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# REVIEW

# Cancer stem cells: lessons from leukaemia

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**Abstract.** Increasing evidence suggests that leukaemias are sustained by leukaemic stem cells. Leukaemia can indeed be viewed as aberrant haematopoietic processes initiated by rare leukaemic stem cells 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 leukaemic stem cells and the molecular pathways underlying the transformation of normal cells into leukaemic stem cells. This report will review our current knowledge on leukaemic stem cells development and finally demonstrate how these discoveries provide a paradigm for identification of cancer stem cells from solid tumours.

## INTRODUCTION

Leukaemic stem cells (LSCs) represent a subpopulation of cells within a leukaemia, which is capable of initiating and maintaining the disease following a prolonged period of time. This occurs because LSCs have unique properties such as longevity, self-renewal and quiescence, similar to normal haematopoietic stem cells. Two excellent recent reviews present an expanding body of literature addressing the similarities in the biology of stem cells and cancer stem cells and propose once again that cancers may arise from quiescent tissue stem cells (Reya *et al.* 2001; Passegue *et al.* 2003). In the present review, I shall introduce the notion of LSCs, the potential origin of these cells with an emphasis on myeloid leukaemia and finally examine the impacts these discoveries may have clinically and on understanding the organization of cancer of other tissues.

## DEMONSTRATION OF THE EXISTENCE OF LSCS

Elegant experiments by Fialkow *et al*. (1981), in which they used patterns of inactivation in X-linked genes, had shown that leukaemias such as chronic myelogeneous leukaemia (CML) and acute myeloid leukaemia (AML) are clonal in origin. The development of quantitative assays

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enabling measurement of the clonogenicity of malignant haematopoietic cells led to further demonstrations that only a small subset of cancer cells is capable of extensive proliferation *in vitro* (Griffin & Lowenberg 1986). 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–colony forming unit (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.

However, it was not until advances in the identification and separation of discrete leukaemia cell subsets, and the availability of appropriate *in vivo* assays, that the existence of LSCs was first demonstrated. Transplantation of primary AML cells into severe combined immunodeficient (SCID) (Lapidot *et al*. 1994) or non-obese diabetic/severe combined immunodeficient (NOD/SCID) (Sutherland *et al*. 1996; Bonnet & Dick 1997) mice led to the finding that only rare cells, termed SCID leukaemia-initiating cells (SL-IC), 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-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 demonstrated by serial transplantation (Bonnet & Dick 1997). 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 cancer stem cell hypothesis.

## COMPARISON BETWEEN NORMAL AND LSCS

Although LSCs appear to share similar cell surface markers previously identified for normal haematopoietic stem cells (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 (Blair *et al*. 1997, 1998; Blair & Sutherland 2000; Jordan *et al*. 2000). Despite these few phenotypic differences between normal HSC and LSC, recently, further characterization of the SL-IC showed that these cells were not homogeneous and exhibited a heterogeneous phenotype with regard to their timing of engraftment, initiation, lifespan of each graft, proliferation capacity and quiescent state (Hope *et al*. 2004). A few years earlier, the same group demonstrated a similar heterogeneity in the normal HSC compartment based on self-renewal and proliferation capacities (Guenechea *et al*. 2001). 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. This concept is supported by a correlation between the genes required for normal haematopoietic development and those perturbed in leukaemia (Tenen 2003), and by the recent demonstration that Bmi-1 plays a key role in self-renewal determination in both normal and leukaemic murine stem cells (Lessard & Sauvageau 2003; Park *et al*. 2003).

## 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 transformation is still controversial. 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 a result of accumulated mutations. Conversely, LSCs could derive from more committed progenitors or even a differentiated mature cell, which would have to reacquire the self-renewal capacity first before accumulating additional mutations.

There are two reasons to think that normal HSC themselves are the target of leukaemic 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. Second, stem cells persist for a 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 (Lapidot *et al*. 1994; Bonnet & Dick 1997).

Alternatively, evidence indicating that cells devoid of self-renewal activity, such as committed progenitors and mature cells can also be the targets for leukaemic transformation, comes from analyses of leukaemia-associated genes in the mouse. Indeed, using promoter elements of several myeloid-specific human genes (for example MRP8, CD11b, cathepsin G) to target transgene expression specifically to committed myeloid cells, allowed the generation of multiple accurate transgenic mouse models of human leukaemias (Brown *et al*. 1997; Jaiswal *et al*. 2003). More recently, Cozzio *et al*. (2003) have shown that the potent leukaemic fusion gene *MLL-ENL*, which results from the t(11;19) translocation, can induce the exact same leukaemia when transduced into HSCs as well as into common myeloid progenitors (CMPs) and granulocytic/monocytic progenitors (GMPs) but not into megakaryocytic/erythroid progenitors (MEPs). 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 leukaemias induced by these oncogenes can be initiated in committed progenitors because of their intrinsic capacities to confer leukaemogenic self-renewal potential. Using a different fusion partner of *MLL*, *GAS7*, So *et al*. (2003) showed that only the transduction of murine HSC, but not CMP or GMP, resulted in the production of mixed lineage leukaemias in transplanted mice. Although the mouse system provides a valuable tool to study leukaemogenesis, 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 the HSC and the myeloid stage depending mostly on the nature of the oncogenic event.

## CANCER STEM CELLS IN SOLID TUMOURS

Recent studies in solid tumours indicate that the concept of cancer stem cells may have broader implications beyond the field of haematopoiesis. Al-Hajj *et al*. (2003) 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 progeny. A recent report has also suggested the existence of brain cancer stem cells, which are able to generate new tumours *in vivo* that exhibit both self-renewal and differentiation (Singh *et al*. 2003, 2004).

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 of cells are not.

## CONCLUSION

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 of 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 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.

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