

A letter in response to “Cancer stem cells: emerging actors in both basic and clinical cancer research” Turk J Biol (2014) 38: ©TÜBİTAK – doi:10.3906/biy-1406-93
Cancer stem cells (CSCs): targets and strategies for intervention

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Abstract: This letter to the editor highlights the important aspects of cancer stem cells and provides a deeper insight into the origin and characterization of these cells as well as the mechanisms contributing to drug resistance and relapse. The two currently available models (CSC and the clonal evolution model) need not be mutually exclusive to explain the origin of CSCs. The identification of CSCs (based on surface markers, drug efflux, enzyme activity, and signal transduction molecules and proteins involved in EMT processes) can help in discriminating these cells from other cell types. Moreover, CSCs prefer a hypoxic environment. This microenvironmental cue can trigger certain metabolic alterations that can orchestrate a rewiring of the epigenome. Consequently, changes in transcription factors and signaling molecules can contribute to the plasticity/inter-convertibility of the CSCs and non-CSC cellular states. Hence, an improved understanding of the mechanisms involved also provides opportunities for pharmacological manipulations. This letter also underscores the presence of precancerous stem cells as well as very small embryonic-like stem cells (VSELs) (both considered to be precursors of CSCs). These reports warrant a thorough reanalysis of the source of CSCs (lineage tracing studies), subsequent to the development of better targeted intervention approaches.

Key words: Cancer stem cells, precancerous stem cells, very small embryonic-like stem cells, pharmacological strategies, drug resistance

1. Introduction

The alarmingly increasing incidence of cancer as a major public health burden has necessitated the need to reiterate the importance of identifying and selectively targeting cancer cells so that the efficacy of the therapeutic intervention can be improved with minimal side effects.

The research article entitled “Cancer stem cells: emerging actors in both basic and clinical cancer research” highlights the role of cancer stem cells (CSCs) (a pivotal cell type occupying a specialized niche in the tumor microenvironment and playing a major role not only in terms of tumor initiation but also in recurrence and metastasis) as attractive targets for drug developers and provides fairly concrete evidence for the same. This letter will serve to highlight the important aspects cited therein, with a deeper insight in terms of their origin, detection, and characterization. Further, this letter aims to provide a glimpse into some of the current pharmacological approaches of selective elimination, thereby underscoring the reviewed druggable targets.

2. Origin and models

The relative contribution (singly or in combination) of the different mechanisms to the origin of CSCs (mutations, dedifferentiation of differentiated cells or committed progenitor cells, or due to gain-of-function mutations) would help in refining or identifying new targets for drug development. The CSC concept is discussed in the context of tumor heterogeneity as well as the clonal evolution models, even though both processes may be occurring (albeit to different extents in different tumors). It has been shown that there is functional heterogeneity (the clonal evolution model), especially tolerance to chemotherapeutic agents, despite genetic stability, as postulated by the cancer stem cell model (Kreso et al., 2013). On the other hand, in other cancers the subclones are complex and genetically variegated. The acquisition of copy number changes is independent and reiterative in the subclones of individuals, with no preference with respect to the order (“stochastically varying intrinsic factors”) (Anderson et al., 2011). Apart from etiology, the determination of heterogeneity in cancer stem cell subsets would help in better tailoring the dosage regimen. There

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is evidence that precancerous stem cells (associated with precancerous lesions) may be the precursors of cancer stem cells (found in primary cancer foci) and coevolve with disease progression in terms of pathological features. These primary cells acquire migratory capabilities, and hence can metastasize to sites distant from their origin. These phenotypically heterogeneous cells exhibit differential sensitivity to radio and chemotherapies, and hence present challenges to the oncologist/drug developer in terms of identifying, localizing, and selectively targeting these cells (horizontal versus vertical hierarchy) (Liu et al., 2011). These cells express embryonic stem cell-related genes (Pou1/Otc4, TDGF1, Zfp42/REX1 and Mili). They develop into benign and malignant tumors in immunocompetent and SCID mice, respectively. This concept presents exciting possibilities (for the drug developer) since the malignant versus the benign status of the tumor depends on the potentially modulatable tumor microenvironment, with the possible regulation of piwil2 (Mili) playing a role in this process (Chen et al., 2007).

3. Identification and isolation of CSCs

3.1. Cell-surface markers

It is well recognized that flow cytometry-based surface marker analysis is the method of choice for the identification of CSCs. However, (as recognized by the authors) due to the overlap in marker expression between CSCs and other cell types (plasticity and heterogeneity), it is now known that other proteins would also have to be measured for their unique identification, apart from correlating their expression with proliferation assays (Cheli et al., 2014). On the other hand, label-free (based on microfluidics and/or nanotechnology and/or biophysics) methods for the identification of cells and CSCs are increasingly being reported in the literature to circumvent the need to define and accurately identify a biomarker (Gossett et al., 2010; Uckermann et al., 2014; Lee and Chang, 2014) using immunolabeling methods that have been reported to be unreliable. Apart from their utility in terms of their identification, they can be actively targeted using monoclonal antibodies. For example, monoclonal antibodies against CD44+, expressed in a subset of cells in a 3D culture from the MCF-7 breast cancer cell line, were conjugated to gold nanorods to selectively ablate these cells exploiting the “receptor-seeking” capabilities of the MAB and the photothermal properties of the nanorods at the target site (Lee et al., 2012).

The aforesaid label-free methods can be extended to identify circulating tumor cells (CTCs) (subpopulations of which have been shown to have stem cell-like features (Weller et al., 2014)) and can possibly replace immunolabeling studies, following their validation using conventional immunocytochemical and in situ

hybridization-based approaches. In this regard, it is increasingly being recognized the 4D models may be better models for drug testing (Vishnoi et al., 2014) and their transcriptional, translational, and in vitro characteristics are different from those obtained from their 2D counterparts (Mishra et al., 2015).

3.2. Dye exclusion assays

Dye efflux assays (due to the increased expression of drug efflux transporters, especially in the side population fraction) and flow cytometry-based identification of surface markers are among the currently available methods to identify CSCs. These assays are complemented by the colony and sphere-forming assays. However, the SP fraction is not a unique property of cancer stem cells. Hence, the authors have recognized the need for a marker that is universal for stem cells of all types as well as an “identification tag” unique for CSCs. Further, a blockade of drug efflux transporters [ABCG2 (BCRP), known to be expressed in side population (SP) cells by nanomolar concentration of Tariquidar analogues, causes reversal of drug resistance (Kühnle et al., 2009).

3.3. ALDH activity

The need to identify a universal marker for all types of stem cells, including CSCs, has prompted aldehyde dehydrogenase (ALDH) isozyme profiling (e.g., ALDH1) and correlatable functional studies such as proliferation, adhesion, and apoptosis-based assays (Ma and Allan, 2011). In another neurosphere study, ALDH1A2 was correlated with a less differentiated phenotype that was resistant to 13-cis-RA, and was involved in CSC regulation; it also correlated with poor prognosis (Hartomo et al., 2014). Such approaches would complement the ongoing efforts to use a combination of markers to more accurately identify the source of CSCs and discriminate them from other types of stem cells.

3.4. Anchorage-independent cell culture

As stated by the authors, spheroid cultures are considered to be better models in comparison with 2D cultures, both for modeling cancer as well as for drug testing. Specifically, mammospheres (either from MCF-7 or MDA-MB-231) with an increase in Nrf2 demonstrated anchorage-independent growth, lower intracellular ROS levels, and a relatively increased resistance to chemotherapeutic agents. Brusatol has been shown to inhibit Nrf2, increase sensitivity to drugs, and suppress anchorage-independent growth (Wu et al., 2014). However, as stated by the authors, the obtained results should be validated in vivo since the artificial environment imposes selection pressures that do not accurately mimic the internal milieu of the tumor and its microenvironment.

3.5. Signaling pathways and currently available drug targeting strategies

The authors have summarized the importance of targeting Notch, Wnt/ β -catenin, and Sonic Hedgehog in cancer stem cells. For example, cyclopamine, a SHH antagonist, has been shown to selectively deplete glioblastoma cancer stem cells in contrast to radiation treatment, which targets the more differentiated neoplastic cells, but enriches these stem cells (Barr et al., 2007)!!!

3.6. CSC niche and hypoxia

The authors have stated that the cancer stem cells prefer to reside in a hypoxic environment. This aspect is significant since metabolic alterations (one of the emerging hallmarks of cancer (Hanahan and Weinberg, 2011)) can be important events contributing to the rewiring of the epigenome (including a more permissive chromatin conformation), thereby facilitating the acquisition of the CSC phenotype. This metastemness property is amenable to pharmacological manipulation (Menendez and Alarcón, 2014). More specifically, the Warburg effect may be acting as a metabolic facilitator for the transcription factors and signaling molecules that orchestrate the signals (intrinsic and/or microenvironmental – for example, hypoxia as stated by the authors) for convertability into a cellular CSC state (Menendez et al., 2013). Apart from hypoxia, a blockade of angiogenesis (one of the hallmarks of cancer) using MAB-based biopharmaceutical approaches (anti-VEGF antibody) can contribute to a decrease in the survival of CSCs and reduce drug resistance. In this regard, Bevacizumab, the only FDA-approved drug targeting VEGF, is useful in terms of contributing towards progression-free survival without any other beneficial changes in the overall survival (Patel et al., 2012).

3.7. CSCs and EMT

The authors have reiterated that the acquisition of stemness can correlated with the expression of mesenchymal markers like vimentin, fibronectin, and N-cadherin, and the loss of E-cadherin expression as observed in cancer cells (Suresh and Nathawat, 2014). It has been opined that one of the mechanisms for Her2-positive breast cancer stem cells resistance to trastuzumab therapy involves EMT processes in this cell type. Hence, this provides a sound, scientific basis for developing novel HER2-based therapies to circumvent this type of drug resistance (Bedard et al., 2009).

4. VESLs: descendants of PGCs and precursors to stem cells and cancer stem cells?

This paper has overlooked the existence (albeit controversial) of VESLs, which are less than 5 μ m in size. These cells exist in adult body organs and the expansion of these cells may obviate the need to reprogram adult cells. These cells do not satisfy all the criteria for stem cells. They

do not form teratomas in immunocompromised mice and do not complement blastocysts. They are relatively quiescent and their inability to form teratomas may be due to their differential methylation patterns (erasure of paternal imprinting – H19-Igf2, RasGRF1 loci, and hypermethylation of maternally imprinted genes). These cells were first discovered in the murine bone marrow (CXCR4⁺/Oct-4⁺/SSEA-1⁺/Sca-1⁺/Lin⁻/CD45⁻) by scientists led by Dr Ratajczak's research group from the University of Louisville, Kentucky (USA), even though corroborative evidence has to be obtained in suitable model systems. Apart from the bone marrow, these cells have also been found in several adult organs (brain, kidneys, muscles, and the pancreas) in the same model system (Ratajczak et al., 2008; Zuba-Surma et al., 2008). They are thought to have originated from the migratory primordial germ cells and were deposited in various organs during the development of the embryo. Mobilization of these cells has been reported in various disease conditions and could be involved in neoplasia development. This can be inferred from the fact that VSEL express markers are common with primordial germ cells (PGCs) (Suresh, 2014) as well as those cells found in the epiblast/germ line. Formation of germinal tumors may have occurred due to an error in the normal migratory patterns of developmentally early PGCs (closely related to VSELs) to the genital ridges. Further, VSELs, like cells in various tumor cell types, express cancer testis (C/T) antigens that have a restricted germ line expression. Hence, these cells could be the origin for the C/T expressing tumors. Germline VSELs, like the epiblast, the primordial stem cells, and the embryonic stem cells, also express Oct-4, a transcription factor that is also expressed in several tumors. Hence, it can be inferred that the VSELs could be a source for the development of malignant cells. The acquisition of the aforesaid critical epigenetic changes (hypermethylation of the DMR in the Igf2-H19 locus or Rasgrf1) in VSELs may lead to the development of certain pediatric sarcomas. This can be inferred from the reported coincidence of such Oct4⁺ cells postnatally in pediatric and young adult patients with tumors. Fairly conclusive evidence has been obtained in patients with the Beckwith–Wiedemann syndrome, who frequently develop sarcomas (e.g., neuroblastoma or rhabdomyosarcoma). Molecular analysis has shown a loss of imprinting of the H19-Igf2 locus. An increase in pro-proliferative Igf2 levels, concomitant with a decrease in growth inhibitory H19 protein levels, may be a contributory factor to the initiation and development of malignancy. Further, these patients have a cell cycle kinase inhibitor (p57^{KIP2}) that is downregulated. This overexpressed protein has to be important for controlling quiescence in VSELs. It has also been hypothesized that Oct4⁺ VSELs may fuse with somatic cells (heterokaryon formation at the wrong time),

and precede the development and selection of aneuploid, immortal, malignant cell clones with an unstable karyotype. These stem cells may provide early development markers (e.g., Oct4) while their somatic partners may be responsible for proper genomic imprinting. Last but not least, the authors' statement that CSCs prefer a hypoxic environment, unlike stem cells that use glycolysis for a lot of their energy needs, may also be reconcilable with the existence of VSELs. It is possible the VSELs could be recruited to the hypoxic microenvironment, wherein the

vasculature and the stroma may provide the necessary inductive signals for their expansion. In both examples, VSELs (normally recruited for regenerative purposes) are erroneously mobilized to the areas of chronic inflammation and tumor microenvironment, respectively.

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