

* CHAPTER 2

Biological properties of haematopoietic stem cells

A. Wodnar-Filipowicz

1. Introduction

Until a few years ago, an interest in stem cells, mostly of haematopoietic origin, was limited to a relatively small representation of scientists and clinicians seeking to understand the role of these rare cells in tissue homeostasis and to utilise their remarkable potential to regenerate an adult tissue following transplantation. Over the last few years, stem cells have captured the imagination of scientists, clinicians and the lay public alike with the promise of representing a future remedy for the major degenerative diseases of our civilisation and even dysfunctions associated with normal ageing. The progress in technologies to detect and enumerate stem cells *in vivo* led to discovery of stem cells residing in most mammalian tissues, contributing to their generation, homeostasis and probably repair. A major breakthrough has been achieved in the development of methods to propagate human embryonic stem (ES) cells in culture and to drive their *in vitro* differentiation into specialised human tissues. The concept of cancer stem cells has emerged, as cells responsible for generation and persistence of tumours. Here we discuss the stem cell field by summarising the current knowledge of the phenotype of these cells, their interactions with the microenvironment in which they reside, and the mechanisms regulating their functions. The central place will be taken by haematopoietic stem cells (HSCs), representing the first-discovered, the best-understood and, at present, the only clinically-applicable population of stem cells. We also summarise the current state of knowledge on the functional plasticity of somatic tissue stem cells and on human ES cells. The therapeutic relevance gained from the basic research findings will be emphasised.

2. Stem cell definition

Stem cells are defined as a population of undifferentiated cells with the capacity to divide for indefinite periods, to self-renew and to generate a functional progeny of highly specialised cells. This common definition includes cells present in different physical locations and having fundamentally different proliferative properties and functions (Table 1).

A fertilised egg (zygote) represents a *totipotent stem cell*, a cell with unrestricted differentiation potential and the only cell with the capacity to give rise to all cells necessary for the development of foetal and adult organs. ES cells forming a cluster of cells inside the blastocyst are *pluripotent stem cells*, capable of generating a variety of specialised cell types, but limited in their differentiation potential by the inability to support the development of a foetus. Further specialisation results in generation of *multipotent stem cells* residing in adult somatic tissues. Their physiological functions are to replenish mature cell populations of the given tissue

Table 1: Definition of stem cell types

Stem cell	Developmental properties
Fertilised egg	- Totipotent - Unrestricted differentiation potential
ES cells	- Pluripotent - Give rise to a variety of specialised cell types
Adult somatic stem cells	- Multipotent - Limited to specific tissues

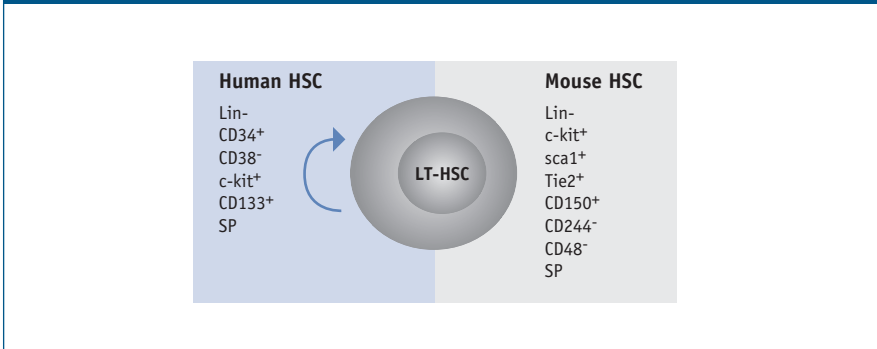
or organ, and to respond to stress by repairing the damage. HSCs represent the prototype of multipotent adult tissue stem cells (1). In humans, HSCs can be found in cord blood as a result of stem cell migratory properties during foetal development, whereas post-natally, the only organ harbouring HSCs and pursuing active multilineage haematopoiesis is the bone marrow.

3. Characteristics of stem cells in the bone marrow

More than 40 years of research on bone marrow-derived stem cells, initiated in the 1960s by Till and McCulloch, marked an ongoing improvement in methods to quantitate and isolate these cells. Assays for clonogenic precursors of the myeloerythroid lineages *in vitro*, defined as long term culture-initiating cells (LTC-ICs) and committed colony forming units (CFUs) were followed by the development of a model of immunocompromised non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice, which allows the study of the repopulating ability of human haematopoietic cells *in vivo* (2). These functional assays are paralleled by progress in the phenotypic characterisation of haematopoietic cells by flow-cytometry, owing to monoclonal antibodies specifically recognizing cell surface molecules (Figure 1). The phenotypic properties of murine HSCs have been precisely defined as cells devoid of lineage markers and expressing the stem cell antigen (sca1) and the receptor c-kit. The Lin-sca1⁺c-kit⁺ (LSK) cell population has self-renewing and long-term repopulating activity *in vivo*. Other cell surface markers defining the HSC compartment in mice include the tie2 and flt3 receptors and CD150. Characteristically, c-kit, flt3 and tie2 function as receptors for early-acting haematopoietic growth factors: stem cell factor, flt3 ligand and angiopoietin, which act as key positive regulators of haematopoiesis (3).

The most primitive human HSCs express CD34 and lack CD38 cell surface antigens and have the capacity to reconstitute a sublethally irradiated NOD/SCID host. The CD34⁺CD38⁻ HSC compartment, which constitutes $\pm 0.1\%$ of bone marrow cells, is

Figure 1: The immunophenotypic characteristics of human and mouse long-term repopulating haematopoietic stem cells (LT-HSC)



Lin: lineage; SP: side population cells

heterogeneous and contains also c-kit⁻, flt3⁻, and CD133-expressing cells. Both mouse and human HSCs are present among the side population (SP) cells which express the drug transporter protein Abcg2 and therefore have the ability to actively efflux the DNA-intercalating dye Hoechst. Human HSCs remain at present less well defined than murine HSCs. Despite the availability of methods which greatly facilitate and enhance the precision of studies with well defined cell populations, it is most likely that pure human HSCs have not yet reached the hands of the scientists. Even less defined remain the properties of stem cells from tissues other than bone marrow, primarily because the isolation of stem cells of skin, muscle, brain or liver remains difficult. Nevertheless, the flow cytometry-based characterisation of somatic tissue stem cells indicates that several cell surface markers are shared, including CD34, c-kit, sca1 and CD133, underlying common features of these rare and not easily-accessible stem cell populations.

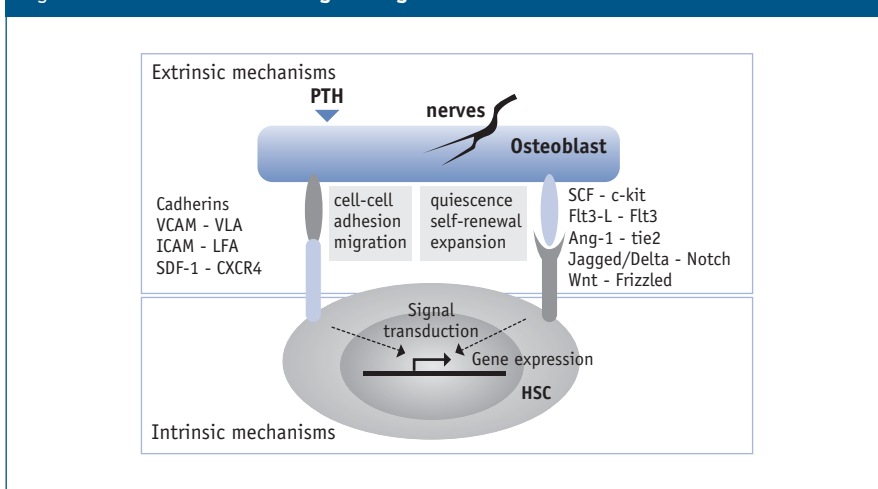
Haematopoiesis occurs in close physical contact with stroma lining the bone marrow niches. Recently, much attention has focused on the differentiation properties of stroma cells themselves. A marrow stromal cell population, termed mesenchymal stem cells, has been shown to give rise to numerous tissue types, including cartilage, bone, fat, and muscle (4). In addition, a minor population of adherent multipotent adult progenitor cells (MAPCs) was found to be capable of differentiation into functional hepatocytes, endothelial cells, skeletal myeloblasts, osteoblasts, chondrocytes and, importantly, haematopoietic cells (5). This suggests the capacity of the bone marrow for multilineage tissue regeneration is present in

both haematopoietic and non-haematopoietic stem cell populations. The therapeutic implications of non-haematopoietic bone marrow cells have already been clinically tested in the treatment of *osteogenesis imperfecta* in transplanted children (6) (see also Chapter 34). Improvement of clinical parameters suggests that bone marrow derived mesenchymal progenitor cells carry a potential for repair of bone and cartilage tissue.

4. Stem cell niches

The concept of the stem cell niche defines a microenvironment, where important interactions between adhesion molecules and their ligands, and between cytokines, chemokines and their corresponding receptors control the fate of stem cells and their progeny (7). In the adult bone marrow, HSCs are located in the trabecular endosteum, where osteoblastic cells are critical components sustaining the quiescence or self-renewal of HSCs, the properties essential for long-term haematopoiesis (8). Both intrinsic and extrinsic mechanisms define the state of either quiescence or cycling and differentiation of HSCs (Figure 2). The intrinsic mechanisms include transcription

Figure 2: The mechanisms regulating the HSC niche



A graphical representation of extrinsic and intrinsic mechanisms in the niche based on the functional interaction of the parathyroid hormone (PTH) and nervous system with osteoblasts, the homing of HSCs in response to chemokines (SDF-1), the physical interactions involving the cadherins and integrins (VLA), and regulation of HSC quiescence, self-renewal and expansion by cytokines: stem cell factor (SCF), Flt3 ligand (Flt3-L), angiopoietin-1 (ang-1), notch ligands (jagged and delta) and wnt ligands, followed by signaling downstream from the cognate receptors and initiating the gene expression

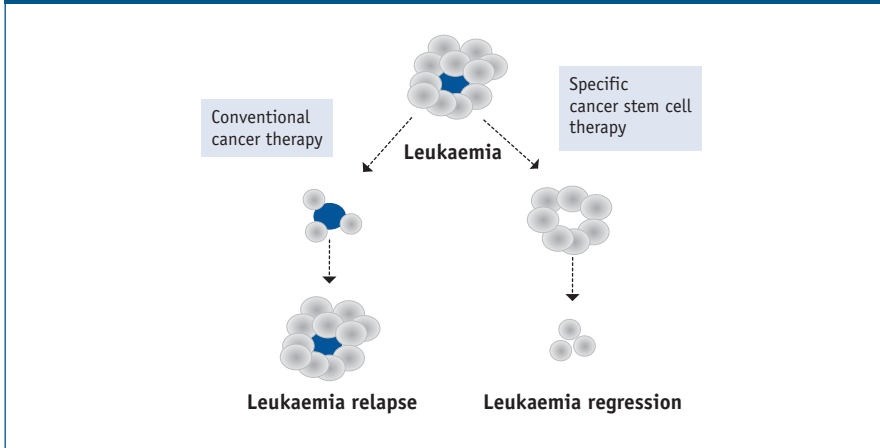
factors and epigenetic regulators acting through chromatin remodelling. The extrinsic mechanisms are dictated by the environment of stromal and osteoblastic cells. Chemokines are responsible for HSC homing into the niche. The direct physical interaction between HSCs and the niche cells are mediated by adhesion molecules, such as integrins and cadherins. The membrane-bound and locally secreted cytokines define the HSC fate by initiating specific signalling pathways within the cell. The most prominent examples are stem cell factor, flt3 ligand, angiopoietin, Notch ligands and wnt ligands, which act synergistically. Also *ex vivo*, these cytokines allow an expansion of HSC numbers. According to the most recent findings, the extrinsic environmental cues in the HSC niche include hormonal regulators, such as parathyroid hormone, and the influence of a sympathetic nervous system, both signalling through the osteoblastic niche component, as well as the regulation by oxidative conditions in the niche. The information on the structure and cellular composition of the niche is only beginning to be revealed, and particularly in the human system the knowledge is only just emerging.

5. Leukaemic stem cells

The available data suggest that leukaemia is a stem cell disease, in which the stem cell self-renewal mechanisms are preserved but the tight growth control is lost due to malignant transformation. As an additional mechanism, the oncogenic events might be imposing the self-renewal capacity at the level of committed progenitor cells (9). Consequently, leukaemic stem cells (LSCs) share many molecular mechanisms that regulate the function of normal HSCs. At present, phenotypic features specific for LSCs are not defined. LSCs are thought to reside within the CD34⁺CD38⁻ cell population, which contains transplantable cells giving rise to human leukaemia in NOD/SCID mice. HSC-characteristic adhesion molecules, such as integrins, are involved in LSC interaction with stroma. Hence, dissimilarities between normal and leukaemic cells most likely have their origin in differences in intracellular signal transduction pathways (10). As an example, oncogenic lesions in the cytokine receptors, c-kit and flt3, are responsible for constitutive activation of downstream signalling in cells at the earliest stages of haematopoietic differentiation, resulting in HSC to LSC transition.

A better understanding of the properties of LSCs is of major therapeutic relevance for the design of LSC-targeted therapies (Figure 3). Conventional chemotherapy-based treatment of leukaemia, and cancer in general, is primarily directed against the bulk of malignant cells, and thus does not eliminate the abnormal stem cells. These cells are the origin of cancer recurrence and are responsible for relapse. Current efforts are focussed on the development of methodology to isolate these cells to better

Figure 3: The therapeutic relevance of LSCs



Leukaemic blasts are depicted in grey, and LSCs in blue

homogeneity, and to dissect the differences in molecular mechanisms used by normal HSC and LSC for their self-renewal and interaction with the microenvironment in the bone marrow (11).

6. Embryonic stem cells

Human ES cells can be isolated from the blastocyst 4–5 days after fertilisation, and cultured *in vitro* to give rise to immortalised cell lines. Depending on the culture conditions, differentiation into cells bearing characteristics of various somatic tissue types including haematopoietic, neural, muscle and other tissues, can be achieved. This work, initiated in 1998 by Thompson et al. (12) who defined methods to isolate and propagate human ES cells from the fertilised oocyte at the 30 cell stage, is of significant value for studies on human developmental biology. Importantly, *in vitro* cultured human ES cells represent valuable tools for drug screening. The most publicised and controversial aspect of ES-related research is associated with the origin of these cells, being a donated surplus human blastocyst from *in vitro* fertilisation procedures. Destruction of human embryos in order to obtain the ES cells has been of serious ethical concern. Novel findings describe derivation of human ES cell lines from single blastomeres at the 8-cell stage, without embryo destruction. ES cells are the subject of intense research which aims at understanding the molecular basis of “stemness”. In parallel, cellular biology techniques have been

developed which allow manipulations such as nuclear transfer from a somatic cell to the enucleated oocyte, and the further generation of ES cell lines bearing genetic information defined by the donated nucleus. The multilineage developmental potential of human ES cells opens new therapeutic avenues for the restoration of damaged or diseased tissue. The possibility to provide these cells with genetic information from the patient by nuclear transfer, is an approach which – in the future – might yield transplantable tissue of a full immunological compatibility. Ultimately, the choice of an approach will require the consideration of advantages and disadvantages associated with the cell source for transplantation (Table 2).

Table 2: Potential therapeutic value of ES cells and adult somatic stem cells

Stem cell source	Advantages	Disadvantages
ES cells	<ul style="list-style-type: none"> - perfect plasticity - easy to programme - stable in culture - no risk of infection transmission 	<ul style="list-style-type: none"> - ethical concern - limited source - rejection danger - carcinogenicity?
Adult somatic stem cells	<ul style="list-style-type: none"> - accessible source (HSCs) - no rejection (autologous) - no ethical concern - no carcinogenicity 	<ul style="list-style-type: none"> - less/no plasticity?

7. Stem cell plasticity

The term “adult stem cell plasticity” defines the ability of tissue-specific stem cells to acquire, under certain microenvironmental conditions, the fate of cell types different from the tissue of origin and belonging to all three germ layers, i.e. similar to the differentiation ability of ES cells. Traditionally, the development of adult stem cells has been depicted along a well-defined path of a linear and irreversible progression concluding in terminally differentiated cell types. Furthermore, the differentiation and regenerative potential of adult stem cells has been regarded as restricted to tissues in which they reside. These traditional concepts have been challenged in the recent years by numerous studies performed by transplantation of stem cells derived from bone marrow and other organs, and which demonstrated the presence of cells of interest in tissues other than those in which they normally reside. The findings from murine studies, which suggested that stem cells may be recruited out of a circulation and engaged in regeneration of diverse tissues at distal sites, have initiated a search for “unusual” locations of donor-derived stem cells

in patients receiving organ transplants. In some cases transitions have been documented, and are likely to reflect a healing response by cells summoned to the site of injury and instructed by the local environment of the damaged tissue. However, donor-host cell fusions rather than functional stem cell plasticity may represent the underlying mechanism (13).

This concept of plasticity of somatic tissue stem cells has a potential clinical impact and may revolutionise tissue transplantation therapies and regenerative medicine. According to this novel view, at least a subset of stem cells may alter their fate in a manner that is more plastic and dynamic than previously thought, causing a fascination with these cells that has spread to nearly all clinical disciplines. There are more questions raised than answers. Following a phase of excitement and rapid accumulation of results in favour of stem cell plasticity, research in this field is now going through a less spectacular phase of verification of the existing data with refined techniques. It is too soon to discard the basic paradigm of developmental biology of the mesodermal, endodermal and ectodermal germ layer origin of mammalian organs, but a need for possible revisions to the unidirectional view of cell fate in post-embryonic development may arise.

8. Conclusions and future perspectives

The ultimate goal for regenerative medicine is to channel the multipotent and/or pluripotent stem cells with high proliferative capacity into specified differentiation programs within the body for a multitude of therapeutic uses. These envisaged uses may include the generation of neurons for treatment of Alzheimer's disease, Parkinson's disease or spinal cord injuries, the generation of insulin-secreting pancreatic cells for the treatment of diabetes, or the generation of heart muscle cells for treatment of congenital disorders or heart attacks. Recent major technical advancements in the isolation, expansion and controlled differentiation of human ES cells and adult stem cells from at least some tissues, and additionally, the establishment of nuclear transfer techniques from the somatic cell to an enucleated donor oocyte opened a number of potential new therapeutic approaches for the restoration of damaged or diseased tissue. The new challenge in stem cell biology is related to understanding the molecular and the functional programmes of leukaemic versus normal stem cells.

The principle of self-renewal and lineage-making decisions of stem cells of different tissue-origin requires understanding in molecular terms. Gene expression profiles bring evidence of the overlapping genetic programs of ES cells, haematopoietic and other adult tissue stem cells, both normal and transformed (14). Determining how epigenetic features relate to the transcriptional signatures of ES and various types

of adult stem cells, is a new key challenge for the future (15). Understanding of the stem cell niche is essential for advancing the approaches to control developmental pathways by both cell-autonomous and microenvironmental cues. All this information will be used as guidance for specifically targeting the fate of normal and malignant stem cells in clinical settings.

Acknowledgments

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Multiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/ebmt-handbook2008answers.htm>

1. Haematopoiesis takes place in the following adult human organs:

- a) Bone marrow
- b) Peripheral blood
- c) Spleen
- d) Liver

2. The stem cell compartment in the bone marrow consists of:

- a) Clonogenic CFU and LTC-IC progenitors only
- b) Haematopoietic, mesenchymal and endothelial cell progenitors
- c) NOD/SCID repopulating cells only
- d) Haematopoietic, liver and neural stem cells

3. Cell surface antigen CD34 is expressed on:

- a) Long-term repopulating haematopoietic stem cells
- b) Short-term repopulating haematopoietic stem cells
- c) Haematopoietic and non-haematopoietic stem and progenitor cells
- d) Lineage-committed progenitors

4. Embryonic stem cells are characterised by:

- a) Lineage-restricted differentiation potential
- b) Potential to become a variety of specialised cell types
- c) Ability to generate placenta
- d) Ability to form a blastocyt

5. Osteoblast components of the stem cell niche are involved in:

- a) Supporting stem cell self-renewal
- b) Supporting the stem cell differentiation
- c) Inhibiting the bone marrow stroma
- d) Promoting the stem cell exit to the peripheral blood

NOTES