Tissue-specific cancer stem cells: reality or a mirage?

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Abstract

Equo ne credite, Teucri. Quidquid id est, timeo Danaos et dona ferentes (*Do not trust the horse, Trojans! Whatever it is, I fear the Greeks, even bringing gifts*) said Laocoön (Virgil, the *Aeneid book*). Cancer stem cells (CSCs) are populations of cancer cells that can be found in different cancerous tissues and organs, and have properties that are similar to normal stem cells. They are thought to be chemo-resistant and radio-resistant and are therefore responsible for cancer recurrence and relapse encountered in cancer patients following chemotherapy and radiotherapy. Although significant progress has been made to characterise CSCs, it is becoming clear that the failure of cancer therapies directed against certain types of aggressive cancers is due to the presence of these malignant cells. Cancer therapies that will rely on a combination of CSCs-targeted therapies, chemotherapy and radiotherapy are more likely to succeed in eradicating aggressive cancers and prevent recurrence in treated patients.

Introduction: the Trojan horse

One of the most challenging aspects of successful cancer therapies relates to the fact that within cancerous tissues, different populations of cancer cells exist and contribute to the heterogeneous feature of tumors and, consequently, offer different potential target molecules for treatment. However, there is an increasing body of evidence that a small percentage of cancer cells known as cancer stem cells (CSCs) or cancer initiating cells have a profound impact on the development of aggressive life-threatening disease. CSCs are considered by many scientists to i) be the primary drivers of cancer initiation by promoting the transition from pre-malignant to malignant tissue; ii) play a key role in invasion and metastasis that are associated with cancer progression; iii) be responsible for the disease recurrence in patients with cancer following chemotherapy or radiotherapy. Since the first experimental identification of CSCs in acute myeloid leukaemia cells,[1] several studies have identified CSCs in solid tumors including breast,[2] brain,[3] melanoma,[4] prostate,[5] ovarian,[6] gastric,[7] lung,[8] and pancreatic[9] cancers. Like their normal stem cell counterparts, CSCs are characterised by their capacity for self-renewal and for the generation of differentiated progenies. These processes are responsible for driving tumorigenesis; and promoting tumor growth and metastasis.[10] Another important aspect that is associated with CSCs, is the presence of oncogenic-related mechanisms that allow CSCs to resist cancer therapies and therefore contributes to tumor aggressiveness.[11,12] CSCs can enter a dormancy state which limits the effect of chemotherapeutic compounds that target proliferating cells. They also express several molecules such as the ATP-binding cassette family of transporters (e.g. ABCG2) that pump-out chemotherapeutic compounds from cells. They have elevated levels of ALDH1 (Aldehyde Dehydrogenase) enzymatic activity. This cytosolic enzyme oxidises aldehydes and converts them into carboxylic acids providing CSCs with further chemoresistence. CSCs also have a high DNA damage response, which allows them to resist radiotherapy- and chemotherapy-mediated damage. Another mechanism relies on high expression of the pro-survival BCL-2 protein family members that bind to the pro-apoptotic proteins BCL2-associated-X-protein (BAX) and BCL-2 homologous antagonist killer (BAK) and impair their ability to release apoptogenic proteins such as cytochrome c from the mitochondria. To prevent CSC-mediated chemoresitance and recurrence in patients suffering from aggressive cancers, it is essential to combine traditional chemotherapy and radiotherapy with efficient CSC-targeted therapies.

Stem cells *versus* cancer stem cells

To define cancer stem cells, it is essential to provide a definition of what are stem cells. Stem cells are undifferentiated and pluripotent cells that are capable of self-renewal and of generating differentiated cells. They are at the origin of all tissues and organs within the body and, during adulthood, contribute to tissues homeostasis and maintenance. In early embryogenesis, embryonic stem cells (ESCs) that are found in the inner cell mass give rise to the three germ layers (ectoderm, mesoderm and endoderm) that in turn, generate tissue-specific stem cells. These cells are at the origin of somatic stem cells (SSCs) that play an essential role in tissue regeneration following cell death or organ damage. Although SSCs possess stem cell properties, they are only capable of generating tissue-specific progenies. They can also be hierarchically organised into a stem cell, progenitor cells and mature cells (Figure 1). The progression from a stem cell to mature cells is tightly regulated by tissue-specific environmental cues that act in a *Start-Break* fashion, and depending on tissue requirement for maintenance and regeneration. In cancer, the process of transformation of healthy cells into cancer cells is triggered by the accumulation of genetic mutations affecting key pathways that are involved in the control of cell proliferation, differentiation and apoptosis. In a similar fashion, mutations affecting somatic stem cells would result in the generation of cancer stem cells (Figure 1). It is not unlikely that mutations that may affect non-CSC could also generate CSCs-like cells as a result of oncogenic acquisition of stem cell-like properties. In addition, it has been shown that non-CSCs could convert into CSCs through inflammatory stroma that activates NF B signalling, which enhances Wnt signalling and induces dedifferentiation of non-CSCs with gain of tumor-initiating capacity.[13] Another aspect to consider is the potential role of the microenvironment in promoting the transformation of normal stem cells into cancer stem cells. Recent studies have implicated

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epigenetic alterations that are initiated by signals from the tumor microenvironment and which may exert oncogenic *selection* on normal stem cells leading to a dysfunctional emergence of CSCs.[14] Although further investigations are required to better characterise CSCs, these malignant cells seem to be generated from mutations or epigenetic alterations that affect normal stem cells and cancer cells.

Cancer stem cells, tumor microenvironment and cancer progression

CSCs exhibit many of the characteristics associated with cancer stem cells that have undergone epithelial-mesenchymal transition (EMT).[15] The role of EMT in tumor invasion and metastasis has been well studied and it is now evident that this event is initiated by contextual signals present in the tumor microenvironment. Epithelial cells are characterised by their baso-apical polarity that is maintained by cell-cell junctions including desmosomes, adherens and gap junctions. When EMT is engaged, epithelial cells lose their polarity via junctions' disassembly that is mediated by the downregulation of epithelial gene expression (such as E-Cadherin) and upregulation of mesenchymal gene expression (such as Vimentin and N-Cadherin). This process results in the generation of mesenchymal-like cells capable of migration, invasion and metastasis. In cancer, EMT is promoted by the tumor microenvironment, where cellular constituents such as CAFs (cancer associated fibroblasts), MDSCs (myeloid-derived suppressor cells), soluble growth factors and cytokines, and hypoxic and acidic conditions, induce pro-EMT intracellular signalling pathways (TGFβ, Wnt/β-catenin, NFκB and Notch). This results in the transdifferentiation of carcinoma cells into mesenchymal cancer cells. Interestingly, many of these signals are also found associated with CSCs. As an example, the activation of EMT by TGFβ and the transcriptional activators Snail1, Twist1 and Zeb1 in normal epithelial cells results in the acquisition of CSCs traits.[16] Another example is the Wnt/β-catenin pathway, which plays an important role in the maintenance of CSCs, is also a critical inducer of EMT in carcinomas. Although the transformation of epithelial cells into mesenchymal ones necessitates EMT, this process may not be the only cellular mechanism that is involved in CSCs-associated invasion and metastasis. CSC-generated hierarchy of stem-like and differentiated tumor cells, also known as tumor cell plasticity, can initiate metastatic growth and is seen in late-stage cancers and at metastatic sites.[17] It is not impossible that CSCs may generate progenies that lack cell-cell adhesions and that are capable of migration, invasion and metastasis. It will be interesting to investigate if some tumor circulating cells have characteristics that are not associated with the mesenchymal phenotype. Finally, cancer cell autonomous functions such as invadopodia formation, paracrine factors as VEGF and Epidermal growth factor (EGF) family members, proteases as MMPs and cathepsins, and recruitment of stromal components and immunosuppressive cells as TAMs can also promote CSCs dissemination.[18]

Burning the Trojan horse: are we there yet?

Although significant advances in defining markers that can identify CSCs in solid tumors have been made, issues remain

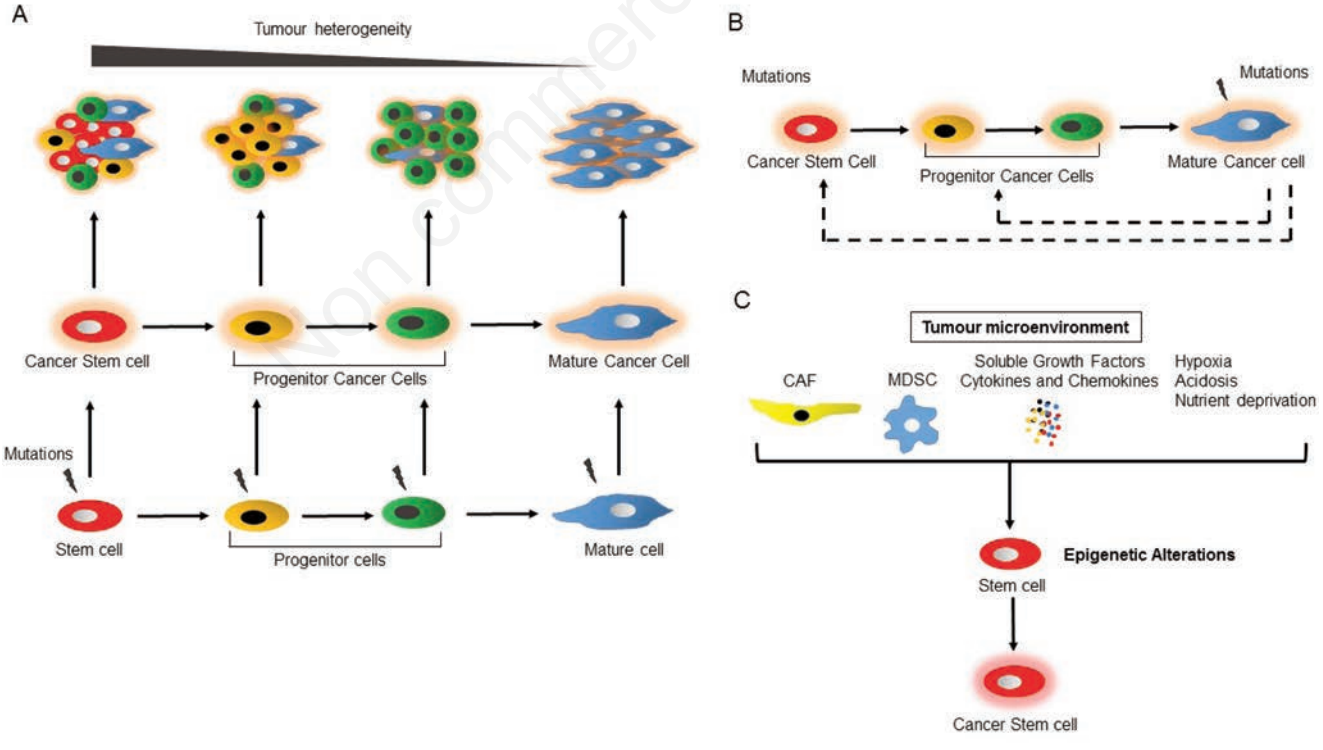

Figure 1. Potential cellular mechanisms involved in the generation of cancer stem cells (CSCs). A) Stem cells generate progenitor cells that produce mature cells. Mutations in stem cells or their progenitor cells may result in the generation of CSCs or progenitor cancer cells. Both types may have the capacity for self-renewal and for the generation of differentiated progenies. B) Mutations in mature cells may initiate their dedifferentiation in CSCs-like cells. C) The tumor microenvironment could initiate epigenetic alterations in normal stem cells inducing their transformation into CSCs.

about their specificity for CSCs.[19] For example, although CD44 and CD133 have been used as markers of CSCs, these markers are also expressed by multiple cell types, including sub-populations of stromal cells and interstitial cells such as immunostimulatory cells. These observations raise questions about their suitability as CSCs markers and limit their potential use as targets for cancer therapies among scientists and clinicians working in the field. Several therapeutic approaches[20] have been used to target CSCs. First, antibodies targeting cell surface markers that are widely expressed by CSCs and play a role in cancer proliferation, survival, migration and invasion. These include molecules such as the cell surface glycoprotein prominin-1 (CD133), the hyaluronic acid receptor CD44 or the transmembrane protein receptor (CD47). Second, targeting signalling cascades that are involved in self-renewal and differentiation and define *stemness* such as Notch, Hedgehog or Wnt/ -catenin pathways. Third, manipulation of the expression of miRNAs that are involved in the maintenance of stemness and tumor progression (e.g. miR-21, miR-34, miR-124). Fourth, targeting ABC cassette transporters. These molecules are abnormally expressed by CSCs and are responsible for driving drug efflux and therefore play an important role in CSC-associated chemoresistence. Fifth, inducing CSC differentiation using molecules such as retinoic acid and histone deacetylase inhibitors. Sixth, targeting the CSC microenvironment. This relies on targeting chemokines and their receptors (e.g. CXCL12-CXCR4), or molecules such as the vascular-endothelial growth factor (VEGF). Seventh, induction of CSC apoptosis by activating cell death receptors (e.g. TRAIL, CD95) or inhibiting survival pathways. Common limitations of these new advances are: i) the molecules and pathways targeted are not specific to CSCs and are found in healthy tissue and normal stem cells, hence the higher toxicity encountered using these therapies; ii) lack of thorough understanding of cellular pathways regulating CSCs role in cancer initiation/progression, and their interactions with the microenvironment (such as immune system, angiogenesis, hypoxia). It is therefore essential to identify CSC-specific targets that are key to successful development of therapies and that are based on robust and reliable methods of identification. This step is inseparable from a better understanding of the CSC *niche*.

Conclusions

Although the existence of CSCs in multiple human tumors has been firmly established, unique biomarkers that could be used to identify CSCs for patient clinical evaluation (diagnostic, prognostic and predictive), and that could also be used for targeted therapies is still a major challenge for scientists and clinicians working in this exciting field. CSCs express many antigens that are present in normal stem cells and in other tissues of the body, and attempts that aim at targeting them would result in increased toxicity with potential and life-threatening consequences for treated patients. The identification of new biomarkers that are truly specific to CSCs will certainly lead to more efficient therapies. In this regard, large scale analysis of tumor samples from different patients and cohorts using genomics, proteomics and data mining approaches (Bioinformatics) could lead to the identification of CSCs-specific genes or antigens signatures that will open new avenues for the development of CSCs targeted therapies. Although *in vivo* studies that rely on mice xenotransplantation from patient isolated cancer cells have certainly made significant advances for our understanding of cancer stem cell behavior, these studies can only provide partial information if identified biomarkers are not clinically validated. Finally, it is an exciting time for many scientists to be working in this challenging field of cancer research and progress will certainly be made in the future with significant impact on cancer therapy.

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