Cancer stem cells: AMLs show the way

D. Bonnet
Hematopoietic Stem Cell Laboratory, Cancer Research U.K., London Research Institute, 44 Lincoln’s Inn Fields, London WC2A 3PX, U.K.

Abstract
The blood-related cancer leukaemia was the first disease where human CSCs (cancer stem cells), or LSCs (leukaemic stem cells), were isolated. The haematopoietic system is one of the best tissues for investigating CSCs, since the developmental hierarchy of normal blood formation is well defined. Leukaemia can now be viewed as aberrant haematopoietic processes initiated by rare LSCs 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 LSCs and the mechanisms responsible for their emergence in the course of the disease. This report will review our current knowledge on LSC development and finally demonstrate how these discoveries provide a paradigm for identification of CSCs from solid tumours.

The hallmark properties of HSCs (haematopoietic stem cells) are the ability to balance self-renewal versus differentiation cell fate decisions to provide sufficient primitive cells to sustain haematopoiesis, 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 [1]. 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 AML (acute myeloid leukaemia) and chronic myeloid leukaemia [2,3]. 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 LSC (leukaemic stem cell) development, and to advance clinical applications of transplantation, gene therapy, stem cell expansion and tumour cell purging. This review will introduce the notion of LSCs, discuss the potential origin of these cells with an emphasis on myeloid leukaemia and finally examine the impacts these discoveries may have clinically and in understanding the organization of cancer of other tissues.

Functional heterogeneity in tumours
The development of quantitative assays enabling measurement of the clonogenicity of malignant haematopoietic cells led to the first demonstrations that only a small subset of cancer cells is capable of extensive proliferation in vitro [4]. Such studies revealed the existence of functional heterogeneity within tumours, and introduced the concept of tumour stem cells. Subsequently, studies in AML have been key in elucidating the biological basis of tumour heterogeneity. AML is a clonal disorder of aberrant haematopoiesis 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 leukaemic blasts (AML-CFU) is able to give rise to colonies in vitro. This observation suggested that, as in normal haematopoiesis, the leukaemic clone in AML is organized as a hierarchy, in which a small number of proliferating progenitors continuously replenish the bulk population of non-cycling leukaemic blasts.

Concept of LSCs
Emerging evidence has provided new insights into cancer biology by emphasizing the relationship between stem cells and tumour cells and by proposing the notion that cancer cells might contain some CSCs (cancer stem cells), which are rare cells with indefinite self-renewal potential that drive the formation and growth of the tumours. The existence of CSCs was revealed first in leukaemia [5–7] but has now extended to other cancer types [8–10]. Transplantation of primary AML cells into SCID (severe immunodeficient) [6] or NOD (non-obese diabetic)/SCID [7] mice led to the finding that only rare cells, termed SL-ICs (SCID leukaemia-initiating cells), are capable of initiating and sustaining growth of the leukaemic clone in vivo. In addition to their ability to differentiate and proliferate, serial transplantation experiments showed that SL-ICs possess high self-renewal capacity, and thus can be considered to be AML stem cells. Importantly, SL-ICs 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 demonstrated by serial transplantation [7]. These findings show that, like the normal...
haematopoietic system, AML is organized as a hierarchy of distinct, functionally heterogeneous classes of cells that is ultimately sustained by a small number of LSCs. These studies provided the first direct evidence for the CSC hypothesis.

**Comparison between normal HSCs and LSCs**

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 (interleukin 3) receptor [11–14]. Despite these few phenotypic differences between normal HSCs and LSCs, 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 composed of individual stem cell classes that differ in their repopulating and self-renewal capacities [15,16]. Overall, these findings suggest that the pathways that regulate normal commitment/differentiation and self-renewal processes in haematopoietic cells are not completely abolished in LSC. Rather, the effects of transforming mutations are layered on to 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 haematopoietic development and those perturbed in leukaemia [17], and by the recent demonstration that Bmi-1 plays a key role in self-renewal determination in both normal and leukemic murine stem cells [18,19].

**Gene expression pattern of LSCs versus normal HSCs**

The phenotypic description of LSCs now enables their purification and will facilitate identification of genes that are preferentially expressed in these cells compared with normal HSCs. However, gene expression profiling is usually conducted on mononuclear cells of AML patients from either peripheral blood and/or bone marrow. Gene expression profiling of highly purified LSCs would allow the identification of genes that reflect the biology of the cells that are actually driving the leukaemia. Hence, in addition to being a more efficient way to further understand the biology of LSCs, 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 leukaemic as the result of accumulated mutations. Conversely, LSCs could derive from more committed progenitors or even a differentiated mature cell, which would first have to reacquire the self-renewal capacity before accumulating additional mutations.

There are two reasons to think that normal HSCs 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 leukaemia (AML-M3) subtype, the only cells capable of transplanting leukaemia in NOD/SCID mice have a CD34+CD38+ phenotype, similar to that of normal HSCs, whereas more mature CD34+CD38+ leukaemic blasts cannot transfer the disease to mice [5,7].

On the other hand, evidence indicating that cells devoid of self-renewal activity, such as committed progenitors and mature cells, can also be the targets for leukemic transformation comes from analyses of leukaemia-associated genes in the mouse. Indeed, using promoter elements of several myeloid-specific human genes [like those encoding MRP8 (multidrug resistance protein 8), CD11b and cathepsin G] to target transgene expression specifically to committed myeloid cells allowed the generation of multiple accurate transgenic mouse models of human leukaemias [20,21]. More recently, Cozzio et al. [22] have shown that the potent leukemic fusion gene MLL-ENL (mixed lineage leukaemia-eleven nineteen leukaemia), which results from the t(11; 19) translocation, can induce the exact same leukaemia when transduced into HSCs as well as into CMPs (common myeloid progenitors) and GMPs (granulocyte/monocyte progenitors). Another fusion gene MOZ-TIF2 has also recently been shown to contribute to the transformation of both HSCs and more committed myeloid progenitors [23]. These results imply that myeloid leukemias induced by these oncogenes can be initiated in committed progenitors due to their intrinsic capacities to confer leukaemogenic self-renewal potential.

Using a different fusion partner of MLL, GAS7 (growth arrest specific gene 7), So et al. [24] showed that only the transduction of murine HSCs 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 leukaemogenesis, the results obtained for 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 HSC and myeloid stages, depending mostly on the nature of the associated fusion gene.

**CSCs in solid tumours**

Recent studies in solid tumours 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 haematopoiesis. Al-Hajj et al. [10] were able to prospectively isolate a minor phenotypically distinct subset of breast cancer cells that was able to recapitulate the tumours 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 produce differentiated non-tumorigenic progenies. A recent report has also suggested the existence of brain CSCs, which are able to generate new tumours in vivo that exhibit both self-renewal and differentiation [8,9].

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 CSCs are biologically distinct from other cells in the tumour, and are able to initiate and sustain tumour growth in vivo, whereas the bulk cells are not.

Conclusions
The identification of CSCs 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 tumour. 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 to be related to the resistance and persistence of CSCs. Future studies must focus instead on identifying and characterizing the rare cancer-initiating cells, and cancer treatments must be designed to specifically target these CSCs if they are to effectively cure and prevent disease relapse.

References

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