CELL REGENERATION

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Return to the hematopoietic stem cell origin

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Abstract

Studying embryonic hematopoiesis is complicated by diversity of its locations in the constantly changing anatomy and by the mobility of blood cell precursors. Embryonic hematopoietic progenitors are identified in traditional *in vivo* and *in vitro* cell potential assays. Profound epigenetic plasticity of mammalian embryonic cells combined with significant inductive capacity of the potential assays suggest that our understanding of hematopoietic ontogenesis is substantially distorted. Non-invasive *in vivo* cell tracing methodology offers a better insight into complex processes of blood cell specification. In contrast to the widely accepted view based on the cell potential assays, the genetic tracing approach identified the yolk sac as the source of adult hematopoietic stem cell lineage. Realistic knowledge of the blood origin is critical for safe and efficient recapitulation of hematopoietic development in culture.

Keywords: Hematopoiesis, Development, Hematopoietic stem cell, Repopulation, Cell potential, Cell tracing

Introduction

All blood cells of adult organism are produced in the process of hematopoiesis which depends entirely on the ability of hematopoietic stem cells (HSCs) to self-renew and differentiate into all hematopoietic cell lineages. In mice, the whole population of adult HSCs is formed during embryogenesis and first neonatal weeks [1]. This process has been described in a number of detailed reviews which reflect the current understanding of the HSC development [2-5]. However, a closer look at experimental data reveals significant contradictions in the current paradigm of hematopoietic ontogenesis, and demands reassessment in view of the recent findings [6-8]. This is especially important if the lessons learned from studying the HSC development are meant to be used for future clinical applications. This review is an attempt of alternative interpretation of published experimental data.

Early mammalian development is highly regulative [9] and it is difficult to determine how long this capacity is operational in early ontogenesis. Isolated parts or tissues of precirculation conceptuses have the ability to adapt to the changed microenvironment by switching to an alternative developmental pathway tissues. This capacity therefore substantially complicates finding the anatomical origin of mammalian hematopoiesis by standard hematological approaches. However, practically all information about development of hematopoietic progenitors and HSCs is based on the analysis of embryonic cell potentials in a number of *in vivo* and *ex vivo* assays. Accumulating evidences on the extent of cell plasticity [10-13] and striking ease of cell reprogramming [14-16] suggest that there might be a significant gap between measured potential of a cell and its fate in developing conceptus. The assays may lead to artefactual inductions that change initial commitment of a cell and promote specification in an alternative direction. Moreover, the assays can selectively kill certain subsets of hematopoietic progenitors by imposing a strong non-physiological stress. Mere dissociation of embryonic tissue before the assay can induce profound changes in cell behavior [17]. Due to their unsettled epigenetic status embryonic cells are more vulnerable than adult cells to various inducing events during the potential assays. And from a practical point of view, it is difficult to detect the induced cell plasticity event in embryonic system due to the absence of defined starting cell types.

Another critical problem is that analyzing cell potentiality is often skewed towards conditionally pluripotent or highly multipotent cells which may be present in conceptus tissues. These cells can give rise to hematopoietic

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progeny in a process similar to embryonic stem (ES) cell differentiation, and the term of the hematopoietic progenitor or even the HSC might be falsely awarded to a cell which is not committed to differentiate into blood *in vivo*.

For molecular studies, the classical hematological assays are valuable since they outline the emerging potentials during progressive mesoderm specification. However, the cell potential dynamics are unlikely to accurately describe developmental processes. The potential data may prove misleading if we are to recapitulate *in vitro* the natural sequence of embryonic cell inductions and maturations from such epiblast cell surrogates as ES or iPS cells.

Terms and definitions

Controversy surrounding the issue of mammalian blood origin to a large extent results from the confusion with the understanding and interpretation of basic terms and definitions. One of these, "definitive HSC", has to designate cells which self-renew and produce committed hematopoietic progenitors at a suitable site of hematopoiesis. Definitive HSCs (dHSCs) are detected using their ability to serially repopulate normal, i.e. non-immunodeficient or otherwise genetically compromised, myeloablated recipients. However, it remains an open issue that some fetal cells may display the HSC potential even in a stringent repopulation assay but do not function as dHSCs *in situ*. It has been difficult to determine when dHSCs start to generate blood lineages in conceptus due to retrospective way of the HSC definition. In the repopulation assay tested cells are introduced into the severely conditioned environment of mouse recipient and their progeny is traced for several months to confirm the long-term repopulation potential. It is possible that apparent stem cell potential of a tested cell population is actually induced within the recipient hematopoietic system and a few newly generated HSCs are efficiently selected for survival. First dHSC potential is found at embryonic day 10.5-11.0 (E10.5-E11.0), when the low-level adult-recipient repopulating activity is first detected in the aorta-gonads-mesonephros (AGM) region [18]. However, there is no evidence that these cells actually function as typical HSCs at this stage *in vivo*, i.e. self-renew and clonally differentiate into a number of blood lineages. On the contrary, blood cell generation does not occur [19,20], and cell proliferation in general has been found to be very low in the AGM region at this stage [21]. At E11.5-12.5 dHSCs expand in fetal liver and possibly in placenta. Again, we can say only about the expansion of a potential, but not about the expansion of the stem cell function. The fetal liver hematopoiesis (mainly erythropoiesis) is substantially increased at this stage, although this might be accomplished mainly by unipotent or oligopotent progenitors lacking the crucial self-renewal capacity [2].

To circumvent the difficulty with staging the onset of dHSC function, the term "HSC lineage" can be introduced or redefined. In developmental hematology this term describes all developmental history of strictly defined dHSCs starting from the earliest mesodermal precursor cells. Since early mammalian development is highly regulative it is necessary to exclude the zygote, blastomeres, inner cell mass (ICM) and epiblast cells from the HSC lineage. The lineage includes committed toward hematopoiesis mesodermal cells in early embryo, their progeny leading eventually to mature stem cells and which can be called pre-HSCs (intermediate cells ultimately giving rise to the initial dHSC population) and two major subtypes – fetal and adult – of dHSCs. Many members of the HSC lineage do not possess the HSC potential and cannot function as stem cells; they have to undergo specification, selection and maturation to become fully operative dHSCs. In the conceptus this lineage coexists with others, such as primitive erythroblasts, macrophages and megakaryocytes, and a number of definitive transient hematopoietic progenitor lineages in yolk sac, fetal liver and other fetal hematopoietic organs constituting the first wave of definitive hematopoiesis [22]. The relationship between all these conceptal hematopoietic cell lineages is unclear, though recent cell tracing experiments point to the probability that all these lineages have common mesodermal precursors [6,8].

Another specific term which needs clarification is "*de novo* hematopoiesis". The purpose of the term is to distinguish two distinct processes of blood cell formation: the hematopoiesis itself, i.e. generation of blood cells from an existing hematopoietic precursor, and differentiation of the lateral mesoderm into first cells which can be regarded as belonging to a blood cell lineage. The obvious difficulty in the definition is the uncertainty about criteria which can be used to define emerging cells as hematopoietic. Perhaps induction of molecular signatures similar to the early hematopoietic triade [23] can be chosen to distinguish this type of hematopoiesis. The term reflects an objective process of primary blood generation in the conceptus, whereas the expansion and maturation of newly formed hematopoietic precursors can be defined as secondary developmental hematopoiesis which is distinct from the "classical" hematopoiesis initiated by mature hematopoietic progenitors or dHSCs. *De novo* hematopoiesis can be regarded as segregation of blood-committed cells from other mesodermal precursors. By definition, *de novo* hematopoiesis is always an autonomous process, whereas the secondary and "the classical" types of hematopoiesis are mostly non-