Biology of Normal and Leukemic Stem Cells

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A B S T R A C T

A fundamental problem in cancer research is the identification of the cell type capable of initiating and sustaining the growth of the neoplastic clone *in vivo*. The key to solving this problem lies on the observation made over 40 years ago that tumours are heterogeneous and thus might be maintained only by a rare subset of cells called *cancer stem cells* (CSCs). The blood-related cancer leukaemia was the first disease where human CSCs, or leukaemic stem cells (LSCs), were isolated. 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 LSC and the mechanisms responsible for their emergence in the course of the disease. This report will review our current knowledge on normal and leukaemic stem cell development.

The hallmark properties of the haematopoietic stem cells (HSC) 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 specialised capacities. In order to assure a persistent pool of regenerating cells without outgrowth of immature cell types, a 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 leukaemia (AML) and chronic myeloid leukaemia (CML).[1,2] 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 leukaemic stem cell (LSC) 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 and of the heterogeneity of both normal and leukaemic stem cell compartment, the potential role of the microenvironment for the regulation of the stem cell and how dysregulation of this process might be involved in leukaemogenesis.

Normal haematopoietic stem cell

Haematopoiesis is thought to occur via progressive lineage restriction as a successive hierarchy of differentiation from a single pluripotent stem cell to multipotent progenitors to bipotent and unipotent progenitors and ultimately to many different committed mature cell types.

The ideal way to assess haematopoietic stem cells is to test their self-renewal and multilineage capacity *in vivo* using lethally irradiated recipient mice. It is not possible to perform these experiments in humans, but xenotransplantation models may provide the closest paradigm for the human haematopoietic environment.

Immuno-compromised mouse models offer the most suitable environment available for the study of human haematopoietic cells and allow to access the self-renewal, proliferation and differentiation of human haematopoietic stem cells.[3]

Identification/isolation of haematopoietic stem cells

To fully understand HSC biology and the hierarchy of haematopoiesis one must be able to separate the extremely rare HSC from the rest of haematopoiesis and ideally be able to identify/isolate their progenies.

Many approaches have been employed to isolate haematopoietic stem cells via flow cytometry. For almost 20 years, the CD34 antigen has been the marker of choice for the identification and isolation of the human haematopoietic stem cell. The $CD34^+$ cell population is a heterogeneous subset consisting of most of the stem/progenitor cells present in haematopoietic tissue. Self-renewing stem cells capable of long-term reconstitution of conditioned hosts may be distinguished from the more mature progenitors via their lack of CD38 co-expression.[4]

In addition to this well-characterised $CD34^+$ subset, haematopoietic stem cells that do not possess any detectable CD34 expression exist. Direct sorting of cells negative for CD34 as well as a cocktail of lineage antigens into NOD/SCID mice has yielded long-term haematopoietic reconstitution. NOD/SCID repopulation derived from $CD34^-$ cells occurred at a different timepoint to $CD34^+$ cell repopulation and involved the generation of $CD34^+$ cells. These results demonstrate that $lin^-/CD34^-$ cells contain a subset of haematopoietic stem cells that are separate and distinct to those previously identified and actually precede $CD34^+$ cells in the hierarchy of haematopoiesis (ref. #5 and Afonso *et al.* in preparation). This observation has been reproduced the foetal sheep model with very similar results.[6]

HSCs are generally regarded as being devoid of lineage specific antigen.[7] As HSCs commit to specific blood cell lineages, lineage markers are expressed. However, it has been noted that genes associated with specific lineages are expressed in cells with a stem cell phenotype.[8] Recently we show that CD33, CD13 and/or CD123, well-established myeloid markers, are expressed on most human long-term repopulating cells from cord blood and bone marrow.[9] This study changes our views of HSCs and the process of differentiation. SRCs within the $Lin^-CD34^+CD38^-$ population are thus heterogeneous. This heterogeneity is consistent with the heterogeneity observed within SRCs using viral tracking studies.[10] Some repopulating cells do not contribute to haematopoiesis until later time points while others appear to be relatively short lived. The phenotypes of the cells with different repopulation characteristics have not yet been defined. The presence of myeloid markers on HSCs is consistent with the lineage-priming hypothesis mentioned previously.[11,12] This hypothesis states that HSCs transcribe lineage specific genes prior to commitment, in readiness for differentiation (HSCs are 'primed').

Leukaemic stem cell

Whereas it was previously believed that most or all cancer cells possess the property to self-renew and replenish new cancer cells, it has recently become clear that cancers, as most normal tissues, are organised in a hierarchical fashion, and that only a small fraction of tumour cells have the ability to reconstitute a new tumour.[1] The existence of such cancer stem cells (CSCs) with self-renewal potential was first documented in leukaemias,[13,14] but has later been extended to solid tumours, including breast and brain raising the possibility that such cells are the apex of all neoplastic systems.[15,16] As these rare CSCs are both required and sufficient to reconstitute a new tumour, they have immediate and important clinical implications. A better identification and characterisation of CSCs should provide a better understanding of tumour developmental biology and the genetic events involved in the transformation process.

Despite of the clear importance of CSCs in the genesis and perpetuation of cancers, little is currently known about the biological and molecular properties that make CSCs distinct from normal stem cells, the developmental/cellular origin of CSCs, the mechanisms responsible for their emergence in the course of the disease, and identification of candidate molecular targets for therapeutic intervention.

The adaptation of the available quantitative assays for normal human stem cells capable of repopulating haematopoiesis *in vivo* allowed the identification of leukaemic initiating cells. Transplantation of primary AML cells into NOD/SCID mice led to the finding that only rare cells, termed AML-initiating cells (AML-IC), are capable of initiating and sustaining growth of the leukaemic clone in vivo, and serial transplantation experiments showed that AML-IC possess high self-renewal capacity, and thus can be considered to be the leukaemic stem cells. By LSC we refer to a cell that has self-renewal and differentiation potential and is able to reinitiate the leukaemia when transplanted into NOD/SCID. This definition does not preclude the nature of the cells that is being transformed (i.e. normal HSC, progenitors or mature cells).

Importantly, AML-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 represented the only AML cells capable of self-renewal.[13] This phenotype has been extended via the use of immunodeficient mice to include an absence of CD71,

HLA-DR and CD117, but include expression of CD123.[17-20] In a recent study we have extended this phenotype further to include expression of CD33 and CD13 on AML-IC from the vast majority of patients.[21] Hence, the extended immunophenotype of the leukaemic stem cell as defined by in vivo propagation is $CD34^+/CD38^-/CD71^-/HLA\text{-}DR^-/CD117^-/CD33^+/CD13^+/CD123^+$.

The cell of origin in leukaemia

A controversial question in the LSC field is to establish what is the nature of the cells from which the leukaemic transformation arises. The importance of establishing this is not only to better understand how the functional properties of the cellular targets of primary and subsequent transforming genetic events can impact on the biology of the disease, but also on therapeutic strategies. The fundamental point is whether the leukaemogenic event dictates the cell phenotype or whether the cell type in which this mutation occurs may influence the phenotype of the abnormality. This question has been addressed in a direct fashion through the use of retroviral-mediated gene transfer and cell fractionation in mice. Direct analysis of the leukaemogenic potential of certain fusion proteins has determined that it is possible to generate AML from either HSCs or myeloid progenitors (22, 23). Whether this is possible *in vivo* and is how AML is generated in humans, still remains to be determined.

Key mechanisms that regulate HSC development

An understanding of the factors that regulate normal haematopoietic cell development is essential for a more complete comprehension of leukaemogenesis and leukaemia. As discussed in the previous section, AML-ICs are very similar to HSCs in terms of phenotype and self-renewal/proliferation, many of the mechanisms/factors that are associated with the growth of HSCs are important for the development of AML-ICs and the propagation of leukaemia. The role of positive and negative factors that influence the self-renewal and/or commitment of HSC and LSC will not be discussed here based on space constrain but can be found in one of my recent review.[24]

The haematopoietic stem cell niche

Stem cells and their immediate progeny interact with the haematopoietic microenvironment. Both cellular as well as extracellular matrix components of the stem cell microenvironment or niche are critical in stem cell regulation. The microenvironment has been described to influence survival, proliferation, and differentiation.[25,26] Recently, advanced imaging studies have demonstrated that the HSCs reside in close proximity to the bone lining osteoblasts[27] as well as blood vessels, which may constitute an alternative niche (also called vascular niche).[28] The molecules that regulate stem cell niche interactions and how these may influence the balance between self-renewal and differentiation are just being revealed.[29,30]

Overall, it appears that the regulation of haematopoiesis is the result of multiple processes involving cell-cell and cell-extracellular matrix interactions, the action of specific growth factors and others cytokines as well as intrinsic modulators of haematopoietic development.

Conclusions

Identification of AML-ICs 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 leukaemia, research should focus more on AML-ICs and not on the blast population that makes up the majority of the leukaemic clone. Existing therapies have been developed largely against the bulk blasts population. Thus, the future rests on the development of new therapies targeting more specifically the LSC compartment.

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