically resembled B cells and appeared to be responsible for the propagation of an HL cell line in vitro. Our group recently confirmed these findings in several other HL cell lines [27]. Moreover, clonotypic memory B cells with a similar phenotype to myeloma CSCs could be isolated from the peripheral blood of most newly diagnosed HL patients, regardless of stage, and these B cells and the patients' HRS cells exhibited identical clonal immunoglobulin gene rearrangements. Clonotypic $CD19^+CD5^+ALDH^{high}$ B cells were also identified in human mantle cell lymphoma (MCL) cell lines, as well as in patients with newly diagnosed MCL [25]. These cells were found to be relatively quiescent and resistant to many classic chemotherapeutic agents used to treat this condition.

Solid Tumors

Identification and characterization of CSCs from hematologic malignancies was founded on decades of biologic experience in human hematopoiesis, including well-understood purification methodology and both in vivo and in vitro functional assays. Limited understanding of the biology of their normal counterparts has hampered the study of solid tumor CSCs, if they indeed exist. Thus, initial research into CSCs in solid

Table 2. Phenotype of cancer stem cell in various human solid malignancies

Cancer type	Phenotype	Xenograft model used	References
Breast	CD44+CD24−Lin−	NOD/SCID	Al-Hajj et al. [28]
	ALDH1+	NOD/SCID	Ginestier et al. [29]
Brain	CD133+	NOD/SCID	Singh et al. [30]
Glioblastoma	CD133+	nu/nu	Bao et al. [31]
Lung	CD133+Ep-CAM+	NOD/SCID	Eramo et al. [32]
Prostate	Side population	NOD/SCID	Patrawala et al. [33]
	CD44+	NOD/SCID	Patrawala et al. [34]
	CD44+/$\alpha 2\beta 1$+/CD133+	Methylcellulose progenitor assay	Collins et al. [35]
Colon	CD133+	NOD/SCID	O'Brien et al. [36]
	CD44+/Ep-CAM+	NOD/SCID	Ricci-Vitiani et al. [37]
Melanoma	ABCB5+	NOD/SCID	Schatton et al. [38]
	1:4 unselected cells	NOD/SCID/IL2Rγ-	Quitana et al. [39]
Liver	CD90+CD44+	SCID/Beige, BALB/c	Yang et al. [40]
Pancreas	ALDH1+	NOD/SCID	Rasheed et al. [41]
	CD133+	NMRI-nu/nu	Hermann et al. [42]
	CD44+CD24+ESA+	NOD/SCID	Li et al. [43]
Head and neck	CD44+Cytokeratin 5/14+	NOD/SCID	Prince et al. [44]

Abbreviations: ABCB5, ATP-binding cassette subfamily B member 5; ALDH1, aldeflour dehydrogenases 1; CD, cluster of differentiation, Ep-CAM, epithelial cell adhesion molecule; ESA, epithelial specific antigen; IL2Rγ−/−, interleukin 2 receptor gamma knock out; Lin, lineage; NOD/SCID, nonobese diabetes/severe combined immunodeficiency; NMRI, Naval Medical Research Institute; nu/nu mice, homozygous nude mice.

tumors was based on findings in liquid malignancies (Table 2). Accordingly, breast CSCs, initially described as $CD44^{+}CD24^{low}$, were identified by their ability to generate tumors in immunodeficient mice [28]. This description was followed quickly by the discovery of CSCs expressing CD133 in brain cancers [45]. Since then, although the importance of any specific marker for CSC identification remains unclear, multiple malignancies have been shown to contain a stem-cell like population capable of initiating tumors in a xenograft model (Table 2). Similar to hematologic CSCs, solid tumor CSCs have been found to be relatively more resistant to cytotoxic therapy than the differentiated cells that make up the bulk of the tumor mass [31].

Controversy

Although cells meeting the definition for CSCs have now been described in many malignancies, there remains healthy skepticism about their true biologic significance. In fact, many investigators have proposed that CSCs may be nothing more than laboratory curiosities, simply reflecting the limitations of NOD/SCID mice for assessing tumorigenic potential [39, 46, 47]. This controversy is highlighted by a study which compared the growth of primary melanoma cells in NOD/SCID mice with the more immunocompromised NOD/SCID interleukin-2 receptor gamma chain null (NOG) mice. Although only about 1:100,000 unselected melanoma cells produced tumors in NOD/SCID mice, as few as 1:4 melanoma cells were tumorigenic when transplanted into NOG mice [39]. However, despite being considered the gold standard assay for CSCs by many in the field, there is no reason to assume that growth in immunocompromised mice is in fact a relevant assay for CSC activity.

Analogous to HSCs' dependence on their interactions with the stem cell niche [48], the microenvironment is likely critical for CSCs. For instance, a malignant niche rich in proinflammatory cytokines (e.g., interleukin [IL]-1, IL-6, tumor necrosis factor α) might promote and maintain cells from a variety of cancers [49]. CSCs are likely to cultivate continuous interactions with their microenvironment via a variety of surface molecules, including CD44, epithelial cell adhesion molecule, CD24, and CXCR4. It has also been shown that high levels of IL-4 and IL-10 in the malignant niche can protect CSCs from Fas–Fas ligand-mediated apoptosis [50]. It is within this nurturing microenvironment that CSCs grow, endure chemotherapy, and possibly evade immune surveillance to initially form a tumor and later cause disease relapse. However, essential interactions between CSCs and their malignant niche are likely disrupted in xenograft models. Accordingly, it is possible that injecting human tumor cells into a mouse primarily tests metastasis-initiating cells rather than cancer-initiating cells. Recent findings have also implicated the microenvironment in determining the pattern of metastatic spread [51]. Interestingly, while circulating cancer cells can be found early in the clinical course of malignancies [27], most cases of relapse occur at the site of the original tumor. The lack of an adequate "premetastatic niche" may explain why metastases are not more of a regular occurrence in the presence of circulating tumor cells.

Minimal Residual Disease and CSCs

Presumably, the most clinically important cancer cells are those that survive therapy and lead to relapse, whether they are tumorigenic in immunocompromised mice or not. Even if every cell in a cancer possessed tumorigenic potential, the presence of a discrete subset responsible for treatment resistance—perhaps as a result of stem cell properties—would have undeniable clinical significance. The CSC concept potentially explains not only the low clonogenic capacity of most malignancies but also why complete treatment responses rarely translate into cures for cancer patients: initial responses in cancer represent therapeutic effectiveness against the bulk cancer cells, while rarer but more resistant CSCs theoretically are responsible for relapse. However, even in the case of leukemia where the most evidence for the CSC concept exists, there is little proof that CSCs have any relevance to clinical practice.

If CSCs are indeed more resistant to therapy than the bulk tumor cells and thus responsible for relapse, then minimal residual disease (MRD) after treatment should be enriched for these cells. Furthermore, the presence of CSCs after therapy should predict recurrence. Indeed, it has recently been found that residual breast tumor cell populations persisting after

conventional treatment are enriched for phenotypic breast CSCs [52]. Similarly, patients with deletion 5q myelodysplastic syndrome (MDS) continue to have a population of phenotypically distinct MDS stem cells ($CD34^{+}CD38^{low}CD90^{+}$), even in complete clinical and cytogenetic remissions [53]; these cells appear resistant to lenalidomide treatment and may account for disease relapse. Our group also showed that there was a strong and significant association between myeloma CSC numbers and progression-free survival in patients after treatment with rituximab [54]. Interestingly, rituximab was detected on the surface of circulating myeloma CSCs in patients who progressed; thus, rituximab was able to target but not kill the myeloma CSCs in those patients. Our recent data also demonstrate that MRD in AML has a stem cell phenotype, and the presence or absence of AML CSCs after therapy correlates with progression-free survival [55].

These data, perhaps for the first time, provide evidence of clinical relevance for CSC's. They also suggest that studying MRD may prove to be an excellent tool for better defining CSCs. Screening for CSCs after treatment might provide an early window into prognosis and help personalize treatment.

Conclusions

There remains a healthy skepticism regarding the CSC concept. The uncertainty is based on discrepant phenotypic findings, conflicting results from the current gold standard xenograft transplant assay, and limited evidence for clinical significance. However, CSCs need not phenotypically mirror normal stem cells or be homogeneous within a tumor type. Moreover, xenograft transplantation may not be the optimal model for testing cancer initiation and may more aptly measure metastasis-initiating cells.

Importantly, new data suggest that cancer cells with stem cell characteristics are enriched in the MRD responsible for disease relapse. If CSCs are indeed proven to be clinically relevant, targeting these cells holds substantial translational potential. First, there may be a role for intensification of treatment in patients with persistent CSCs after initial therapy. Second, emerging data suggest that CSCs across a wide spectrum of malignancies exhibit similar stem cell biology and rely on similar mechanisms to outcompete the normal tissue (e.g., efflux pumps, Hedgehog signaling [56], and telomerase expression [57]). While the bulk cells of various tumors have distinct biology and thus require distinct treatments, the therapies targeting CSCs of different malignancies may prove to be more universally applicable. Using such treatments either in addition to debulking therapy in the upfront setting, or as subsequent maintenance therapy, may improve cure rates. Third, similar to normal tissue stem cells, the microenvironment may play a crucial role in the behavior of CSCs. Accordingly, microenvironment-directed therapies may impact disease biology and improve clinical outcomes. Finally, tools developed through CSC research may allow a better understanding of key cancer-initiating events, such as the influence of chronic inflammation, environmental exposures, and nutrition. Such studies have proved difficult when looking only at the bulk tumor. Ultimately, the clinical translation of ongoing investigations into CSC biology will provide the final verdict as to whether CSCs are really just laboratory curiosities or truly represent a relevant part of cancer biology.

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Disclosure of Potential Conflict of Interest

The authors indicate no potential conflicts of interest.

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