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REVIEW

Progress and challenges in generating functional hematopoietic stem/progenitor cells from human pluripotent stem cells

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Abstract

The generation of hematopoietic stem cells (HSCs) from human pluripotent stem cells (hPSCs) *in vitro* holds great potential for providing alternative sources of donor cells for clinical HSC transplantation. However, the low efficiency of current protocols for generating blood lineages and the dysfunction identified in hPSC-derived hematopoietic cells limit their use for full hematopoietic reconstitution in clinics. This review outlines the current understanding of *in vitro* hematopoietic differentiation from hPSCs, emphasizes the intrinsic and extrinsic molecular mechanisms that are attributed to the aberrant phenotype and function in hPSC-derived hematopoietic cells, pinpoints the current challenges to develop the truly functional HSCs from hPSCs for clinical applications and explores their potential solutions.

Key Words: *hematopoietic differentiation, hematopoietic reconstitution, hematopoietic stem cells, human embryonic stem cells, induced pluripotent stem cells*

Introduction

Hematopoietic stem cell (HSC) transplantation has been broadly used for the treatment of life-threatening hematopoietic disorders, including deadly genetic diseases and malignant leukemia, with matchless therapeutic benefits. Since the early 1960s, autologous HSCs obtained from bone marrow (BM) or mobilized peripheral blood (M-PB) have been used for transplantations in clinics to treat a wide variety of hematopoietic diseases, such as myeloma and autoimmune diseases [1,2]. As an alternative and extension, allogeneic HSCs isolated from tissue-matched BM, M-PB or umbilical cord blood (UCB) have been used and proven to be beneficial in treating a wider range of pathological conditions such as leukemia, myeloproliferative disorders, lymphoma, myeloma and other solid tumors [3,4]. However, although much effort has been put into banking BM and cord blood cells, the limited availability of suitable donors still severely restricts the application of this powerful approach in clinical therapies because of the heavy demand of large numbers of HSCs for transplantation. Hence, establishing new sources and generating a large number of transplantable HSCs has been a long sought-after goal for treating hematologic conditions.

A recent advance in stem cell research has opened a new avenue for HSC transplantation. A novel source, human pluripotent stem cells (hPSCs), can be potentially used in hematopoietic therapies to develop HSCs. hPSCs include human embryonic stem cells (hESCs) derived from early embryos at the blastocyst stage and induced pluripotent stem cells (hiPSCs) generated from somatic cells through epigenetic reprogramming. hESCs and hiPSCs exhibit highly similar, if not identical, features, having the ability to differentiate into various somatic cell types while

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undergoing robust self-renewal in culture [5,6]. Hence, they provide a renewable source for generating blood lineage cells. iPSCs reprogrammed from somatic cells in particular broaden the horizons in generating patient-specific stem cells, thus having enormous potential for the treatment of hematologic diseases without restriction from immune incompatibility and ethical concerns (reviewed in van Bekkum *et al.* [7]). Currently, hematopoietic differentiation strategies have enabled the generation of multiple desired blood cell types [8]. However, *in vitro* derivation of human HSCs with robust capacity to sustain multilineage engraftment has not been achieved. Therefore, understanding the differences between hPSC-derived hematopoietic cells and their *in vivo* counterparts and uncovering the reasons why hPSC-derived HSC-like cells lack the multilineage engraftment potential have become major issues for the research in this field. In this Review, we provide an overview of the recent progress in improving hematopoietic differentiation from hPSCs, pinpoint the current challenges to the further application of these cells and discuss potential solutions that may facilitate the development of technology for the *de novo* generation of clinically-applicable HSCs.

HSC development at a glance

Mammalian hematopoietic development comprises a sequential series of cell fate decisions, which results in the progressive loss of cellular plasticity as well as gradual specification into single or multiple lineages (Figure 1). In embryos at the blastocyst stage, pluripotent inner cell mass first segregates into primitive endoderm and epiblast. The posterior epiblast then gives rise to a transient structure called the primitive streak (PS), which extends and differentiates into three primary germ layers (ectoderm, mesoderm and endoderm) through gastrulation [9]. Hematopoietic cells originate from the mesoderm, emerging as blood islands in the yolk sac (YS) that primarily support the primitive hematopoiesis at early embryonic stages (E 7.5 in mouse) [10,11]. Around day 10 of mouse gestation, hematopoiesis shifts to an intra-embryonic site—the aorta-gonad-mesonephros (AGM) region—where definitive hematopoietic

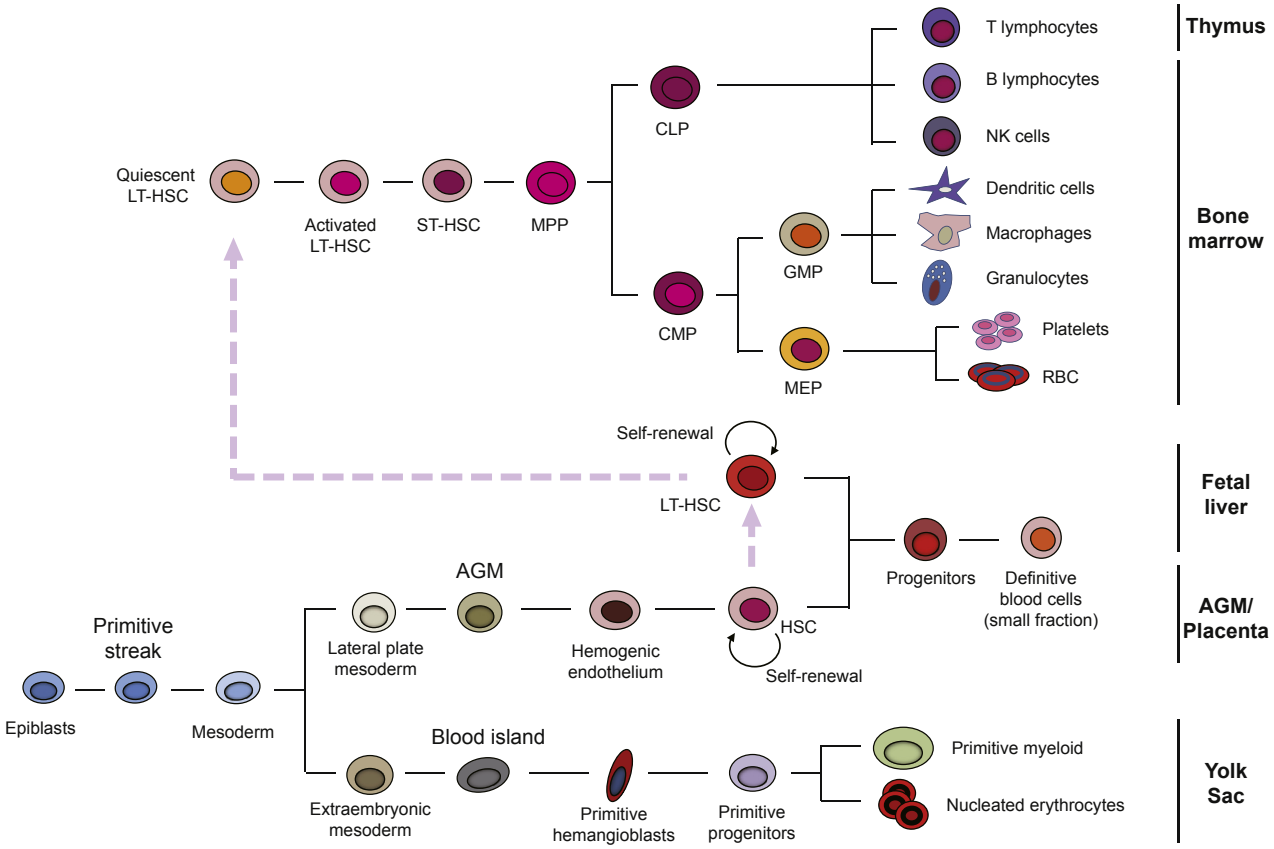

Figure 1. Overview of normal hematopoietic development *in vivo*. This model depicts normal hematopoietic development in the mammalian system. Primitive hematopoietic progenitors in yolk sac initiate transient hematopoiesis for the developing embryo [11,28]. Definitive HSCs originate in AGM and move to bone marrow [12,29]. Long-term HSCs in bone marrow sustain life-long hematopoiesis and provide a continuous supply of various blood cell types [15,16]. ST-HSC, short-term HSCs; MPP, multipotent progenitors; CLP, common lymphoid progenitors; CMP, common myeloid progenitors; MEP, megakaryocyte/erythrocyte progenitors; GMP, granulocyte/macrophage progenitors; RBC, red blood cells.