

REVIEW ARTICLE

Cancer stem cells in oncology

I. Cetin¹, M. Topcu²

¹Istanbul University, Institute of Science, Department of General Biology, Istanbul; ²Istanbul University, Faculty of Science, Department of Biology, Istanbul, Turkey

Summary

Cancer stem cells (CSCs) possess several characteristics including self-renewal, pluripotency and tumorigenicity and constitute a rare population in a tumor mass. Because conventional cancer therapies can not kill CSCs, these cells are responsible for tumor relapse and metastasis. Currently, with advances in the identification of CSCs, the importance

of these cells is increasing in the field of cancer diagnosis and prognosis. In addition, clarifying the mechanisms responsible for the maintenance of CSCs properties led to the development of CSC-targeted therapies.

Key words: cancer, cancer stem cells, CSC-targeted therapies, diagnosis, prognosis, therapeutic resistance

Introduction

Cancer is an abnormal growth of cells caused by multiple changes in gene expression leading to dysregulated balance of cell proliferation and cell death and ultimately evolving into a population of cells that can invade tissues and metastasize to distant sites, causing significant morbidity and if untreated, death of the host [1].

Cancer is a heterogeneous disease, involving differences between tumors as well as between cancer cells within the same tumor [2]. These cells can be distinguished from each other by several characteristics such as size, morphology, and antigen expression, as well as by behaviors like cell turnover, cell-cell interaction, angiogenic, immunogenic, invasive and metastatic ability, and sensitivity to pharmacologic interventions [3,4]. Clonal evolution contributes to this heterogeneity as cancer cells undergo irreversible genetic changes over time, leading to functional and phenotypic differences. Another explanation for the heterogeneity within tumors comes from the CSC model [2]. According to this model, not all cells within a tumor are equal [5] and only a specific subset of the cancer cell population is able to sustain in vivo tumor growth, whereas all oth-

er cell subsets are not. Indeed, this assumption has now been repeatedly confirmed in several tumor systems [6]. CSCs exist in leukemia and several solid tumors, including breast, brain and lung cancers [7]. Stem cell markers and their incidence in different malignancies are shown in Table 1 [8].

In this review we discuss about the general properties of CSCs, why they do not die with conventional therapies and also about targeted therapies aiming at killing these cells.

Table 1. Cell surface phenotypes of cancer stem cells

Tumor type	Phenotype	Fraction (%)
Breast	CD44 ⁺ CD24 ⁻	11-35
Brain	CD133 ⁺	5-30
Prostate	CD44 ⁺ CD133 ⁺ α2β1 ^{hi} or CD44 ⁺ CD24 ⁻	0.1-3
Pancreatic	CD44 ⁺ CD24 ⁻ ESA ⁺	0.2-0.8
Hepatocellular	CD133 ⁺	1-3
Colon	CD133 ⁺ or ESA ^{hi} CD44 ⁺	1.8-24.5
Head and neck	CD44 ⁺	<10
Lung	CD133 ⁺	0.3-22
AML	CD34 ⁺ CD38 ⁺	0.2-1
Multiple myeloma	CD138 ⁺	2-5
Melanoma	CD20 ⁺	~20

Characteristics of CSCs

All CSCs must display several characteristics including self-renewal, pluripotency and tumorigenicity. The term “self-renewal” represents the ability of the CSC to give rise to another CSC. Self-renewal maintains a CSC pool when these cells undergo either symmetrical or asymmetrical division. Symmetrical division results in the CSC forming either two differentiated cells or two CSCs. Asymmetric division forms one more differentiated cell and one CSC. This behavior is critical because it allows the CSC to expand its numbers [8].

During the behavior of self-renewal, while CSCs generate phenotypically similar tumorigenic daughter cells, on the other hand they differentiate into phenotypically different non-tumorigenic daughter cells [9]. Differentiation into non-tumorigenic cells results in formation of cancer cells within the heterogeneous tumor mass. These cells do not possess tumor initiating ability. The ability of differentiation reflects pluripotency feature of these cells.

Another characteristic, tumorigenicity, is the ability of CSCs to regenerate the tumor mass. CSCs are highly tumorigenic and can generate a serially transplantable phenocopy of the primary human malignancy in immunocompromised mice [10]. The differences between normal somatic stem cells and CSCs are shown in Table 2 [11].

The occurrence of CSCs

If stem cell differentiation potential becomes impaired and their proliferative capacity becomes uncontrolled, these mutated, potentially tumorigenic, self-renewable stem cells have the potential to cause cancer. Although this is still the subject of considerable debate, it is possible that the development of CSCs might involve at least two of the following events: (i) a change

in the microenvironment of the stem cell niche within a tissue; (ii) alterations in cellular metabolism, cell cycle control and/or progression and signaling pathways as a result of mutations and epigenetic changes; and (iii) amplification of cell populations with an altered molecular phenotype that give rise to heterogeneous primary tumors and metastases [12].

CSC niche

Niche, a specialized physiological microenvironment in which stem cells reside, plays a crucial role in the maintenance of stem cell characteristics such as pluripotency and self-renewal [13]. The niche is thus a physical anchoring site for stem cells and generates factors including certain extracellular matrix (ECM) components and signaling molecules that control stem cell number, proliferation, and fate determination [14]. The stem cell niche exhibits structural asymmetry, and asymmetric division of stem cells is one of the proposed mechanisms controlling the balance between self-renewal and differentiation [15].

Recent data suggest that CSCs also rely on a similar niche, dubbed the “CSC niche,” which controls their self-renewal and differentiation. Moreover, CSCs can be generated by the microenvironment through induction of CSC features in more differentiated tumor cells. In addition to a role in CSC maintenance, the microenvironment is hypothesized to be involved in metastasis by induction of the epithelial-mesenchymal transition, leading to dissemination and invasion of tumor cells [10].

The importance of CSCs in diagnosis and prognosis

Current data about the relationship between CSC and prognosis indicate that the clinical behavior of a

Table 2. Normal stem cell properties vs. cancer stem cell properties

<i>Normal somatic stem cells</i>	<i>Cancer stem cells</i>
Extensive but limited self-renewal capacity	Extensive and indefinite self-renewal capacity
Organogenic capacity	Tumorigenic capacity
Capacity to generate differentiated progeny with limited proliferative potential, often phenotypically diverse	Capacity to generate abnormal progeny with limited proliferative potential, often phenotypically diverse
Highly regulated self-renewal and differentiation	Highly dysregulated self-renewal and differentiation
Rare in normal adult tissues	Infrequent or rare within tumors
Identifiable based on surface markers	Often express similar surface markers as normal stem cells in the same tissue
Normal karyotype	Abnormal karyotype
Quiescent most of the time	Less mitotically active than other cancer cells

cancer is largely dependent on the characteristics of its CSC population [16]. In recent years, an effort has been made to successfully identify stem cells in many human malignancies, including hematological, breast, colorectal, brain, pancreatic, and maxillofacial cancer [17]. Identifying CSCs by their outer appearance or cell surface markers has been focused on by many researchers. The concept of identifying CSCs by these markers is a rational one. The challenge in targeting CSCs is identifying which cell surface markers are going to be the distinguishing factors that will make them a suitable target [18].

Recently, several CD markers have been identified as solid CSC markers. CD133, also known as PROML1 or prominin, is a stem cell surface antigen that has been recently identified as a potential CSC marker in brain, colon and prostate cancer. CD44, also known as homing cell adhesion molecule, is a cell surface glycoprotein expressed on lymphocytes, monocytes and granulocytes, which has been identified as a stem cell marker in breast and head and neck cancer [19]. The cell surface markers CD44, CD24, and epithelial-specific antigen (ESA) are expressed by the pancreatic cancer cells [20].

Therapeutic resistance of CSCs

The standard treatment modalities for patients with cancer include surgery, radiotherapy, and chemotherapy [21]. It is well established that a fraction of cells in a tumor frequently survives anticancer treatment when exposed to radiation and cytotoxic drugs. This drug resistant subpopulation of tumor cells may constitute CSCs, and in this way, these cells may be responsible for the failure of most, if not all, anticancer therapies, as these cells are postulated to be inherently resistant to anticancer agents [22]. Novel insights into CSC-mediated resistance mechanisms in solid tumors are given by recent findings in various cancers. For example, in breast cancer patients tumors from women who had received chemotherapy showed increased levels of CD44⁺CD24^{-/low} CSC, indicating that this subset may be selectively resistant to eradication [23]. In another study with glioblastoma patients, treatment with radiation increased the percentage of CD133⁺ cancer cells by 2- to 4-fold, depending on the model [24]. This study suggests that radioresistance may be a general property of CSCs and that this may be due to their ability to repair DNA more efficiently than non-CSCs [25].

Conventional therapies, such as chemotherapy, radiotherapy and antiangiogenic therapies, also act on the proliferating cancer transit-amplifying cells. When these therapies are discontinued, the cancer will reform

from the therapy-resistant CSCs [26]. There are several molecular mechanisms that may account for CSC resistance to therapy. Many CSCs are not cycling and are in G₀ phase and thus resistant to cell cycle-specific chemotherapy agents [27]. They express several ATP binding cassette (ABC) transporters [28]. Expression and activity of ABC-transporters leads to multiple drug resistance (MDR), and this is a major obstacle to antineoplastic therapy [29]. CSCs express higher levels of antiapoptotic proteins, such as members of the Bcl-2 family and inhibitors of apoptosis [27]. Increased tolerance to radiation-induced DNA damage and enhanced DNA repair activity enables the CSCs radioresistance [16].

CSC-targeting therapies

The successful eradication of cancer requires anticancer therapy that affects the differentiated cancer cells and the potential CSC population. At present, conventional anticancer therapies, including chemotherapy, radiation and immunotherapy kill rapidly-growing differentiated tumor cells, thus reducing tumor mass but leaving behind potentially cancer-initiating cells. Therapies that exclusively address the pool of differentiated cancer cells but fail to eradicate the CSC compartment might ultimately result in relapse and the proliferation of therapy-resistant and more aggressive tumor cells [30].

If tumor-initiating cells are indeed the major culprits of tumor development and progression in humans, the design of cancer therapies that target markers or signaling pathways specific to the CSC compartment could potentially increase the efficacy of current treatment regimens and might reduce the risk of relapse and metastasis [23].

Although the goal for any CSC-targeted therapy is the eradication of all CSCs, the efficacy of single-agents may be limited by several factors. First, currently defined CSCs may not be homogeneously sensitive to any given therapy. Second, CSC immunophenotypes may not be homogeneous, which may limit the efficacy of monoclonal antibody therapies directed at cell surface markers. Third, pathways shared by CSCs and normal stem cells may limit dosing due to toxicity to normal stem cells. Finally, and perhaps most important, there is no reason to expect that CSCs will be free from selection pressures and therefore therapy-resistant CSC clones may emerge [31]. Therefore, various therapeutic approaches which target especially CSCs are being developed.

CSC-targeted therapies are aimed at destroying them, either directly or indirectly. Direct approaches are destruction therapies targeting pathways or mechanisms essential for their survival. Destruction therapies include

self-renewal pathways alterations that target NOTCH, Hedgehog, WNT, Polycomb, HOX, and PTEN/PI3K/Akt signaling pathways, and modulation of chemoresistance that target ABC-transport proteins, anti-DNA repair mechanisms, and inhibition of antiapoptotic pathways. Other mechanisms of destruction therapies are telomerase inactivation, modulation level of reactive oxygen species (ROS) and inhibition of tumor vasculature. Indirect approaches are differentiation therapies that force the CSCs out from their stem status into more differentiated, proliferating cells that can then be destroyed using more conventional therapeutic approaches carried out with the help of various chemical substances such as vitamin A and epigenetic alterations [32].

Conclusions

Currently cancer is a disease that can not be cured completely due to various reasons. Unlike rapidly dividing cancer cells within the tumor mass, CSCs have a slower cycle under the effect of various factors such as microenvironment in which they reside and therefore following conventional cancer therapies that kill rapidly dividing cells, CSCs can survive. Under proper conditions, CSCs proliferate with symmetric and asymmetric divisions and because CSCs provide remodeling of the tumor mass, they are responsible for tumor relapse. In order to overcome this problem studies with CSCs attract considerable interest in cancer research. Identification of these cells is important in terms of early diagnosis and disease prognosis. Defining mechanisms and the molecules that are responsible for the stem cell characteristics is also important in overcoming the resistance of these cells by exploring novel agents that could target CSCs. The development of CSC targeted therapies is an area that gains increasing importance and is considered that new therapeutic approaches aimed at killing these cells that are responsible for tumor relapse can fill critical missing points in oncology.

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References

- Ruddon RW. Characteristics of human cancer. In: Ruddon RW(Ed): *Cancer Biology* (4th Edn). Oxford University Press, New York, 2007, pp 3-61.
- Elsa Q, Mark S, Hannah RF et al. Phenotypic Heterogeneity among Tumorigenic Melanoma Cells from Patients that Is Reversible and Not Hierarchically Organized. *Cancer Cell* 2010; 18: 510-523.
- Franziska M, Kornelia P. The origins and implications of intratumor heterogeneity. *Cancer Prev Res* 2010; 3: 1361-1364.
- Yoh I, Franziska M. Evolutionary dynamics of intratumor heterogeneity. *PLoS ONE*, www.plosone.org. 2011; 6: 1-8.
- Wei G, Joseph LL, Hong W. Cancer stem cells. *Pediatric Res* 2006; 59: 59-64.
- Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. *Stem Cells* 2008; 1: 267-284.
- O'Brien CA, Kreso A, Jamieson CHM. Cancer stem cells and self-renewal. *Clin Cancer Res* 2010; 16: 3113-3120.
- Hurt EM, Farrar WL. Purification and characterization of cancer stem cells. In: Farrar WL (Ed): *Cancer Stem Cells*. Cambridge University Press, Cambridge, 2010, pp 1-14.
- Pardal R, Clarke MF Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer* 2003; 3: 895-902.
- Borovski T, De Sousa E Melo F, Vermeulen L et al. Cancer stem cell niche: the place to be. *Cancer Res* 2011; 71: 634-639.
- Buchstaller J, Quintana E, Morrison SJ. Cancer stem cells. In: Mendelsohn AC, Howley A, Israel S, Gray JE, Lindsten T (Eds): *The molecular basis of cancer* (3rd Edn). Elsevier, Philadelphia, 2008, pp 141-154.
- Klonisch T, Wiechec E, Hombach-Klonisch S, et al. Cancer stem cell markers in common cancers - therapeutic implications, *Trends Mol Med* 2008; 14: 450-460.
- Honoki K, Fujii H, Tsujiuchi T. Cancer stem cell niche: the role of mesenchymal stem cells in tumor microenvironment. In: Shostak S(Ed): *Cancer Stem Cells-The Cutting Edge*. InTech, Croatia, 2011, pp 189-206.
- Bapat S, Collins A, Dean M, Nephew K, Rasheed S. Cancer stem cells: similarities and variations in the theme of normal stem cells. In: Bapat S (Ed): *Cancer Stem Cells Identification and Targets*. John Wiley & Sons, New Jersey, Canada, 2009, pp 1-26.
- Li L, Xie T. Stem cell niche: structure and function. *Annu Rev Cell Develop Biol* 2005; 21: 605-631.
- Cheng L, Zhang S, Davidson DD, Montironi R, Lopez-Beltran A. Implications of cancer stem cells for cancer therapy. In: Bagley RG, Teicher BA (Eds): *Stem Cells and Cancer*. Humana Press, New York, 2009, pp 255-262.
- Morrison R, Schleicher SM, Sun Y et al. Targeting the mechanisms of resistance to chemotherapy and radiotherapy with the cancer stem cell hypothesis. *J Oncol* 2011; 2011: 941876. Epub 2010 Oct 12.
- Clayton S, Mousa SA. Therapeutics formulated to target cancer stem cells: Is it in our future? *Cancer Cell International* 2011; 11: 1-9.
- Choi D, Lee HW, Hur KY et al. Cancer stem cell markers CD133 and CD24 correlate with invasiveness and differentiation in colorectal adenocarcinoma. *World J Gastroenterol* 2009; 15: 2258-2264.
- Li C, Heidt DG, Dalerba P et al. Identification of pancreatic cancer stem cells. *Cancer Res* 2007; 67: 1030-1037.
- Efstathiou E, Logothetis CJ. Clinical challenges for treatment and cure. In: Pardee AB, Stein GS (Eds): *The Biology and Treatment of Cancer; Understanding Cancer*. John Wiley & Sons, New Jersey, Canada, 2009, pp 155-167.
- Sabisz M, Skladanowski A. Cancer Stem Cells in Drug Resistance and Drug Screening: Can We Exploit the Cancer

- Stem Cell Paradigm in Search for New Antitumor Agents? In: Shostak S (Ed): *Cancer Stem Cells Theories and Practice*. In-Tech, India, 2011, pp 423-442.
23. Schatton T, Frank NY, Frank MH. Solid tumor stem cells - implications for cancer therapy. In: Rajasekhar VK, Phil M, Vemuri MC (Eds): *Regulatory Networks in Stem Cells*. Humana Press, New York, 2009, pp 527-543.
 24. Rich JN. Cancer stem cells in radiation resistance. *Cancer Res* 2007; 67: 8980-8984.
 25. Diehn M, Clarke MF. Cancer stem cells and radiotherapy: new insights into tumor radioresistance. *J Natl Cancer Inst* 2006; 98: 1755-1757.
 26. Sell S, Glinsky G. Preventative and therapeutic strategies for cancer stem cells. In: Farrar WL (Eds): *Cancer Stem Cells*. Cambridge University Press, Cambridge, 2010, pp 68-92.
 27. Wicha MS, Liu S, Dontu G. Cancer stem cells: an old idea--a paradigm shift. *Cancer Res* 2006; 66: 1883-1890.
 28. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer* 2005; 5: 275-284.
 29. Donnenberg VS, Meyer EM, Donnenberg AD. Measurement of multiple drug resistance transporter activity in putative cancer stem/progenitor cells. In: Yu JS (Ed): *Cancer Stem Cells Methods and Protocols*. Humana Press, New York, 2009, pp 261-279.
 30. Naoki O, Xin WW. Novel Therapeutic Strategies for Targeting Liver Cancer Stem Cells. *Int J Biol Sci* 2011; 7: 517-535.
 31. Christopher YP, Diane T, Irving LW. Cancer Stem Cell-Directed Therapies: Recent Data From the Laboratory and Clinic. *Mol Therapy* 2009; 17: 219-230.
 32. Sagrera A, Pérez-Losada J, Pérez-Caro M, Jiménez R, Sánchez-García I, Cobaleda C. Elimination of cancer stem cells. In: Dittmar T, Zänker KS (Eds): *Stem Cell Biology in Health and Disease*. Springer, London, New York, 2009, pp 364-377.