CANCER STEM CELLS: A MYTH OR REAL TARGET

Snezana Matic

Center for Molecular Medicine, Faculty of Medicine, University of Kragujevac, Serbia

KANCERSKA STEM ĆELIJA, OD MITA DO STVARNOSTI

Snežana Matić

Centar za Molekulsku Medicinu, Medicinski Fakultet, Univerzitet u Kragujevcu, Srbija

ABSTRACT

Ever since the 17th century, determining which cells are able to produce tumours has been a key question in cancer biology. The answer seemingly lies somewhere between the postulates of the stochastic and hierarchical hypotheses, which is to say that hierarchical order exists in both normal tissue and tumours, that both stem cells and differentiated cells (after dedifferentiation) can give rise to several cell lineages, and that both stem cells and mature cells can mutate. It has been found that tumours of various types contain a small percentage of chemotherapy- and radiotherapy-resistant tumour cells, which are long-lived and capable of self-renewal, much like normal stem cells. These cells, which are capable of regrowing tumours, were named "cancer stem cells" (CSCs). According to recent findings, CSCs are genetically and phenotypically similar to normal stem cells and represent the only cell population within tumours that can completely regenerate the original tumour following transplantation. They are the only malignant cells that can be grown as spheres in non-adherent, serum free cultures (a unique characteristic of stem cells). Normal stem cells and CSCs both reside anchored in specifically organized microenvironments, or niches, which modulate their behaviour and determine their destiny. CSCs are highly metastatic and resistant to conventional tumour therapies.

Conclusion. CSCs and normal tissue stem cells share most of their signalling and self-renewal pathways. The only way to overcome CSCs may be to discover their unique regulatory mechanisms and attempt to block these pathways using targeted therapies. Given this conclusion, additional investigation should be performed.

Key words: cancer stem cells, isolation, stem cell niche

Zaključak. Kancerske stem ćelije i stem ćelije normalnog tkiva su slične u vezi s biologijom. Možda je jedini način da se kancerske stem ćelije savladaju, terapija usmerena na jedinstvene regulatorne mehanizme ovih ćelija. Iz tog razloga je neophodno nastaviti istraživanja u ovom pravcu.

Ključne reči: kancerske stem ćelije, izolacija, stem cell niche

Correspondence: Snezana Matic, Center for Molecular Medicine, Medical Faculty, University in Kragujevac, Svetozara Markovica Street 69, 34000 Kragujevac, Serbia, tel: +381 64 303 53 98

Received / Primljen: 11. 09. 2010. Accepted / Prihvaćen: 22. 11. 2010.
INTRODUCTION

Determining the cell type with the capacity for carcinogenesis is a central concern in cancer biology. There are two hypotheses that attempt to address this issue: the stochastic model proposes that specific events in a tumour cell population have the potential to transform any tumour cell into a tumour-initiating cell, while the hierarchy model proposes that a limited number of cells, termed cancer stem cells (CSCs), are capable of initiating a heterogeneous tumour (1). The latter hypothesis proposes that a small subset of cells is responsible for the initiation, proliferation and metastasis of a tumour. Furthermore, these cells are resistant to radiotherapy and many chemotherapeutical agents and are the basis for tumour regrowth in patients with relapsed disease following therapy (2). It is widely accepted that CSCs are equally capable of arising from either mutated early stem cell progenitors (through the acquisition of epigenetic and genetic changes required for tumourigenicity) or mutated mature, more differentiated cells (through dedifferentiation/trans-differentiation) (3). Given their potential role in tumourigenesis, CSCs are important targets for therapy. The ability to specifically target pathways that are dysregulated in cancers raises the hope of developing therapies with enhanced specificity and decreased toxicity (4). The CSC hypothesis has played an essential role in our understanding of carcinogenesis, and in the development of new approaches for cancer prevention and the treatment of advanced disease.

DEFINITION OF A STEM CELL

Stem cells are cells with the capacity to self-renew (symmetrical division) and to generate daughter cells capable of downstream differentiation into several cell lineages to form all of the cell types that are found in mature tissue (asymmetrical division) (5). There are essentially two types of stem cells: embryonic (pluripotent stem cells with the potential to give rise to any cell of the organism) and adult (multipotent stem cells with multilineage potential that can give rise to any cell from a particular tissue or organ). Adult stem cells can be further divided into stem cells responsible for tissue renewal (cells from the bone marrow, skin, or intestine, which are continually active), and stem cells responsible for tissue repair (satellite cells of muscle, or putative liver stem cells, which are inactive until required in response to environmental factors) (6).

All stem cells have the following unique characteristics: a) they are present in very small numbers within specific tissues; b) they express specific cell surface markers, which enables their isolation; c) they, and their progeny, are organized hierarchically within their tissue of origin; d) they may be phenotypically homogeneous but functionally heterogeneous; e) they are mitotically quiescent; f) they give rise to all the terminally differentiated cell types within a tissue; g) they can self-renew to give rise to new, functionally identical stem cells; h) they have cell fusion properties; and i) they are long lived cells (7, 8, 9).

DISCOVERY OF CSCs

In the 17th century, Georg Ernst Stahl, a German chemist and physician, speculated that cancers contain self-propagating seeds that often remained in the body after surgery (10). In the 19th century, Rudolf Virchow, a German anthropologist and doctor (cited as the first person to recognize leukaemia cells), observed that each cell stems from another cell ("Omnis cellula e cellula"), which provided the basis for the concept that cancer is a disease that originates from an immature cell (11). At the same time, Julius Friedrich Cohnheim, a German pathologist and Virchow’s assistant, proposed the theory of "Embryonal rests". He postulated that excess germ cells from embryonic development subsequently develop into cancers and possibly link the origin of life to its end. He also proposed that cancers retained the embryonic capacity for cell division and unrestrained growth (12). In 1961, Till and McCulloch provided the first experimental models that suggested the existence of normal blood stem cells. Their reports were based on a novel method for the detection and enumeration of multipotent hematopoietic stem cells, and another method for the determination of the number of hematopoietic stem cells required to restore blood production (13). In 1963, Bruce and Van Der Gaag demonstrated that, analogous to normal bone marrow stem cells, only a minority of malignant blood cells could form colonies in the spleen of a mouse (14). In 1967, Fialkow et al. showed a clonal origin of chronic myelocytic leukaemia (CML) (15). In 1990, Fialkow’s studies on CML and acute leukaemia provided the first conclusive evidence that a single progenitor cell can give rise to replicating clones that sequentially acquire additional mutations and create a tumour. The data from these studies suggested that a pluripotent stem cell is initially transformed, and that this transformed cell then produces malignant clonal progeny (16). In 1997, John Dick and colleagues characterised acute myeloid leukaemia (AML) stem cells and showed that only a small subpopulation of leukemic cells was capable of initiating leukaemia upon serial transplantation in the NOD/SCID mouse model. These cells, designated SCID leukaemia-initiating cells (SL-IC), express immunophenotypic markers that distinguish stem cells (e.g., CD34+CD38-), suggesting that the initial transformation event occurred in a stem cell rather than a committed progenitor cell (7, 17). Following the example of the leukaemia studies, investigators have also isolated cells with stem cell-like features from solid tumours. One of the first such studies was conducted in 2003, when Al-Hajj et al. reported that the CD44+CD24–low cell fractions from metastatic pleural effusions and a primary invasive breast carcinoma had significantly higher tumourigenic potential than the CD44+/-CD24+ cell fractions when injected into the mammary fat pad of female NOD/SCID mice (18). In the same year, the Dirks group, using the neurosphere culture
technique, discovered cancer stem cells in the CD133+ fractions of brain tumours of different phenotypes (19). Continuing their investigations, in 2004, the same group reported the functional identification of human brain tumour CSCs (20). By measuring the ability of cells to form tumours in the brains of NOD/SCID mice, they found that only the CD133+ cell fraction was capable of regrowing the original brain tumour. In contrast, the CD133- cell fraction failed to form tumours, even when 1000 times as many cells were injected into the brains of mice (20). These findings have been followed by the isolation of potential CSC fractions from other solid tumours, including lung cancers (Sca-1+CD34+Lin-) (21), ovarian carcinomas (CD44+CD117+) (22), prostate cancers (CD44+/α2β1, CD133+) (23) and colon cancers (CD133+) (24).

Taken together, these data suggest that the CSCs share similar markers with the progenitors from their original tissue, as predicted by Virchow’s law: *Omnis cellula e cellula.*

**STEM CELL AND CANCER STEM CELL NICHES**

Stem cells reside in a specialized supportive microenvironment, or niche, which differs depending on the tissue type (25). The niche serves as a physical anchoring site for stem cells, and interactions between the cells and the extracellular matrix (E-cadherin, β-catenin, integrins) in the niche play a major role in controlling their behaviour. These microenvironments contain several extrinsic factors and developmental regulatory signalling molecules (e.g., Hh, Wnts, BMP, FGF and Notch), which control stem cell number, differentiation and fate determination. Under normal conditions (at least in the hematopoietic, intestinal, and hair follicle systems), the niche inhibits stem cell proliferation and growth (promoting a quiescent state), and this maintenance of the delicate balance between proliferative and antiproliferative signals is a cornerstone of tissue homeostasis (25-27). In flies, genetic ablation of the germ line stem cell niche results in a loss of stem cells (28). In mice, increasing the size of the niche leads to an increased number of hematopoietic cells (29). There is evidence that the niche microenvironment can induce stem cells from nearby daughter cells if the stem cells are depleted (30). It has been shown that deregulation in the mammary gland stem cell niche leads to abnormal expression of TFFa, resulting in the development of breast cancer (31). Though the niche may act to maintain stem cells in a quiescent state for decades, these cells are highly dynamic once activated: an embryo develops from a single cell in 9 months, the intestine regenerates rapidly and constantly, and the liver recreates itself within a few days after partial hepatectomy (32). It therefore appears that stem cells niches may represent microenvironments that control tumourigenesis.

Studies using spontaneously arising tumours in rodents have shown that the number of cells required to successfully transplant a tumour into a syngeneic recipient depends on the specific location and tissue environment of the transplant, and on whether heavily irradiated tumour cells (feeder cells) are injected together with the viable tumour cells (the Ravesz effect) (33). The kidney capsule has been shown to be a highly receptive site for tumour cells, and the induction of inflammation at the injection site can modify the efficacy of tumour cell transplantation (34). Recent studies have demonstrated that efficiency of tumour cell transplantation can be increased when tumour cells are injected with Matrigel (a basement membrane-like substance containing growth factors) (35, 36). The mouse teratocarcinoma model provides a fascinating framework for studying the contribution of the cellular microenvironment to oncogenesis. It is possible to derive normally-functioning cells from teratocarcinoma cells, after the latter are introduced into a normal blastocyst environment (37). Experiments have demonstrated that Rous sarcoma virus causes tumour formation when injected into the wings of adult chickens but does not do so when injected into chick embryos. Viral particles were found to be expressed in most organs of the infected embryos but were not tumourigenic. However, if the infected embryos were dissociated and placed in culture, extensive transformation occurred within 24hr (38). Several studies have identified extracellular signals from the microenvironment as being potentially oncogenic. Inappropriate expression of different metalloproteinases (MMPs) leads to a loss of the tissue microenvironment and the generation of tumours (39). Studies involving the genetic manipulation of stromal cells have indicated that mutations in neighbouring cells, rather than in the tumour cells themselves, can serve as the initial basis for tumour formation (40). Stromal cells have been shown to acquire unique chromosomal rearrangements relative to the tumourigenic epithelium in some mammary carcinomas (41), and the higher incidences of cancer in carriers of certain heritable diseases have been shown to be due to stromal defects (42). Experiments with the HMT-3522 luminal epithelial cell line (isolated from a reduction mammoplasty) have shown that tumourigenic cells can retain their aberrant genome but revert to a normal phenotype if tissue polarity is restored. These cells were used to derive mutated S1 cells that do not have tumour-forming potential when injected into NOD/SCID mice or cultured in 3D laminin-rich basement membrane. Extensive passing of S1 cells in the absence of EGF was able to derive a non-polarized T4-2 cell population that could form tumours in mice. Analysis of these T4-2 cells has shown that they have a number of altered signalling pathways: EGFR, MAPK, PI3 kinase and β1 integrin were highly active, and PTEN was downregulated. Treating T4-2 cells in laminin-rich 3D gels with blocking antibodies or pharmacological agents that reduce signalling through these key pathways causes formation of phenotypically normal acinus-like structures (i.e., the cells become less tumourigenic) (43). It is possible that tumour therapies that disrupt the stem cell niche, through ablation of the surrounding differentiated cells, could lead to the subsequent death of the cancer stem cells (44, 45).
TECHNIQUES FOR ISOLATION OF CSCS

A critical issue in the investigation of CSCs is the isolation of sparing cancer cells, with an unlimited potential for growth, from tumours. There are a few techniques that are commonly used with aim of studying CSCs: a) the side population (SP) technique; b) isolation based on surface marker expression; c) the ALDEFLUOR assay; d) in situ detection; and e) the anchorage-independent cell culture technique.

The SP technique, based on the ability of stem cells to exclude vital dyes, has been used for many years to isolate both normal and tumour stem cells from various organs and species. Normal stem cells and CSCs express transmembrane transporters, such as the ATP-binding cassette (ABC) transporter ABCG2/BCRP1 (breast cancer resistance protein 1). These molecules allow stem cells to exclude dyes, such as Hoechst 33342 and Rhodamine 123, a property not found for these dyes (as detectable by flow cytometry). However, functional studies using Hoechst staining are limited by the toxicity of this agent (49). The expression of cell surface markers has been widely used to isolate stem cells, but the choice of markers can vary greatly depending on the tissue or species. The following phenotypic marker combinations have been used in studies of breast CSCs: CD44+/CD24−/low/Lin− (the first characterised CSC fraction from a solid tumour) (18); CD44+/CD24−/low/Lin−/ALDH1+ (identified as being more tumourigenic than the former fraction) (50); and CD44+/CD24−/low/Lin+, together with combinations of CD10, MUC1 and ESA markers (to determine the compartment from which the isolated cell originated) (51). Other phenotypic markers have been used to identify CSCs in other tissues: CD133 for brain (20, 52) and colon CSCs (1, 24, 53); the CD44+/α2β1 phenotype for prostate CSCs (23, 54); the Stro-1+/CD105+/CD44+ phenotype (with activated STAT3, and expression of Oct3/4 and Nanog) for bone sarcoma CSCs (55); CD44 and CD117 (c-kit) for ovarian carcinoma CSCs (22); CD20 for skin carcinoma CSCs (56) Sca-1/CD34 together with the negative expression of lineage markers for lung carcinoma CSCs (21, 57); and the CD34+/CD38+/CD90+/IL-3R+/CD71+/HLADR+/CD117+ phenotype for AML CSCs (58) (CSC phenotypic markers are summarized in table 1). Taken together, these data suggest that CSCs often share similar markers with the progenitor cells of their original tissue. The ALDEFLUOR assay may fit the universality required for the reliable identification of CSCs across all species and tissues. It is based on the enzymatic activity of aldehyde dehydrogenase 1 (ALDH 1), a detoxifying enzyme responsible for the oxidation of retinol to retinoic acid. ALDH 1 may have a role in early stem cell differentiation. It has been shown that ALDEFLUOR positive CSCs are capable of differentiating into multiple lineages in vitro and have a higher capacity to engraft following transplantation in vivo (in comparison to ALDEFLUOR negative CSCs) (59, 60). In situ detection of CSCs has a future in routine clinical practice for patient treatment and prognosis evaluation. This technique makes it possible to detect ALDH 1 expression in formalin-fixed, paraffin-embedded tissue with immunostaining, while also performing double immunostaining using antibodies specific to CD44 and CD24 (60, 61). The anchorage-independent cell culture technique was adapted to grow cancer cells with the capacity for independent growth under serum-free conditions (a property of stem cells). Cell culture under non-adherent conditions was initially adapted to normal breast tissue obtained from reduction mammoplasty. Human mammary stem and progenitor cells were able to survive in suspension and produce spheroidal colonies (mammospheres) composed of both stem and progenitor cells. To date, culturing cells under non-adherent conditions has been adapted for the cultivation of CSCs from various cancers, including breast cancers (mammospheres), bone sarcomas (sarcospheres) and brain tumours (neuospheres). (62, 63).

Table 1. Cancer stem cell markers in some tumours.

<table>
<thead>
<tr>
<th>Type of tumour</th>
<th>Cancer stem cell marker</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) First characterised</td>
<td>CD44+/CD24−/low/Lin−</td>
<td>18</td>
</tr>
<tr>
<td>b) More tumourigenic phenotype</td>
<td>CD44+/CD24−/low/Lin−/ALDH1+</td>
<td>50</td>
</tr>
<tr>
<td>c) Compartment markers</td>
<td>CD44+/CD24−/low/Lin+, in combination with CD10, MUC1, or ESA</td>
<td>51</td>
</tr>
<tr>
<td>Brain cancer</td>
<td>CD133+/nestin+</td>
<td>20, 52</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>CD133+</td>
<td>1, 24, 53</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>CD44+/α2β1+/CD133+</td>
<td>23, 54</td>
</tr>
<tr>
<td>Bone sarcoma</td>
<td>Stro-1+/CD105+/CD44+ (with activated STAT3, and expression of Oct3/4 and Nanog)</td>
<td>55</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>CD44+/CD117(c-kit)+</td>
<td>22</td>
</tr>
<tr>
<td>Skin carcinoma</td>
<td>CD20+</td>
<td>56</td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td>Sca-1+/CD34+/Lin+</td>
<td>21, 57</td>
</tr>
<tr>
<td>AML</td>
<td>CD34+/CD38+/CD90+/IL-3R+/CD71+/HLADR+/CD117+</td>
<td>58</td>
</tr>
</tbody>
</table>
Figure 1. Signaling pathways in stem cells and CSCs.

- Yellow arrow - signalling pathway through SHH.
- Red arrow - β-catenin/Wnt signalling pathway.
- Blue arrow - NFκB signalling pathway.

Abbreviations:
- SMO – G-protein-coupled receptor (with the PATCH protein), receptor for sonic hedgehog (SHH) proteins.
- Wnt – Wg (wingless) and Int (“wint”), involved in development of Drosophila melanogaster.
- GLI-like – transcription factor.
- β-catenin – cadherin subunit.
- Myc/Mad/Max – group of transcription factors
- LEF-1/Tcf – transcription factor.
- NFκB – transcription factor.
- IκB – inhibitor of NFκB
- cycD – cyclin D-family of proteins controlling cell cycle.
- c-myc – transcription factor.
- bcl-2 – antiapoptotic protein.

It should be noted that all of the above methods are still insufficient to separate these CSC populations, with high specificity, from the rest cells in the tumours. Thus, this field requires further investigation develop methods to precisely characterise and isolate CSCs.

**MOLECULAR SIGNALLING PATHWAYS IN CSCs**

Signaling pathways, such as Hedgehog (Hh), Wnt/β-catenin and Notch, that play a role in embryogenesis and organogenesis, also play a role in the maintenance of adult tissues by regulating the balance between stem cell self-renewal and differentiation (64). Because both normal stem cells and CSCs must renew themselves, it is reason-
able to assume that they share some molecular mechanisms that regulate this critical stem cell function. Mutation of the SHH (Sonic hedgehog) locus causes Gorlin’s syndrome (65), whereas activation of SHH has been implicated in skin, breast and brain carcinogenesis (66, 67). The Wnt/β-catenin pathway is involved in the maintenance of normal intestinal epithelial cells and in regenerative processes during tissue repair. Wnt inhibitors retard hematopoietic reconstitution in vivo. Wnt signalling increases the expression of HoxB4 and Notch-1, both of which have been implicated in self-renewal. Wnt signalling also plays a role in blood diseases, colon (activating mutations of β-catenin, or inactivating mutations of APC) and breast carcinomas (β-catenin accumulation) (68).

The Notch (Notch 1-4) signalling pathway is well conserved from nematodes to humans and regulates homeostatic processes in almost all tissues in organism. Notch mutations can cause T-cell acute lymphoblastic leukaemia and breast carcinoma (in the case of Notch 3 and 4). It was found that Bmi-1 (PCGF4), a member of the Polycomb-group protein family, is responsible for the self-renewal of hematopoietic stem cell (HSC), neural stem cells and leukaemia stem cells (LSCs) in mice (69). In mouse models, aberrant expression of Hox genes affects the proliferation and differentiation of HSCs. Overexpression of HoxB6 culminates in AML, suggesting that genes responsible for stem cell proliferation are directly involved in AML initiation (70). A schematic representation of the pathways involved in CSC and stem cell signalling are presented in figure 1.

RESISTANCE OF CSCS TO CHEMOTHERAPY AND RADIOTHERAPY

Residual CSCs may survive in a quiescent state for many years after cancer remission and result in later relapse and metastasis. Several intrinsic features of CSCs should make them less susceptible to chemotherapy and/or radiotherapy: a) the presence of efflux pumps; b) their relatively low proliferative activity and high levels of anti-apoptotic proteins (bcl-2 and survivin); c) the presence of ALDH, which metabolizes chemotherapeutic drugs such as cyclophosphamide; and d) CSC-derived VEGF and other angiogenic factors that help to maintain the stem cell niche (71- 74).

Recent studies of brain and breast carcinomas have implicated CSC radioresistance (75, 76). It was shown that CD133+ CSCs contribute to glioma radioresistance through preferential activation of the DNA damage checkpoint response and a higher capacity for DNA repair, compared with CD133- tumour cells. The radioresistance of CD133+ glioma stem cells could be reversed with a specific inhibitor of Chk1 and Chk2 checkpoint kinases, which are closely associated with cellular resistance to radiation, thereby providing a therapeutic advantage to reducing brain tumour occurrence (75).

These data make it clear that cancer therapy needs a more targeted approach. Most cancer drugs target signalling pathways, such as hedgehog (cyclopamine) or Wnt/β-catenin (imatinib) (77). Another way to combat CSCs is to force them to differentiate (as can achieved using transretinoic acid, TPA, DMSO, butyric acid, vitamin D and nerve growth factors) (78). There is evidence that certain cancer drugs accomplish their functions by directly blocking ABCG1 pumps (verapamil and cyclosporine) (79) or ABCG2 pumps (the natural compound fumitremorgin C) (80). The pumps mentioned above are unable to remove some substances that are tumorotoxic but not cytotoxic (such as the phytochemical sulforaphan from broccoli), which gives us hope for potential natural, specific antitumour therapies (81). It has been shown that it is possible to prevent primary tumour and metastasis formation in animal models by blocking the homing factor CXCR4 (82).

It is unlikely that there will be a single magic bullet. The future of cancer treatment may require individualized combinations of therapies targeting molecular pathways, perhaps those unique to the appropriate type of CSC.

CONCLUSION

CSCs appear to use the same self-renewal pathways as stem cells from normal tissues. Thus, a compelling approach for studying CSCs is to understand the biology of normal tissue stem cells in order to better characterise CSCs. Ideal CSC markers have yet to be identified, so novel methods to accurately identify and target these cells would represent a significant advance in cancer therapy. Future research must focus on establishing reliable criteria for the identification and isolation of CSCs, and finding ways to briefly disrupt CSC niches without damaging normal stem cell niches.

REFERENCES


