Cancer stem cells: the dark knights of clinical hematology and oncology

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Summary

According to recent epidemiological studies, malignant diseases represent the second cause of mortality worldwide and metastasis is the main cause of morbidity and mortality in most cancers. Even if the concept of “cancer stem cells” (CSCs) was anticipated by the genius of Rudolph Virchow, the father of modern pathology, more than 150 years ago, it is only in last few years that that scientists have begun to develop strategies aimed at inhibiting CSCs at a molecular level, the only way cancer can truly be attacked, by crossing the border between histology and molecular biology. The current concise review aims at emphasizing the main characteristics of tumor initiating cells, bridging the basic science to clinical hematology and oncology.

Key words: cancer stem cells, clinical hematology, clinical oncology

Cancer stem-like cells: the dark knights of clinical hematology and oncology

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Cancer stem cells in malignant initiation and metastasis

According to recent epidemiological studies, malignant diseases represent the second cause of mortality worldwide and metastasis is the main cause of morbidity and mortality in most cancers. The words “cancer” (Latin for “crab”) and “metastasis” (Greek for “change in position”) refer to cell movement, the crab-like invasion of the healthy tissue by cancer and the migration of cancer cell to distant sites from the primary tumor. Since the work of Paget in the XIXth century, pathologists have recognized that the spread of cancer cells follow a well-established pattern and that different types of cancers have different destinations. What was at the moment called the “soil and seed theory” states that different organs provide optimal growth conditions for specific cancers.

From a darwinistic point of view, metastasis is the end product of an evolutionary process in which diverse interactions between cancer cells and their microenvironment yield alterations that allow these cells to transcend their programmed behavior. Tumor cells thus populate and flourish in new tissue habitats and ultimately cause organ dysfunction and death. Understanding the many molecular elements involved in metastasis will lead to effective targeted approaches to prevent and treat neoplastic diseases [1-4]. Evidence supports that many, if not all tumors depend on a small population of CXCR4+ CSCs for their continuous growth, a concept postulated by oncologists and recently experimentally documented for human leukemia, brain, breast, prostate, liver, bone, skin or pancreatic cancer [5-7].

Mesenchymal stem cells and CSCs share many characteristics, the most important of which being the capacity of self-renewal. Both types of cells have long telomeres, high activity of tel-
Cancer stem-like cells in hematology/oncology

The ABC transporter explains why some types of cancers are relatively resistant to certain types of anticancer drugs. At the molecular level, the cells share similar pathways involved in self-renewal and proliferation, including Wnt, Sonic Hedgehog, Notch signaling, Polycomb genes BMI-1 and EZH2.

Last but not least, CSCs and normal stem cells share similar surface antigens, including CXCR4, potentially employed in addition to other antigens, like Sca-1 in mice and CD133 in humans, as markers used to identify tumor stem cells from growing solid cancers or leukemias. The SDF-1-CXCR4 axis influences metastasis by chemoattracting cancer cells to organs that highly express SDF-1, such as lymph nodes, lungs, liver or bone tissue and this is the very reason why breast cancer, ovarian cancer, rhabdomyosarcoma or neuroblastoma metastasize especially to bones and lymph nodes. The axis is also responsible for the retention in the bone marrow of acute lymphoid leukemia and acute myeloid leukemia cells. Facilitating VLA-4-VCAM-1 interactions of cells increases their survival and accounts for anticancer drug failure. Also, SDF-1 is responsible for deregulated adhesion and integrin-dependent bone marrow retention of Philadelphia (Ph+) CD34+ cells in chronic myeloid leukemia. Elevated levels of SDF-1 are associated with increased osteoclast activity and osteolytic bone disease in patients with multiple myeloma [8,9].

Even if the concept of CSCs was anticipated by the genius of Rudolph Virchow, the father of modern pathology, more than 150 years ago, who along with Julius Connheim proposed the idea of "lost" embryonic remnants during developmental organogenesis that lie dormant and may give rise to malignancies [10], it is only in the last few years that scientists have begun to develop strategies aimed at inhibiting CSCs at a molecular level, the only way cancer can truly be attacked, by crossing the border between histology and molecular biology.

From normal histology and physiology to cancer

The goal to elucidate cellular mechanisms that link genetic changes to the development of cancer is a formidable task because it involves a complex process that occurs in the local niche, it develops over a long period of time and involves a multitude of causes, contributory elements and risk factors. Cancer is initiated by Wnt-pathway-activating mutations in genes such as adenomatous polyposis coli (APC) and as in many cancers, the cell of origin remains relatively elusive.

The anatomy of the crypts is uniquely suited to study adult stem cells and their niche. Each crypt contains long lived stem cells intermingled with Paneth cells in the small intestine and with goblet cells in the colon. These cells divide every day, being called crypt base columnar cells, and their daughter cells constitute the transit-amplifying crypt compartment. Transit amplifying cells divide every 12 to 16 hrs, reside within the crypts and undergo several rounds of cell division while migrating upwards. When a transit amplifying cell reaches the crypt-villus junction, it differentiates while continuing its upward migration and initiating mutations in colorectal malignancies targeting Wnt pathway components, most frequently the negative regulator APC. This will result in the constitutive activation of a Wnt target gene that drives the formation of bening adenomas [11,12].

The Vogeltein’s theory, first developed at the Johns Hopkins Hospital in the United States, was improved by later research, especially by the hypothesis of stem cell overpopulation and adenoma development. Morphologically, adenomatous crypts are wider and longer than normal ones, indicating that the total crypt cell population has expanded. Adenomatous epithelium has a relatively immature, undifferentiated phenotype that resembles the proliferative epithelium found in the bottom of normal crypts. This proves that the proliferative cell population expands to replace the entire crypt population and that the distribution of S-phase cells is shifted upward to the luminal surface of adenomatous crypts [13,14]. A further displacement of the rapidly proliferating cell population and a further shift in the distribution of S-phase cells toward the crypt top is testable by immunohistochemical staining of colon tissue sections using antibodies against available colonic markers, such as CD133 (Figure 1), CD44 (Figure 2) or Lgr5 (Figure 3), and against rapidly proliferating cells (i.e. Ki67) [15].

While a few tumors develop very rapidly, most develop over many years or even decades. A malignant tumor requires mutations that confer a tumor cell the ability to overcome the multiple intrinsic and extrinsic mechanisms that the human body possesses to prevent the breakdown of homeostasis. Mutations that cause aberrant cell proliferation are key aspects of oncogenesis and still, most DNA damage is quickly repaired. Also,
because somatic cells are dependent on homeostatic signals, the mutation that disrupts an epithelial cells’ interaction with the basal lamina can trigger apoptosis or replicative senescence. Considering that the immune system also plays a role in cancer progression, we might say that all the anti-cancer initiation and progression systems act as a selective force that promotes somatic evolution of different clonal subspecies [16].

Cancer can be considered a phenotypic selection of multiple advantageous traits by a specific environment rather than an expansion of individual mutant genotypes. In tumor cell biology, as well as in evolutionary biology, selection is applied at the phenotypic level and cellular phenotypes in tumors are the result of the integration of the genotype with the microenvironment. Tumor cells acquire a number of traits as the neoplasm
progresses to malignancy, as described by Baylin and Pupa et al. [17,18], include self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, angiogenesis, limitless replicative potential and the ability to invade the surrounding tissues.

A rather unconventional but as close to reality as possible way to see cancer is the “game theory”, a ecological mathematical tool used by ecologists to address cancer, evolution of cooperation between the tumor cell and the surrounding environment and the network of metastasis. The game theory, initially introduced by the Nobel laureate John Nash in the 1950s to study human behavior and economy [19], was expanded to study interactions between individuals in the same species to determine the evolutionary dynamics of the population. In the past decade mathematical oncologists have started using the evolutionary game theory to study somatic evolution in cancer, which requires formally defining the relevant cell phenotypes present during invasion and metastasis and the interplay between these elements in relation to a fitness payoff. The theory has successfully demonstrated the emergence of phenotypes capable of unconstrained growth, avoiding apoptosis, acidifying the microenvironment, and promoting angiogenesis and invasion [20-22].

**Stem cell niche and metastasis**

The undifferentiated cells in a niche are a cell population undergoing a constant process of regeneration. In order to strictly regulate this process, there has to be a balance between proliferation, differentiation, senescence and apoptosis, a central role being played by the stem cells located at this level [23], as cancer stem-like cells have a very high proliferation rate. Figures 4A and B show the proliferation rate of the cells within 48 hours.

In the colon for example, these cells are situated in the basal compartment of the crypts. Just like the other adult stem cells, it is assumed that these cells are surrounded by a niche consisting of mesenchymal structures, which ensure the preservation of their characteristic properties as stem cells [24]. The studies conducted in the attempt to determine the clonal origin of colonic crypts have indicated that although during their embryological development the cells that constitute the crypts are polyclonal, in a later stage they become monoclonal, possibly through the positive selection of a dominant clone or following the segregation of the cell lines caused by the fission of the crypts, which occurs quite frequently during the development of the colon. This observation has led to the notion of a crypt cycle. Thus, crypts have a limited lifespan which, in the case of mice, has been established at two years [25,26]. Because the fission of the crypts begins at the base, where we also find the colonic stem cells, it is assumed that they play a major role in this process. Thus, customarily, the cell population generated by one stem cell would lead to the formation of an entire colonic crypt. Normally, competition between the cell populations coming from different stem cells is moderate and leads to an increase in the number of niches. In the case of a malignant transformation, the increased proliferation of the cells coming from the tumor stem cell leads to an aggressive, unbalanced competition with the other cells, which come from normal stem cells. This time, the consequence is not the formation of other stem cells, but an anarchic formation of a malignant niche, leading to architecture typical of malignant tumors [27-30].

This behavior can explain the architecture of colonic metastases in which crypts were identified, provided that the migrated cell has properties similar to those of the colonic stem cells capable of generating an entire crypt [31]. One of the important characteristics that define a stem cell is asymmetrical division. In this case, the division of a stem cell generates a cell identical to the original one and a cell that would differentiate. Following this division, one of the cells would take the place of the initial cell in the niche, and the other would leave the niche and differentiate [32], as shown in Figure 5.

During a malignant transformation, this nor-
The natural history of stem-like cells of the gastrointestinal tract, showing the property of asymmetrical cell division, as well as the pathophysiology of malignant transformation.

**Targeted molecular therapies**

A key focus of cancer research is the understanding of molecular changes that underlie tumor initiation and progression. One of the most promising pathways to targeting CSCs is the induction of differentiation with the loss of self-renewal capacity. This is the case of acute promyelocytic leukemia, where retinoic acid has significantly increased the efficacy of chemotherapy. This proof principle is that differentiation agents may be the most promising path to developing effective, non-toxic therapies. Inhibitors of Wnt signaling, such as ICG-001, particularly the CREB-Binding Protein (CBP) pathway, show promising in vivo and in vitro efficacy without toxicity, as well as arsenic trioxide that sensitizes CSCs to standard chemotherapy [41,42].

CD44 is a widely expressed protein with an important role in cell-to-cell and cell-to-matrix interactions. Though normally expressed at the base of dividing crypts in the proliferative zone, during inflammation and malignant proliferation the distribution of CD44 expression extends to the luminal surface. In a mouse model of acute myeloid leukemia, Jin et al. used a CD44-activat-
Cancer stem-like cells in hematology/oncology

FOLFOX [45].

Curcumin (diferuloylmethane) – the major active ingredient of Curcuma longa. Curcumin inhibits the growth of transformed cells, and suppresses initiation, promotion and progression of colon carcinogenesis in combination with either 5-FU or oxaliplatin, the ability to decrease tumorigenic growth was increased when cells were first treated with anti-IL-4 monoclonal antibodies. Colon CSCs may also be targeted by natural products, such as curcumin (diferuloylmethane) – the major active ingredient of Curcuma longa. Curcumin inhibits the growth of transformed cells, and suppresses initiation, promotion and progression of colon carcinogenesis in combination with either 5-FU or FOLFOX [45].

Cancers are extremely heterogeneous in terms of the frequency and types of mutations, and the high degree of diversity among human colorectal cancers suggests that individualized treatment strategies hold great promise in future clinical experiments. Radioimmunotherapy is an emerging approach with only two FDA-approved protocols thus far, both targeted against non-Hodgkin’s lymphoma. Abraham et al. reported a set of 9 different decapptides which, when labeled with the β-emitter 32P, bound to cell lines derived from a panel of different colorectal adenocarcinomas [46]. The most efficient 32P-labeled decapptide resulted in permanent incorporation of radioisotope into colon adenocarcinoma cellular proteins at a rate over 100 times greater than in cell lines derived from other cancers.

Telomerase, the research of which has won Blackburn, Greider and Szostak the 2009 Nobel Prize in Physiology or Medicine, is a ribonucleoprotein considered as a potential target of cancer therapy because of its preferential expression in tumors. Telomerase, whose activity is generally undetectable in normal somatic cells and expressed in most tumors, has two major components and several associated proteins essential for proper cell function. The first major component is the human telomerase RNA (hTER) that serves as the template on which telomeric repeats are added to the ends of the chromosomes, and the second component is the human telomerase reverse transcriptase (hTERT) catalytic sub-unit. In anticancer therapy, the promoter of the hTERT gene is used to restrict the expression of suicide gene or toxin to telomerase positive tumor cells. Using hTERT-derived peptides or hTERT RNA, cytotoxic T cell responses and tumor immunity are elicited. Due to the fact that normal cells lack telomerase activity they are kept intact and adverse effects are minimal [47,48].

Gene-based therapeutics based on the concept on oncolytic virus therapy also hold considerable promise. Viruses have adapted through millennia of evolution to effectively invade and affect cells through various mechanisms. The research team lead by Galanis at Mayo Clinic in Rochester uses strains of the attenuated measles virus Edmonton (MV-Edm) vaccine lineage that can preferentially infect and destroy cancer cells while sparing the healthy tissue. To facilitate monitoring of the viral gene expression and replication, the oncolytic strains are engineered to express soluble marker peptides, such as the human carcinoembryonic antigen [49,50].

Another approach that has been introduced recently in the clinic to reduce primary tumor and metastasis load is targeting the angiogenesis process. As neovascularization depends largely on VEGF signaling, several strategies have been developed to disrupt this signaling pathway. Small molecule tyrosin kinase inhibitors and antibodies against VEGF receptors can block activation. A phase III clinical trial showed that blocking VEGF with a humanized anti-VEGF antibody in combination with standard chemotherapy will increase overall and progression-free survival of patients with metastatic colorectal cancer [51].

Plerixafor, also known as AMD3100, is a bicyclam molecule which binds reversibly to CXCR4. Even though initially developed as a potential therapeutic agent against HIV, preclinical data have shown that AMD3100 blocks CXCL12 binding of CXCR4 and as a result it inhibits SDF-1α-induced calcium flux and chemotaxis. Clinical trials have demonstrated that Plerixafor is effective for the mobilization of peripheral blood stem cells for use in autologous hematopoietic stem cell transplantation, but blocking the same CXCR4-CXCL12 axis can also play an important role in the control of CSC dissemination from the primary prostate cancer lesion and metastasis. New drugs, such as CTCE-9908, are currently undergoing preclinical confirmation in this setting [52].

Conclusion

Although the treatment of cancer has seen considerable progress during the past few years, the mortality associated with this disease remains high. The integration of paradigms coming from various fields, such as evolutionary dynamics and mathematical oncology, has increased our understanding of the manner in which tissues are organized and operate. This has opened new perspectives in what concerns the genesis of various
pathological processes, especially of malignant tumors. Nowadays we are witnessing a new approach to treatment, and this time the target is a small cell population – tumor stem cells – deemed responsible for the resistance to treatment and for metastasis. That is why current efforts are directed towards the understanding of the biology of this type of cells and of the differences between them and normal stem cells, in an attempt to find more effective treatments that would also have less significant side-effects.

Acknowledgements

The research on cancer stem cells was carried out at the Research Center for Functional Genomics and Translational Medicine, Iuliu Hatieganu University of Medicine and Pharmacy in Cluj Napoca, Romania. Funding was received from internal grants of the Iuliu Hatieganu University awarded to Dr. Ciprian Tomuleasa and Dr. Florin Zaharie.

References