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Analysis of concentration-dependent functions of PU.1 in hematopoiesis using mouse models

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Abstract

The Ets family transcription factor PU.1, encoded by the gene *Sfp1*, is essential for normal hematopoiesis. A number of studies have suggested that changes in PU.1 concentration play a role in directing cell fate decisions during hematopoiesis. However, the stages of hematopoietic development at which changes in PU.1 concentration are important have not been defined until recently. Experiments using conditional null alleles, reporter alleles, and hypomorphic alleles of the *Sfp1* gene in mice demonstrate that PU.1 concentration is uniformly high during early stages of hematopoietic development. However, reduction of PU.1 concentration is required for normal development of megakaryocyte-erythroid progenitors, B cell progenitors, and T cell progenitors. PU.1 concentration increases in granulocyte-macrophage progenitors. Furthermore, experimental reduction of PU.1 concentration in the myeloid lineages leads to failed differentiation, abnormal proliferation, and leukemia. In this review, we synthesize recent studies to develop a new model of PU.1 function in hematopoiesis.

Introduction

The transcription factor PU.1 was first described in 1988 as a gene dysregulated by proviral insertion of the spleen focus forming virus (SFFV) in murine erythroleukemia [1]. This gene, called *Sfp1* in mice and *Spi-1* in humans, was found to encode a transcription factor of the Ets family [2]. PU.1 is highly expressed in the immune system, and regulates many genes in cell types such as macrophages and B cells. The importance of PU.1 in hematopoiesis is underscored by the observation that null mutation of the *Sfp1* gene in mice results in fetal or perinatal lethality and multiple defects in blood cell development [3,4]. While PU.1 is not essential for erythrocyte or megakaryocyte development, mice homozygous for a null allele of *Sfp1* fail to generate B lymphocytes, macrophages, or neutrophils. Transplantation and organ culture experiments demonstrate that PU.1 is also required for the normal development of T lymphocytes and natural killer cells [5,6]. Abnormal levels of PU.1 expression are associated with acute myeloid leukemia in human patients [7] as well as in mouse models [8,9,10]. These studies suggest that appropriate concentration of PU.1 protein is critically important for normal hematopoiesis.

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Concentration-dependent function of PU.1 in hematopoietic cell fate determination

Soon after its discovery, PU.1 was recognized to be expressed at significantly higher levels in myeloid lineage cells than in B cells [11]. Subsequently, experiments using retroviral transduction of PU.1 cDNA into *Sfpil* null hematopoietic progenitors resulted in several striking observations. First, B cell progenitors (pro-B cells) generated in culture after infection of *Sfpil*^{-/-} fetal liver progenitors with PU.1 cDNA expressed exclusively low levels of PU.1 transcripts and protein. Second, macrophages generated in such cultures expressed exclusively high levels of PU.1 transcripts and protein. Finally, infection of *Sfpil*^{+/-} fetal liver progenitors with PU.1 cDNA efficiently blocked the generation of pro-B cells and instead appeared to divert differentiation of infected cells into macrophages [12]. These observations led to the hypothesis that low concentration of PU.1 might direct differentiation of hematopoietic stem cells (HSCs) to the lymphoid lineages, whereas high concentration of PU.1 might direct differentiation of HSCs to the myeloid lineages [12]. This hypothesis has recently been tested directly, using mice with inducible null alleles, reporter alleles, or hypomorphic alleles of the *Sfpil* gene. The results of these studies are described below.

Function of PU.1 during hematopoiesis revealed by studies using conditional null alleles

Two alleles of *Sfpil* have recently been described in which the PU.1 DNA binding domain can be conditionally deleted using Cre-mediated excision of loxP elements [13,14]. These model systems allow analysis of the consequences of deletion of *Sfpil* in purified progenitor cells by infection with viral vectors encoding Cre recombinase; deletion in committed B cells (by crossing to CD19-Cre knock-in mice), or deletion in HSCs (by crossing to Mx1-Cre transgenic mice). Experiments in which *Sfpil* is deleted in common lymphoid progenitors (CLPs) or in B cell progenitors (pro-B cells) suggest that PU.1 is not essential for the generation of B cells from committed CLPs. In fact, B cells lacking PU.1 can secrete immunoglobulin and participate in immune responses [14,15,16]. Experiments in which *Sfpil* is deleted in purified common myeloid progenitors (CMPs) or granulocyte-macrophage progenitors (GMPs) demonstrate that PU.1 is essential for the normal differentiation of macrophages and granulocytes at all stages tested [13,14]. Finally, deletion of *Sfpil* in adult HSCs results in the loss of phenotypically recognizable CLPs, CMPs, and B cell development [13,14]. However, in one model, deletion of *Sfpil* in adult HSCs resulted in an unexpected expansion of immature myeloid cells [13]. Taken together, these studies suggest that PU.1 is essential for specification of the earliest lymphoid progenitors, but not essential for the specification of myeloid progenitors from HSCs. PU.1 is not essential for generation of B cells from committed lymphoid progenitors, but is required for generation of macrophages and neutrophils from committed myeloid progenitors. These experiments should not be interpreted as showing that PU.1 has no function in B cells, since gene expression in *Sfpil*^{-/-} pro-B cells is profoundly different from wild type B cells [17].

Use of reporter alleles to measure levels of PU.1 during hematopoiesis

Changes in PU.1 concentration are important for differentiation of several hematopoietic lineages, but it has been unclear at what stage of development these changes occur. Recently, two laboratories independently reported generation and analysis of mice with reporter alleles of *Sfpil* [18,19]. In both cases, cDNA encoding green fluorescent protein (GFP) was inserted into the *Sfpil* locus by gene targeting, such that the pattern of GFP expression resembles PU.1 expression. These studies suggest that PU.1 is expressed at uniformly high levels in HSCs, CMPs, and CLPs. However, PU.1 expression is substantially upregulated in GMPs and

substantially downregulated in megakaryocyte-erythrocyte progenitors (MEPs), B cell progenitors (pro-B cells), and T cell progenitors (pro-T cells). Therefore, these data are not compatible with the idea that changes in PU.1 concentration direct differentiation of HSCs into CMPs or CLPs. Instead, changes in PU.1 concentration are initiated prior to or during specification of MEPs, GMPs, pro-B cells, and pro-T cells.

Function of PU.1 during hematopoiesis revealed by studies using hypomorphic alleles

The most direct test of whether PU.1 concentration plays a role in lymphoid-myeloid cell fate decisions is to experimentally increase or decrease concentration in developing hematopoietic cells. PU.1 concentration can be experimentally increased by retroviral infection of wild type hematopoietic progenitors with PU.1 cDNA. The results from these types of experiments demonstrate that increased expression of PU.1 blocks development of erythrocytes, B cells, and T cells [12,20,21]. Forced expression of PU.1 can also lead to immortalization of erythroid lineage cells and eventually erythroleukemia [22,23]. Therefore, PU.1 concentration must be reduced for the proper development of these cell types.

One method to experimentally decrease PU.1 concentration is to modify the *Sfpi1* gene to reduce levels of expression. Two groups have recently generated and analyzed alleles of *Sfpi1* with altered levels of PU.1 expression [10,24]. Rosenbauer et al. [10] generated mice with a deletion of an upstream regulatory region (Δ URE) in the *Sfpi1* gene. Δ URE mice express reduced levels of PU.1 in most tissues examined, except for T cells, which exhibit an increase in PU.1 levels [10,25]. Reduced PU.1 expression resulted in a block to B cell development, impaired myeloid differentiation, and acute myeloid leukemia [10,25].

More recently our own laboratory has generated a novel hypomorphic allele of *Sfpi1* termed BN [24]. This allele was generated by insertion of the bacterial β -lactamase gene into exon 1 of the *Sfpi1* gene, replacing two known translation start codons. Analysis of cells homozygous for the BN allele (*Sfpi1*^{BN/BN}) revealed that the BN allele produces ~20% of wild type levels of PU.1 protein as a consequence of reduced transcription and translation initiation at a third start codon at position 32 of the protein. The BN allele generates a PU.1 protein lacking the N-terminal 31 amino acids. It is unclear if there is a biological function for these first 31 amino acids, since Δ N31 PU.1 protein has activity identical to full-length protein in experiments performed to date [24,26].

We have examined hematopoiesis in fetal and neonatal *Sfpi1*^{BN/BN} mice to determine the consequences of reduced PU.1 concentration *in vivo*. *Sfpi1*^{BN/BN} mice are runted and osteopetrotic, and all die by one month of age, suggesting a macrophage deficiency similar to that observed in one line of *Sfpi1*^{-/-} mice [27]. B cells were not detectable in *Sfpi1*^{BN/BN} fetal liver, bone marrow, or spleen at any day of development. Furthermore, *Sfpi1*^{BN/BN} fetal liver progenitors could not reconstitute B cell development when transplanted into immunodeficient recipient mice. These data suggest that reduced concentration of PU.1 results in a failure to specify B cell progenitors *in vivo*. Interestingly, *Sfpi1*^{BN/BN} mice have a thymus, although it is reduced in size and contains T cells with altered frequencies of CD4 and CD8-expressing cells (Figure 1). The development of T cells while B cell development is blocked raises interesting questions about what progenitor cell has seeded the thymus in these mice, and why development of this progenitor is not blocked by the *Sfpi1*^{BN/BN} mutation.

We also analyzed myeloid development in fetal and neonatal *Sfpi1*^{BN/BN} mice. Cells expressing the myeloid-specific markers Gr-1, CD11b or Fc γ RII/III are undetectable in E14.5 *Sfpi1*^{BN/BN} fetal liver. The frequency of myeloid colonies that can be generated from *Sfpi1*^{BN/BN} fetal liver progenitors in response to cytokines such as granulocyte-macrophage

colony-stimulating factor (GM-CSF), M-CSF, and G-CSF is dramatically reduced compared to wild type fetal liver progenitors. Unexpectedly, cells with myeloid cell surface markers appear in the bone marrow and spleen of *Sfpil*^{BN/BN} mice after birth. The number of these cells increases rapidly in the first week after birth, and by two weeks of age the spleens of *Sfpil*^{BN/BN} mice essentially contain only immature myeloid cells. These cells have an abnormal phenotype in which the immature cell marker c-Kit is co-expressed with myeloid cell-specific markers such as Gr-1 and CD11b. From these data, several major conclusions can be drawn. First, high PU.1 concentration is essential for the generation of B cells *in vivo*. This requirement is probably at the level of the generation of CLPs from HSCs [14]. Second, high PU.1 concentration is not essential for the generation of myeloid lineage cells *in vivo*, although such cells fail to differentiate normally. Third, the function of PU.1 in generation of myeloid lineage cells appears different between fetal and adult hematopoiesis. During fetal hematopoiesis, PU.1 seems to be essential for the generation of myeloid cells. However, after birth, PU.1 is not required for the generation of myeloid cells, and low concentration of PU.1 appears to stimulate proliferation of immature myeloid cells.

Role of reduced PU.1 concentration in myeloid leukemia

The results of reporter allele studies suggest that PU.1 concentration is uniformly high during differentiation of the myeloid lineages, and increases further during terminal differentiation [18,19]. Therefore, reductions in PU.1 concentration do not normally occur during myeloid development. If PU.1 concentration is experimentally reduced in myeloid cells, the consequences include abnormal proliferation and myeloid leukemia [10,24,28]. Interestingly, PU.1 expression and/or activity is frequently suppressed in myeloid leukemia by a variety of different mechanisms [reviewed in 29,30]. It will be important to determine whether there is a causal relationship between abnormal myeloid proliferation caused by low PU.1 concentration and neoplastic transformation, which presumably requires additional somatic mutations. Identification of the target genes responsible for abnormal myeloid proliferation when PU.1 concentration is reduced will be an important future area of investigation.

Conclusions: a revised model of PU.1 function in hematopoiesis

The results of studies using conditional null, reporter, and hypomorphic alleles of *Sfpil* provide important insights into the function of PU.1 in hematopoiesis. These studies show that changes in PU.1 concentration probably do not occur during differentiation of HSCs into CMPs or CLPs. However, PU.1 concentration is reduced during the generation of committed progenitor cells for erythrocytes, megakaryocytes, B cells, and T cells (Fig. 2). Reduction of PU.1 concentration from initially high levels is a requirement for megakaryocyte/erythroid and lymphoid differentiation. We speculate that an important function of PU.1 during hematopoiesis might be to repress erythroid and lymphoid cell fates. Identification of the target genes that mediate this cell fate repression will be an important future research direction.

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Fig 1.

Lymphocyte development in mice homozygous for a hypomorphic allele of *Sfpi1*. A) Engraftment of wild type (WT) or *Sfpi1*^{BN/BN} fetal liver progenitors into *RAG2*^{-/-}*il2rγ*^{-/-} immunodeficient mice. The percentage of B220⁺ CD19⁺ B cells in the spleen of recipient mice (*n* = 5) was measured by flow cytometry six weeks after *i.v.* injection of 2×10^6 fetal liver cells. B) Total cellularity of the thymus of wild type (WT) or *Sfpi1*^{BN/BN} mice. C) Flow cytometric analysis of single-cell suspensions from thymus of either wild type (WT) or *Sfpi1*^{BN/BN} mice at 10 days of age. Cells were gated for proper size and granularity and incubated with an antibody to the indicated cell surface markers. Numbers represent percentages of gated cells in each quadrant.

Fig 2.

A revised model for concentration-dependent function of PU.1 in hematopoiesis. The model of hematopoiesis shown is based on a revised model of hematopoiesis proposed by Adolfsson et al. [31]. PU.1 concentration is indicated by a gradient from red (high) to blue (low) in the top to bottom direction. The position of each cell type within this gradient indicates the relative concentration of PU.1. Mature megakaryocytes, erythrocytes, and T cells do not express PU.1. Abbreviations are as follows: HSC, hematopoietic stem cell; MPP, multipotential progenitor; LMPP, lymphoid-primed multipotential progenitor; CLP, common lymphoid progenitor; pro-B, progenitor B cell; B, mature B cell; pro-NK, progenitor natural killer cell; NK, mature natural killer cell; pro-T, progenitor T cell; T, mature T cell; MEP, megakaryocyte-erythroid progenitor; Meg, megakaryocyte; E, erythrocyte; GMP, granulocyte-macrophage progenitor; Mac, macrophage; Neut, neutrophil.