

Leukemic stem cells show the way

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Abstract: The blood-related cancer leukemia was the first disease where human cancer stem cells (CSCs), or leukemic stem cells (LSCs), were isolated. The hematopoietic system is one of the best tissues for investigating cancer stem cells, since the developmental hierarchy of normal blood formation is well defined. Leukemia can now be viewed as aberrant hematopoietic processes initiated by rare leukemic stem cells (LSC) that have maintained or reacquired the capacity for indefinite proliferation through accumulated mutations and/or epigenetic changes. Yet, despite their critical importance, much remains to be learned about the developmental origin of LSC and the mechanisms responsible for their emergence in the course of the disease. This report will review our current knowledge on leukemic stem cell development and finally demonstrate how these discoveries provide a paradigm for identification of Cancer Stem Cell (CSC) from solid tumors.

Key words: Hematopoietic stem cell (HSC) - Xenotransplantation model - Leukemic stem cell (LSC) - Cancer stem cell (CSC) - Self-renewal

Introduction

The hallmark properties of hematopoietic stem cells (HSCs) are the ability to balance self-renewal versus differentiation cell fate decisions to provide sufficient primitive cells to sustain hematopoiesis, while generating more mature cells with specialized capacities. Through the process of asymmetric cell division, a single division can result in the formation of both an identical stem cell and a more mature cell [10]. In order to ensure a persistent pool of regenerating cells without outgrowth of immature cell types, tight regulation of HSC division is required. Unchecked growth of immature cells is thought to represent a paradigm for malignant outgrowth, at least for acute myeloid leukemia (AML) and chronic myeloid leukemia [16, 18]. Thus, determining the composition and relationship of the cell types that constitute the human stem cell compartment may help both to identify the cellular and molecular factors that govern normal and leukemic stem cell (LSC) development, and to advance clinical applications of transplantation, gene therapy, stem cell expansion and tumor cell purging. This review will introduce the notion of LSCs, the potential origin of these cells with an emphasis on myeloid leukemia and finally examine the

impacts these discoveries may have clinically and in understanding the organisation of cancer of other tissues.

Functional heterogeneity in tumors

The development of quantitative assays enabling measurement of the clonogenicity of malignant hematopoietic cells led to the first demonstrations that only a small subset of cancer cells is capable of extensive proliferation *in vitro* [8]. Such studies revealed the existence of functional heterogeneity within tumors, and introduced the concept of tumor stem cells. Subsequently, studies in AML have been key in elucidating the biological basis of tumor heterogeneity. AML is a clonal disorder of aberrant hematopoiesis characterized by an accumulation of functionally immature blasts, which fail to differentiate normally. Despite their morphological homogeneity, the blast cell population is biologically heterogeneous. Only a minority of proliferative leukemic blasts (AML-CFU) is able to give rise to colonies *in vitro*. This observation suggested that, as in normal hematopoiesis, the leukemic clone in AML is organized as a hierarchy, in which a small number of proliferating progenitors continuously replenish the bulk population of non-cycling leukemic blasts.

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Lecture presented at the Third Annual Meeting of the European Stem Cell Therapeutics Excellence Centre, October 6-9, 2005, Cracow, Poland

Concept of leukemic stem cells

Emerging evidence has provided new insights into cancer biology by emphasizing the relationship between stem cells and tumor cells and by proposing the notion that cancer cells might contain some cancer stem cells, which are rare cells with indefinite self-renewal potential that drive the formation and growth of the tumors. The existence of cancer stem cells was revealed first in leukemia [5, 14, 22] but has now extended to other cancer types [1, 19, 20]. Transplantation of primary AML cells into SCID [14] or NOD/SCID [5] mice led to the finding that only rare cells, termed SCID leukemia-initiating cells (SL-IC), are capable of initiating and sustaining growth of the leukemic clone *in vivo*. In addition to their ability to differentiate and proliferate, serial transplantation experiments showed that SL-IC possess high self-renewal capacity, and thus can be considered to be AML stem cells. Importantly, SL-IC can be prospectively identified and purified as CD34⁺CD38⁻ cells in AML patient samples, regardless of the phenotype of the bulk blast population, and are the only cells capable of self-renewal as demonstrate by serial transplantation [5]. These findings show that, like the normal hematopoietic system, AML is organized as a hierarchy of distinct, functionally heterogeneous classes of cells that is ultimately sustained by a small number of leukemic stem cells (LSC). These studies provided the first direct evidence for the cancer stem cell hypothesis.

Comparison between normal and leukemic stem cells

While LSCs appear to share similar cell surface markers previously identified for normal HSCs, such as CD34, CD38, HLA-DR and CD71, several groups have reported that some markers are differentially expressed between the two such as CD90, Thy.1, c-kit and IL-3 receptor [2, 3, 4, 13]. Despite these few phenotypic differences between normal HSC and LSC, a recent work has reported similar heterogeneity in the normal and LSC compartment based on self-renewal and proliferation capacities. Using clonal tracking of retroviral-transduced normal and leukemic cells in NOD/SCID mice, it was demonstrated that both normal and LSC compartments were comprised of individual stem cell classes that differ in their repopulating and self-renewal capacities [9, 11]. Overall, these findings suggest that the pathways that regulate normal commitment/differentiation and self-renewal processes in hematopoietic cells are not completely abolished in LSC. Rather, the effects of transforming mutations are layered onto the normal developmental framework of HSC, resulting in the leukemic clone having an aberrant developmental hierarchy that retains aspects of its normal counterpart.

This concept is supported by a correlation between genes required for normal hematopoietic development and those perturbed in leukemia [23], and by the recent demonstration that Bmi-1 plays a key role in self-renewal determination in both normal and leukemic murine stem cells [15, 17].

Gene expression pattern of LSC versus normal HSC

The phenotypic description of LSC now enables their purification and will facilitate identification of genes that are preferentially expressed in these cells compared to normal HSC. However, gene expression profiling is usually conducted on mononuclear cells of AML patients from either peripheral blood and/or bone marrow. Gene expression profile of highly purified LSC would allow the identification of genes that reflect the biology of the cells that are actually driving the leukemia. Hence, in addition to being a more efficient way to further understand the biology of LSC, this should also provide a more efficient way of identifying new therapeutics and diagnostic targets.

The cell of origin in cancer: studies in AML

A focus of much cancer research is identification of the normal cell within which cancer initiates. The target of cell transforming mutations is still unknown. Because normal stem cells and LSCs share the ability to self-renew, as well as various developmental pathways, it has been postulated that LSCs are HSCs that have become leukemic as the result of accumulated mutations. Conversely, LSCs could derive from more committed progenitors or even a differentiated mature cell, which would have first to reacquire the self-renewal capacity before accumulating additional mutations.

There are two reasons to think that normal HSC themselves are the target of leukemic transformation. First, HSCs have the machinery for self-renewal already activated thus maintaining this activation may be simpler than turning it on *de novo* in a more differentiated cell. Secondly, stem cells persist for long period of time and thus have a greater opportunity to accumulate mutations than more mature short-lived cell types.

There is now evidence that most subtypes of human AML arise from mutations that accumulate in HSCs. For most AML subtypes, except for promyelocytic leukemia (AML-M3) subtype, the only cells capable of transplanting leukemia in nonobese diabetic/severe immunodeficient mice (NOD/SCID) mice have a CD34⁺CD38⁻ phenotype, similar to that of normal HSC, whereas more mature CD34⁺CD38⁺ leukemic blasts cannot transfer the disease to mice [5, 22].

On the other hand, evidence indicating that cells devoid of self-renewal activity, such as committed pro-

genitors and mature cells, can also be the targets for leukemic transformation comes from analyses of leukemia-associated genes in the mouse. Indeed, using promoter elements of several myeloid-specific human genes (MRP8, CD11b, cathepsin G) to target transgene expression specifically to committed myeloid cells, allowed the generation of multiple accurate transgenic mouse models of human leukemias [6, 12]. More recently, Cozzio *et al.* [7] have shown that the potent leukemic fusion gene MLL-ENL, which results from the t(11; 19) translocation, can induce the exact same leukemia when transduced into HSCs as well as into CMPs and GMPs. Another fusion gene MOZ-TIF2 has also recently been shown to contribute to the transformation of both HSC and more committed myeloid progenitors. These data imply that myeloid leukemias induced by these oncogenes can be initiated in committed progenitors due to their intrinsic capacities to confer leukemogenic self-renewal potential. Using a different fusion partner of MLL, GAS7, So *et al.* [21] showed that only the transduction of murine HSC but not CMP, and GMP resulted in the production of mixed lineage leukemias in transplanted mice. Although the mouse system provides a valuable tool to study leukemogenesis, the data obtained in mice do not imply that committed myeloid progenitors will necessarily be the target of transformation in the corresponding human disease. Nevertheless it appears highly probable that human AML might arise from cells at both HSCs and myeloid stage depending mostly on the nature of the associated fusion gene.

Cancer stem cells in solid tumors

Recent studies in solid tumors indicate that the concept of cancer as a hierarchy that is initiated and maintained by a rare population of stem cells may have broader implications beyond the field of hematopoiesis. Al-Hajj *et al.* [1] were able to prospectively isolate a minor phenotypically distinct subset of breast cancer cells that was able to recapitulate the tumors when transplanted into NOD/SCID mice. Thus like AML, breast cancer appears to be driven by a rare subpopulation of cells that demonstrate self-renewal and produced differentiated non-tumorigenic progenies. A recent report has also suggested the existence of brain cancer stem cells, which are able to generate new tumors *in vivo* that exhibit both self-renewal and differentiation [19, 20].

Based on these recent studies, the paradigm of cancer as a hierarchical disease whose growth is sustained by a rare population of stem cells is emerging. Implicit in this model of cancer development is the notion that CSC are biologically distinct from other cells in the tumor, and are able to initiate and sustain tumor growth *in vivo* whereas the bulk cells are not.

Conclusions

The identification of CSC has important implications for future research as well as for the development of novel therapies. In order to learn more about the nature of the events involved in cancer, research should focus more on CSCs and not on the bulk cells that makes up the majority of the tumor. Existing therapies have been developed largely against the bulk population. The lack of durable response in most cases, suggests that the treatment used may not effectively target the CSC population. Indeed, the failure of the current therapeutic regimens is likely related to the resistance and persistence of CSC. Future studies must focus instead on identifying and characterizing the rare cancer-initiating cells, and cancer treatments must be designed to specifically target these CSC if they are to effectively cure and prevent disease relapse.

Acknowledgements: This work was supported by Cancer Research UK and a National Institute of Health Grant No. HL-64856-03 to D. Bonnet.

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Received: June 15, 2005

Accepted: June 20, 2005