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Developmental niches for embryonic erythroid cells

Joan Isern^{a,b}, Stuart T. Fraser^{a,b,c}, Zhiyong He^{a,b}, and Margaret H. Baron^{a,b,c,*}

^a Department of Medicine (Division of Hematology and Medical Oncology), Mount Sinai School of Medicine, New York, NY 10029

^b Tisch Cancer Institute, Mount Sinai School of Medicine, New York, NY 10029

^c Black Family Stem Cell Institute, Mount Sinai School of Medicine, New York, NY 10029

Abstract

Primitive erythroid cells (EryP) are the first differentiated cell type to be specified during mammalian embryogenesis. EryP arise from a pool of lineage-restricted progenitors in the yolk sac (YS) and then enter the newly formed embryonic circulation to mature in a stepwise, synchronous fashion. Numbering in the millions in the mid-gestation mouse embryo, EryP are the dominant circulating blood cell prior to the rapid generation of adult-type definitive erythroid (EryD) cells in the fetal liver. The identification of maturational events in this lineage presented a significant challenge, as EryD begin to outnumber EryP in the bloodstream from ~E14.5 onwards. We used human *epsilon-globin* gene regulatory elements to drive lineage-specific expression of a histone-H2B::EGFP fusion protein, allowing us to label the chromatin of EryP during their development and to track and quantify EryP nuclei following their expulsion from the cell. Using this transgenic fluorescent reporter mouse line, we have monitored primitive erythropoiesis in three distinct niches: the YS, where EryP progenitors arise; the circulation, where EryP continue to divide and mature; and the fetal liver, where EryP complete the terminal stages of their differentiation.

Keywords

primitive erythropoiesis; yolk sac; fetal liver; mouse embryo; transgenic mice

Introduction

Mammalian development requires the rapid, *de novo* formation of embryonic blood cells to support embryonic and fetal growth prior to the establishment of the adult hematopoietic system [1]. Two ontogenically distinct erythroid lineages emerge during embryogenesis [2] (reviewed in [3,4]). The very first red blood cells to appear in the embryonic circulation are the primitive erythroid (EryP) lineage and arise during a restricted developmental window in the extra-embryonic yolk sac (YS) [1,5]. As the embryo develops, YS-derived progenitors and newly formed hematopoietic stem cells found in the fetal liver (FL) generate a second wave of adult-type definitive erythrocytes (EryD), that will enter the circulatory system and eventually replace EryP [1].

*Corresponding author: Dr. Margaret H. Baron, M.D., Ph.D., Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1079, New York, NY 10029, Tel: (212) 659-9691; Fax: (212) 849-2442, margaret.baron@mssm.edu.

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EryP exhibit a number of distinctive features that distinguish them from their definitive counterparts. EryP are considerably larger, circulate for a significant period of the lifespan as nucleated cells and express a unique set of embryonic globin genes. In contrast, EryD are released from the microenvironments in which they form as small, anuclear cells expressing only adult-type globins. The simultaneous presence of both EryP and EryD has previously complicated analysis of the development of both erythroid lineages. To address this issue we have capitalized on the exquisite lineage restriction of globin gene expression in designing transgenic fluorescent reporter mouse lines. Human *epsilon-globin* gene regulatory elements have been used to drive GFP and histone-H2B::EGFP (nuclear-GFP) specifically in the primitive erythroid compartment [4,6,7]. These transgenic models have allowed us to monitor EryP appearance, expansion, maturation and enucleation at a previously unattainable resolution.

Embryonic Erythropoiesis

The Yolk Sac is the first developmental microenvironment for primitive erythropoiesis

In the mouse, the first EryP-committed progenitors appear shortly after gastrulation (approximately E7.5) in the YS blood islands [1,2,8] and, by E8.5, EryP have expanded throughout the primary honeycomb-like vasculature [9,10]. At this developmental stage, EryP comprise 25–40% of all cells in the embryo (our unpublished data), indicating that huge resources have been set aside for their development of this lineage. Previous attempts at purifying EryP progenitors (defined as colony forming cells or EryP-CFC) have relied upon the expression of CD41, a marker of hematopoietic progenitors, in the absence of markers of early mesoderm or mature erythroid cells [10] or of PECAM1 or Tie-2, expressed on hematopoietic and vascular progenitors [11]. However, none of these markers was specific for EryP progenitors and, therefore, had limited utility for purification of this population. We have detected expression of *epsilon-globin::H2B-EGFP* [7] at E7.5 and have used this transgene to enrich for cell populations with EryP progenitor activity (Isern *et al.*, manuscript in preparation). Within 48 hours of the first appearance of EryP progenitors, the circulatory systems of the YS, the embryo and the placenta are connected and the heart functions as an effective pump. By this stage, EryP enter the bloodstream and EryP progenitor activity is abruptly lost [1].

EryP undergo a stepwise maturational transformation after entering the circulation

Upon entering the circulation, EryP begin to mature as a more or less synchronous cohort and progress through a number of morphologically identifiable events, including loss of nucleoli, reduction in cell size, nuclear condensation, and changes in cytoplasmic content [4]. By E12.5–E13.5, all EryP exhibit condensed chromatin and highly pyknotic nuclei and have ceased dividing [4]. It is at this time when the first enucleated EryP begin to appear in the bloodstream [3,4]. We have used a novel approach that combines the cell-permeable DNA-binding fluorescent dye DRAQ5 and the EryP-restricted reporter expression of GFP to follow the time course of enucleation [4]. The numbers of enucleated EryP (GFP+ DRAQ5-negative) increased dramatically from E13.5 to E15.5 [4]. By E15.5, most of the circulating EryP had enucleated [4]. Flow cytometric analysis of cell surface protein expression revealed that enucleated EryP expressed higher levels of Ter119 than do their nucleated precursors and had lost expression of $\alpha 4$ -integrin [4,7]. In contrast with a previously held belief that EryP are cleared from the bloodstream as definitive erythropoiesis takes off in the fetal liver, EryP in fact persist in the circulation through at least the end of gestation [4] (R. Moore, S. Fraser, and M. Baron, unpublished results).

The fetal liver as a site of terminal maturation of EryP

We detected strong GFP fluorescence in the developing livers of ϵ -globin::GFP transgenic embryos [7]. EryP were found to accumulate transiently within the fetal liver (FL) parenchyma and often within erythroblastic islands (EBI), in close contact with fetal liver macrophages (FLMs) [7]. GFP+ cells from the FL showed significantly higher levels of adhesion molecule expression compared to those from peripheral blood (PB) [7]. EryP from PB or FL bind to FLM *in vitro* in a developmentally regulated manner: mid-gestation EryP (E12.5-E14.5) EryP bound to FLM, while early (E9.5) or late stage (E15.5) EryP did not [7]. This binding depends, at least in part, on interactions between $\alpha 4 \beta 1$ -integrin on EryP and VCAM-1 on FLM [7]. Following enucleation, the ability of circulating EryP to adhere to macrophages was lost [7]. Nuclei labeled by expression of H2B-EGFP were found within FLM after co-culture of EryP and FLM and in FLM in the native fetal liver (identified by immunostaining of FL slices or by FACS sorting of FLM), suggesting that EryP nuclei are cleared and degraded by FLM [7].

Interestingly, a population of low side scatter (low granularity) GFP+ particles could be identified by FACS in the circulating blood and fetal liver [7]. When isolated using flow cytometry and analyzed morphologically, these structures proved to be expelled nuclei [7]. The extruded nuclei displayed much higher levels of cell surface adhesion proteins such as $\alpha 4$ -integrin than did nucleated EryP, suggesting asymmetric redistribution of surface molecules during enucleation [7].

Conclusion

We have developed transgenic mouse models to study the biology of primitive erythropoiesis at a resolution not previously possible. Given their stepwise, synchronous maturation and final enucleation, the development of primitive erythroid cells provides a useful model for mammalian red blood cell ontogeny. Comparisons of the similarities and differences between the primitive and definitive erythroid lineages should provide new insights into the general mechanisms underlying erythroid development and terminal differentiation.

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