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REVIEW

Haematopoietic stem cells: past, present and future

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The discovery and characterisation of haematopoietic stem cells has required decades of research. The identification of adult bone marrow as a source of haematopoietic cells capable of protecting an organism from otherwise lethal irradiation led to the intense search for their identity and characteristics. Using functional assays along with evolving techniques for isolation of haematopoietic cells, haematopoietic stem cell populations were able to be enriched and their characteristics analysed. The key haematopoietic stem cell characteristics of pluripotentiality and the ability for self-renewal have emerged as characteristics of several haematopoietic stem cell populations, including those that have recently challenged the conventional concepts of the haematopoietic hierarchy. Human allogeneic stem cell therapy relies on these functional characteristics of haematopoietic stem cells that can be isolated from peripheral blood, bone marrow or cord blood, with the additional requirement that immunological barriers need to be overcome to allow sustained engraftment while minimising risk of graft-versus-host disease developing in the recipient of transplanted stem cells. Current and future research will continue to focus on the identification of haematopoietic stem cell regulators and methods for *in vitro* and *in vivo* stem cell manipulation, including genome editing, to expand the scope, potential and safety of therapy using haematopoietic stem cells.

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KEY-POINTS

- Haematopoietic stem cells are rare cells with characteristics of pluripotency and self-renewal that are capable of generating an entire haematopoeitic system.
- Haematopoietic stem cells have been identified at defined stages of embryonic development and several subsets have been characterised in adult haematopoiesis.
- The relative contribution of haematopoietic stem cells to steady-state and stress haematopoiesis remains controversial.
- Allogeneic haematopoietic stem cell transplantation therapy in human requires significant immunological barriers to be overcome.
- Current and future research aims to identify key stem cell regulators and methods for *in vitro* and *in vivo* stem cell manipulation including genome editing.

Haematopoietic stem cells (HSC) are the architects of definitive haematopoiesis, that is, blood cell production that occurs continuously during the life of an organism. Each HSC is programmed to allow efficient production of the cellular blood components with a manifest purpose that has been shaped by evolution: from red cells that allow efficient carriage of oxygen, megakaryocytes and their platelet progeny that interact with blood vessels and soluble coagulation factors to regulate clotting, to the cells of the innate and acquired immune system that act against microbial attack. HSCs are defined by their pluripotentiality, the capacity for a single HSC to generate any and all of the diverse mature functional haematopoietic cell types. Key genes and select genetic programmes are invoked for the maintenance, or self-renewal of HSCs and for the formation of the specific haematopoietic lineages.[1–5]

Definitive haematopoiesis in the embryo begins with the emergence of the first identifiable HSC in the aorto-gonado-mesonephros region.[6,7] Thereafter, haematopoiesis shifts to the fetal liver, and subsequently to the bone marrow, where HSCs will reside for the life of the mammalian organism[8,9] (Figure 1).

IDENTIFICATION OF HSCS

It became apparent, initially through work that sought to characterise radiation sensitivity, that donor adult bone marrow transplanted into syngeneic irradiated murine recipients was capable of protecting the recipients from lethal irradiation by regenerating (reconstituting) the irradiation-ablated haematopoietic system. This research was crucial in the development of the concept of HSC as cells in the bone marrow capable of generating the complete blood cell system, although at this time the specific cell had yet to be isolated and characterised.

In these early experiments, donor-derived clonogenic colonies of multiple haematopoietic lineages were able to be macroscopically identified in the spleen of transplanted recipients.[10,11] These spleen colony-forming units, although not definitive HSCs, were nevertheless useful in allowing characterisation of progenitor cells responsible for haematopoietic reconstitution. Specific progenitor cells appeared to possess the ability to form multiple haematopoietic lineages from within the one colony (multipotency), while others appeared able to form daughter cells that retained the characteristics of the original parental cell (self-renewal).[12] These two important characteristics ultimately came to be recognised as definitive characteristics of HSC.

The identification and characterisation of HSCs ultimately required strategies to separate these rare bone marrow cells from more numerous cellular components. Functional competitive

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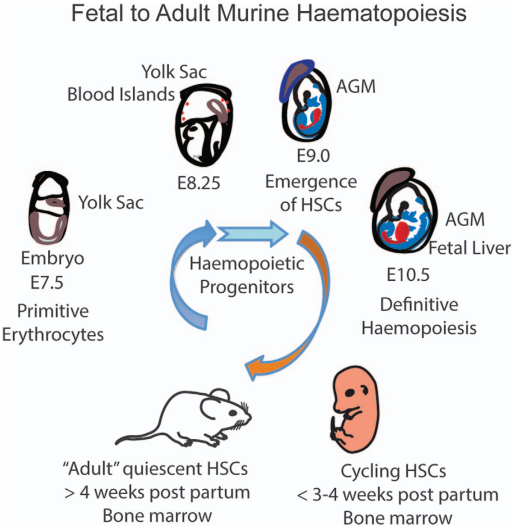

Figure 1. The journey from fetal to adult haematopoiesis adapted from Dzierzak and Speck.[8] AGM, aorto-gonado-mesonephros; PsP, para-aortic-splanchnopleura. See references in main text.

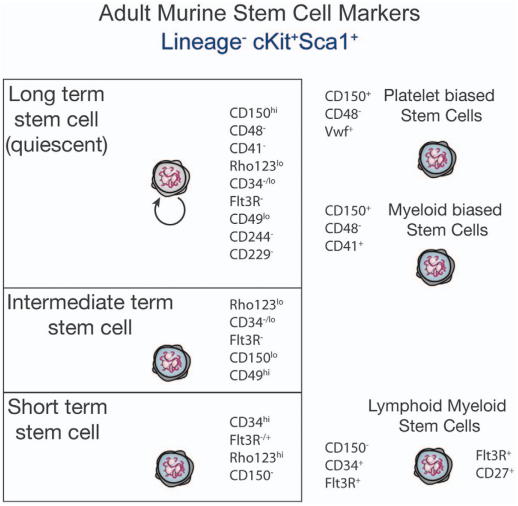

Figure 2. Immunophenotypic markers of adult murine HSCs and 'lineage-restricted' HSC populations. See references in main text.

repopulating unit assays estimated the frequency of these rare cells as one in 10 000 cells in bone marrow.[13] Like the proverbial search for the needle in a haystack, HSCs were eventually isolated with increasing purity based on physical properties, such as Hoescht 33342 supravital dye exclusion,[14] resistance to 5-fluorouracil[15] or γ-irradiation.[16] Ultimately, however, it was the application of flow cytometry and the use of specific cell surface antigen markers[17] that led to the ability to prospectively identify cell populations able to reconstitute multiple lineages upon transplantation, and capable of self-renewal as judged by serial transplantation assays. These cell populations, enriched for HSCs, were notable for their lack of mature lineage antigen expression, and expression of antigens such as cKit, the cellular receptor for the cytokine stem cell factor.[18,19]

HSCs were found to possess unique properties that set them apart from other blood-forming progenitor cells. In addition to the properties of pluripotency and self-renewal, adult long-term HSCs were found to reside in a specific niche environment in the bone marrow, which was closely associated with endosteum,[20] and where they exist in conditions of relative hypoxia.[21] Here, HSC exists predominantly in a non-replicative and quiescent state,[22] in which signalling by the cytokine thrombopoietin[23,24] and the presence of megakaryocytes are recognised to have an important role.[25–27] In contexts that place the haematopoietic system under stress, such as chronic infection, these quiescent stem cells are recruited into cell cycle, for example, via interferon signalling, which is associated with a numerical increase in downstream progenitor cells.[28] Evidence increasingly suggests that lineage specification can occur very early in the haematopoietic hierarchy in immunophenotypically defined 'stem cell' populations[29–32] (Figure 2), supporting findings that self-renewing lineage-restricted progenitors may emerge directly from HSC.[33] A significant degree of complexity may therefore exist in the pathways via which mature haematopoietic lineages develop from HSC and progenitor populations. More direct pathways from HSC to specific mature cells may coexist alongside traditional models of progenitor population hierarchies. Recent evidence from clonal dynamic studies tracing the origin of blood cells over time has suggested that steady-state blood cell maintenance does not incessantly call upon the quiescent HSCs to enter into cell cycle, but rather, successive recruitment of long-lived progenitor populations appears to primarily maintain blood cells at steady state.[34] However, other studies have yielded apparently conflicting results on the relative contribution of HSCs to steady state and stress haematopoiesis,[35,36] a current controversy that remains to be resolved.

In the setting of bone marrow transplantation, which is dependent on HSCs to sustainably reconstitute haematopoiesis, further refinement of cell surface markers have also identified specific subsets of HSCs with more limited capacity for self-renewal, yet important for maintaining haematopoiesis in the short and intermediate terms after transplantation.[37,38] Stem cells capable of true long-term reconstitution with durable self-renewal potential do appear to be a very rare but essential cell population required for long-term haematopoietic engraftment[39–41] (Figure 2).

BONE MARROW TRANSPLANTATION AS THERAPY

Although murine haematopoiesis reflects human haematopoiesis in many ways, the immunophenotypic markers of human HSCs ($Lineage^{-}CD34^{+}CD38^{-}$) differ from functionally similar murine counterparts.[42] Unlike inbred genetically and immunologically identical mouse strains, successful allogeneic transplantation therapy in humans requires significant immunological barriers to be overcome. The discovery of the HLA system of MHC class I and II receptors, which engage T-cell antigen receptors, allows histocompatible matching of donors and recipients. This is supplemented with the use of immunosuppression during and after the transplantation of allogeneic stem cells from volunteer-related and -unrelated donors. Advancement of stem cell transplantation therapy has focussed on research to broaden the availability of donors to patients. Use of cord blood units as a source of stem cells[43,44] and recently developed conditioning and immunosuppressive regimens have allowed haploidentical transplantation[45] to become a therapeutic reality while limiting the immunological consequence of graft-versus-host disease. These approaches are increasingly making the option of allogeneic transplantation available to patients who otherwise do not have a matched-related or volunteer-unrelated donor source of stem cells (Figure 3).

THE FUTURE

Research defining the nature and regulation of HSCs has allowed regulators of blood cell production to be manipulated

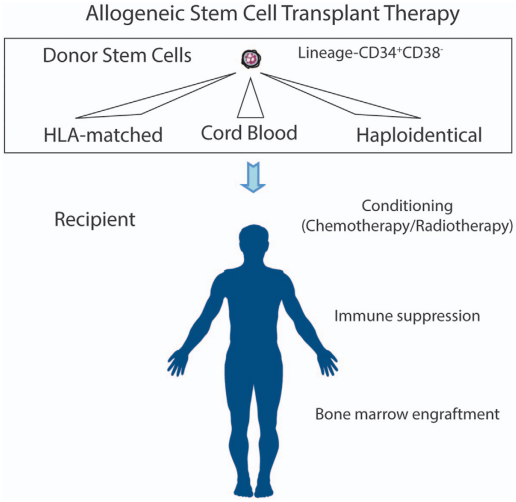

Figure 3. Human HSC transplantation therapy. HLA-matched adult, cord blood or haploidentical adult donor stem and progenitor cells are transplanted intravenously into a recipient following conditioning therapy to permit engraftment of donor marrow into the recipient. Immune suppression is administered to prevent acute graft-versus-host disease.

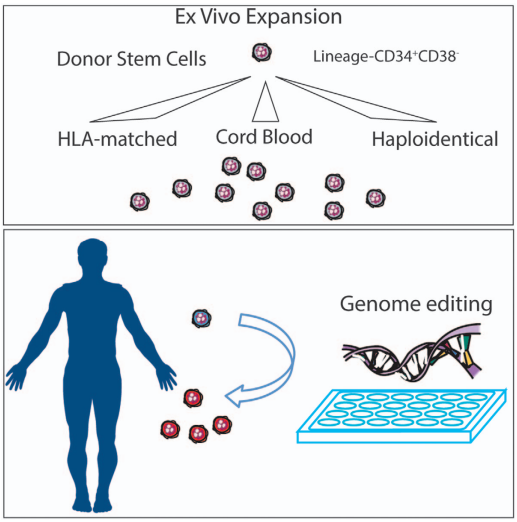

Figure 4. Future directions and applications of HSC research. (Top panel) *Ex vivo* stem cell expansion. (Bottom panel) Genome editing of HSCs.

in ways that have revolutionised treatment of blood disorders and the use of stem cell transplants. Novel outcomes from ongoing stem cell research continue to refine this understanding and provides the avenue to continual treatment improvements.

Important challenges remain. These include: developing robust methods to maintain HSCs *in vitro* both to enhance research and to expand cell numbers for therapy; developing a deeper understanding of the HSC niche and intrinsic and extrinsic HSC regulators; and to develop safely the future capacity to 'reprogramme' cells to HSCs, correct genetically defective HSCs that would allow transplantation of 'corrected' syngeneic patient cells or transplant reprogrammed haematopoietic cells for directed therapy against specific patient diseases (Figure 4).

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COMPETING INTERESTS

The authors declare no conflict of interest.

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