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Therapeutic implications of cancer stem cells

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Most cancers comprise a heterogeneous population of cells with marked differences in their proliferative potential as well as the ability to reconstitute the tumor upon transplantation. Cancer stem cells are a minor population of tumor cells that possess the stem cell property of self-renewal. In addition, dysregulation of stem cell self-renewal is a likely requirement for the development of cancer. This new model for cancer will have significant ramifications for the way we study and treat cancer. In addition, through targeting the cancer stem cell and its dysregulated self-renewal, our therapies for treating cancer are likely to improve.

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Current Opinion in Genetics & Development 2004, **14**:43–47

This review comes from a themed issue on
Oncogenes and cell proliferation
Edited by Zena Werb and Gerard Evan

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DOI 10.1016/j.gde.2003.11.007

Abbreviations

AML	acute myeloid leukemia
CML	chronic myeloid leukemia
FISH	fluorescence <i>in situ</i> hybridization
HSC	hematopoietic stem cell
LIC	leukemia-initiating cell
TIC	tumor-initiating cell

Introduction

Recent evidence has demonstrated that in leukemia and solid tumors only a minority of cancer cells have the capacity to proliferate extensively and form new tumors. These tumorigenic, or tumor-initiating cells (TICs), have been identified and enriched on the basis of their expression of cell-surface markers. Upon transplantation, TICs give rise to tumors comprising both new TICs as well as heterogeneous populations of non-tumorigenic cells reminiscent of the developmental hierarchy in the tissues from which the tumors arise. Most adult tissues are maintained over the lifetime of the host by normal stem cells that undergo expansion and differentiation to yield the functional elements of the organ. Through the tightly regulated process of self-renewal, stem cells are able to

function over the lifespan of the host. The genetic constraints on self-renewal restrict the expansion of stem cells in normal tissues. Breakdowns in the regulation of self-renewal is likely a key event in the development of cancer as demonstrated by the fact that several pathways implicated in carcinogenesis also play a key role in normal stem cell self-renewal decisions. Thus, malignant tumors can be viewed as an abnormal organ in which a minority population of tumorigenic cancer cells have escaped the normal constraints on self-renewal giving rise to abnormally differentiated cancer cells that have lost the ability to form tumors. This new model for cancer has important implications for the study and treatment of malignancies.

What is a stem cell?

Normal tissue stem cells are defined by three common properties: first, the presence of an extensive capacity for self-renewal that allows maintenance of the undifferentiated stem cell pool over the lifetime of the host; second, strict regulation of stem-cell number; and third, the ability to undergo a broad range of differentiation events to clonally reconstitute all of the functional elements within the tissue. Importantly, the stem cells in each tissue differ with respect to their intrinsic ability to both self-renew and to differentiate into particular mature cell types [1].

Self-renewal and cancer

Self-renewal is a cell division in which one or both of the resulting daughter cells remain undifferentiated and retain the ability to give rise to another stem cell with the same capacity to proliferate as the parental cell. Proliferation, unlike self-renewal, does not require either daughter cell to be a stem cell nor to retain the ability to give rise to a differentiated progeny. The committed progenitor cell is destined to stop proliferating as with each cell division its potential to proliferate decreases. In normal tissues, such as the blood, both stem cells and committed progenitor cells have an extensive capacity to proliferate. Although committed progenitor populations can maintain hematopoiesis for up to 6 to 8 weeks [2–4], a single hematopoietic stem cell (HSC) can restore the blood system for the life of the animal. This tremendous potential is a direct result of its capacity to self-renew.

Most tumors develop over a period of months to years and like normal tissues consist of heterogeneous populations of cells. In previous models of cancer, the unregulated growth of tumors was attributed to the serial acquisition of genetic events that resulted in the turning on of genes promoting proliferation, silencing of genes involved in inhibiting proliferation, and circumventing of genes

involved in programmed cell death. In the stem-cell model for cancer, another key event in tumorigenesis is the disruption of genes involved in the regulation of stem-cell self-renewal. Thus, some of the cancer cells within a tumor share with normal stem cells the ability to replicate without losing the capacity to proliferate.

It is not surprising, then, that several genes initially identified for their role in carcinogenesis have been implicated in normal stem-cell self-renewal decisions. Genes that have been demonstrated to be involved in regulation of self-renewal in normal stem cells include the Bmi-1, Notch, Wnt and Sonic hedgehog pathways [5–8]. The importance of the Notch and Wnt pathways are reviewed elsewhere in this issue. Recently, Reya *et al.* [9[•]] demonstrated the dependence of normal HSC self-renewal decisions on Wnt-signaling through the canonical pathway. Willert *et al.* demonstrated the ability of purified Wnt3a to permit the *in vitro* expansion of transplantable HSCs [10[•]]. Additional studies implicate the Wnt/ β -catenin pathway in the maintenance of stem-cell self-renewal in other tissues as well [11,12].

The Polycomb and trithorax groups are transcriptional repressors and activators that are part of multimeric complexes that interact with chromatin leading to either a repressed or activated state of gene expression, respectively. Bmi-1, a member of the Polycomb group, targets the *INK4a* locus and overexpression of Bmi-1 results in downregulation of both p16 and p19Arf [13]. Post-natal mice deficient in the expression of Bmi-1 display failure of hematopoiesis and fetal liver and bone marrow stem cells from *Bmi*^{-/-} mice are able to contribute to recipient hematopoiesis only transiently indicating a primary defect in adult HSC self-renewal [14[•],15,16[•]]. Bmi-1 also plays a key role in malignant hematopoiesis as HOXA9/MEIS 1 induced murine leukemia is dependent on the expression of Bmi-1 [16[•]].

The importance of epigenetic events, such as modification of chromatin, in normal and malignant tissues is likely to remain a key focus of research. Preliminary studies have examined the ability to reverse these epigenetic changes through the transfer of nuclei from cells in a differentiated tissue into enucleated oocytes. Nuclei obtained from medulloblastoma tumor cells arising in Ptc1 heterozygous mice were transferred into enucleated oocytes [17]. Blastocysts derived from medulloblastoma were morphologically indistinguishable from those derived from control spleen cell nuclei without evidence of the uncontrolled proliferation. This study suggests that epigenetic reprogramming was responsible for the loss of the tumor cells' capacity to form tumors.

Cellular origin of cancer stem cells

The term 'cancer stem cell' is an operational term defined as a cancer cell that has the ability to self-renew giving rise

to another malignant stem cell as well as undergo differentiation to give rise to the phenotypically diverse non-tumorigenic cancer cells. The cell-of-origin for cancer stem cells remains unclear: they may or may not be derived from their normal stem cell counterpart. The fact that multiple mutations are necessary for a cell to become cancerous [18] has implications for the cellular origin of cancer cells. As both progenitor cells and mature cells have a very limited lifespan, it is unlikely that all of the mutations could occur during the life of these relatively short-lived cells. In addition, to maintain the disease, cancer cells must overcome the tight genetic constraints on both self-renewal as well as proliferation [19]. Because cancer stem cells must possess the ability to self-renew, it follows that they are derived either from self-renewing normal stem cells — which could be transformed by altering only proliferative pathways — or from progenitor cells that have acquired the ability to self-renew as a result of oncogenic mutations.

Work by Dick and others has identified a common phenotype for the leukemia-initiating cells (LICs) [20–23]. Although the phenotype of the LIC is very similar to that of the normal HSC, there are differences, including the differential expression of Thy1 and IL3 receptor α chain. [22–24]. These differences suggest that early mutations occurred in the HSCs and the final transforming events either alter the phenotype of the stem cells or occur in early downstream progenitors. In patients with chronic myeloid leukemia (CML), the BCR/ABL transcript is expressed in multiple hematopoietic lineages consistent with HSC involvement [25,26]. Although the stem cell population likely contains the genetic lesion, other studies (e.g. [27]) demonstrate that the BCR/ABL transcript is not expressed until the level of the committed progenitors. A model of CML was reported recently in which the expression of the fusion product was targeted to myeloid/monocytic progenitor cells using the hMRP-8 promoter. A subset of the hMRP8p210^{BCR/ABL} mice develop a CML-like disease with elevated white cell counts and splenomegaly [28]. When crossed with hMRP8bcl-2 mice, a proportion of the mutant mice developed a disease resembling acute myeloid leukemia (AML). One explanation for this finding is that targeting the expression of the fusion protein to the committed progenitor instills in this population the capacity for self-renewal. Alternatively, expression of the fusion gene by the committed progenitor increases the proliferative potential of the progenitor population that is maintained by the abnormal stem cell. Additional studies examining the ability of purified hMRP8p210^{BCR/ABL} progenitors to reconstitute the disease upon transplantation into primary as well as secondary recipients will be necessary to distinguish between these two possibilities. As with AML, the phenotype of breast cancer TICs may be similar to that of normal breast epithelial stem or progenitor cells because early multipotent epithelial cells have been

reported to exhibit a similar phenotype to that seen in the tumorigenic breast cancer cells [29–31].

Implications of the stem cell model for cancer

There are major implications for the way we study, diagnose and treat cancer if the same populations of cancer cells are tumorigenic in humans and the xenograft model. If only a rare subset of tumor stem cells drives tumor formation then the goal of therapy should be to identify this population and then develop therapies that target it. Current therapeutic strategies fail to account for potential differences in drug sensitivity or target expression between the TICs and the more frequent non-tumorigenic cells. A differential sensitivity to treatment between the tumorigenic and non-tumorigenic populations may account for the difficulty in developing therapies that are consistently able to eradicate solid tumors other than testicular cancer.

Traditionally, treatments for cancer have relied on the ability to non-discriminately kill proliferating cells. In both the AML and breast studies, the tumorigenic cells represent a minor population of the total tumor bulk [20–23,32*]. Therefore, agents selectively killing the cancer stem cells are likely overlooked in screening methods that rely on rapid reduction of tumor size. If an agent spares the TICs, then relapse is likely as residual TICs reinitiate tumor growth. To date only a few studies have addressed potential differences in drug sensitivity between the tumorigenic and non-tumorigenic populations. In 2000, Costello *et al.* demonstrated that CD34+CD38– leukemic cells were significantly less sensitive to daunorubicin than the more committed CD34-CD38+ cells [33]. These results were extended in a recent study where Jordan and co-workers showed the LICs are relatively resistant to cytarabine, whereas the remaining leukemic blasts were easily killed by this agent [34].

This new model for cancer will also likely impact our understanding of the mechanisms of drug resistance. A wide variety of transporters, including members of the ABC transporter family, have been demonstrated on normal stem cells [35,36] and several of these transporters have well-established roles in drug efflux [37]. The ability of leukemic progenitors to efflux mitoxantrone and daunorubicin, two agents commonly used in treatment of AML, has also been shown [38]. Thus, our understanding of drug sensitivity as well as the development of drug resistance by tumors might be significantly impacted by the fact that only a rare subset of tumor stem cells drives tumorigenesis.

Much of cancer research is focused on the identification of therapies that target essential genes or pathways critical to the development of cancer. The stem-cell model for cancer predicts that therapies whose target

is expressed on or by the TICs are more likely to be successful. In CML, Gleevec[®] targets the ATP-binding domain of the Abl kinase and most patients treated with Gleevec[®] experience complete cytogenetic responses [39,40]. When sensitive methods such as PCR are used to follow the disease, however, the majority of patients remain positive for the presence of the fusion transcript [41]. The persistence of these rare transcripts is concerning because it suggests that this therapy may not be curative. The stem cell model for cancer provides a potential explanation for this finding. Bhatia *et al.* demonstrated the presence of the BCR/ABL fusion by FISH analysis in between 6.5 to 13% of CD34+ progenitor cells isolated from a subset of patients in remission and negative for the translocation by FISH on unfractionated bone marrow [42]. Other studies suggest that although the HSC contains the abnormal gene, it is not expressed until the level of early committed progenitors. The absence of target expression by the abnormal stem cell population is a potential explanation for the failure to eradicate the disease. Alternatively, inherent properties of the LIC for CML might confer resistance to Gleevec[®] and the responses seen are the result of the preferential targeting of the non-tumorigenic population.

In addition to its impact on our understanding of the efficacy of available therapies, the stem-cell model for cancer probably has an impact on the identification of future therapeutic targets. The expression patterns of normal stem cells and their more differentiated progeny can differ significantly [43]. DNA and tissue microarrays of tumors to date have failed to account for the cellular heterogeneity as well as differences in the proliferative potential of these different populations. By directing expression analyses to enriched populations of tumorigenic cancer cells, the identification of novel diagnostic markers and novel therapeutic targets should be more effective. In addition, it is becoming apparent that treatments that directly target those pathways involved in malignant stem-cell self-renewal would have a significantly greater chance of success.

Finally, this new cancer model has significant implications for the design of future studies aimed at improving our ability to diagnose cancer and our ability to identify individuals at risk for metastasis. Studies have shown that disseminated cytokeratin-positive breast cancer cells can be detected in the bone marrow of patients that never relapse [44,45]. One possible explanation of this observation is that the cancer cells lie dormant until some unknown event triggers them to renew proliferation. An alternative explanation is that the disseminated cancer cells in this group of patients arose from the spread of non-tumorigenic cells, and only when cancer stem cells disseminate and subsequently self-renew will metastatic tumors form. Thus, further study of the tumorigenic

population may shed considerable light on the process of metastasis and allow for the development of diagnostic reagents that allow us to predict which patients will develop metastatic disease. This will allow clinicians to identify patients most likely to benefit from adjuvant treatment and potentially spare many patients from unnecessary therapy.

Conclusions

It is becoming increasingly clear that cancer is a stem-cell disorder. In addition, our ability to identify pathways involved in both normal and malignant self-renewal will be important in furthering our knowledge of the events that lead to the development of cancer. The ability to prospectively identify, isolate and study cancer stem cells will significantly alter the way we think about, study, and treat cancer.

Acknowledgements

We would like to thank Laurie Luzynski for help in the preparation of this manuscript.

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