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REVIEW

The Transcription Factor PU.1 is a Critical Regulator of Cellular Communication in the Immune System

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Abstract PU.1 is an E26 transformation-specific family transcription factor that is required for development of the immune system. PU.1 functions at both early and late stages of lymphoid and myeloid differentiation. At least 110 direct target genes of PU.1 have been identified since its discovery in 1988. We used the published literature to determine if aspects of PU.1 function can be inferred from the identity of target genes that are directly activated. This analysis revealed that 61% of described PU.1 target genes encode extracellular proteins or transmembrane proteins, most of which are involved in cellular communication. The genes activated by PU.1 can be grouped into pathways based on function. Specific examples of cellular communication pathways regulated by PU.1 include (1) antibodies and antibody receptors, (2) cytokines and cytokine receptors regulating leukocyte growth and development, and (3) cytokines and cytokine receptors regulating inflammation. As a consequence of mutation or repression of the gene encoding PU.1, hematopoietic progenitors may be generated but there is a "failure to thrive" because they cannot interact with their environment. The loss of cellular communication caused by reduced PU.1 levels can lead to leukemia. In summary, PU.1 is a critical regulator of cellular communication in the immune system.

Keywords Immune development · Transcription factor · PU.1 · Cytokine · Antibody · Leukemia

Abbreviations

ChIP	Chromatin immunoprecipitation
ETS	E26 transformation-specific
IPA	Ingenuity Pathway Analysis
miR	microRNA
Ig	Immunoglobulin
Fc	Fragment constant
IL	Interleukin
AML	Acute myeloid leukemia

Introduction

The transcription factor PU.1 was discovered as the product of a gene transcriptionally upregulated in murine erythroleukemia, as a consequence of proviral integration of the spleen focus forming virus SFFV (Moreau-Gachelin et al. 1988). This gene was called *SFFV proviral integration site 1* (*Sfpi1*) in mice and *SPI1* in humans, and was found to encode a transcription factor of the E26 transformation-specific (ETS) family that was named PU box binding-1 (PU.1) (Klemsz et al. 1990). PU.1 protein has 272 amino acids and contains a transcriptional activation domain in its N-terminal 100 amino acids, as well as a DNA-binding domain (ETS domain) in its C-terminal 112 amino acids (Klemsz and Maki 1996). PU.1 interacts with DNA as a monomer and recognizes DNA sequences

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containing the "PU-box" nucleotide motif GGAA or AGAA (Kodandapani et al. 1996; Ray-Gallet et al. 1995). The function of PU.1 has been extensively studied in mice using *Sfpi1* gene knockout studies. Three germline knockout alleles of *Sfpi1* have been generated and analyzed (Back et al. 2004; McKercher et al. 1996; Scott et al. 1994). More recently, two conditional knockout alleles of *Sfpi1* were generated and analyzed (Carotta et al. 2010; Dakic et al. 2005; Iwasaki et al. 2005). These studies revealed that PU.1 is required to generate most myeloid lineages (including macrophages, neutrophils, and dendritic cells) and is required to generate B cells. PU.1 is partially dispensable to generate NK cells, T cells, and erythroid cells, and is not required to generate megakaryocytes. Therefore, PU.1 is a critical regulator of differentiation within the hematopoietic system, and is particularly important for differentiation of the cells that constitute the innate and adaptive immune systems.

PU.1 Expression

The pattern of PU.1 expression in the hematopoietic system has been studied by a combination of techniques, including most recently, reporter alleles "knocked in" to the *Sfpi1* locus in mice (Back et al. 2005; Colucci et al. 2001; Hromas et al. 1993; Nelsen et al. 1993; Nutt et al. 2005). These studies revealed that PU.1 is expressed in a tissue-specific manner, with the highest levels of expression in the myeloid lineages. PU.1 is expressed at similar levels in hematopoietic stem cells and most hematopoietic progenitor types. PU.1 expression is substantially increased during granulocyte–macrophage differentiation, and decreased during megakaryocyte, erythrocyte, B cell, and T cell lineage differentiation. Erythrocytes and T cells silence PU.1 expression during terminal differentiation, although PU.1 can become re-expressed in a subset of T cells (Back et al. 2005; Chang et al. 2010; Nutt et al. 2005). In summary, PU.1 is highly expressed in cells of the immune system, particularly B cells and myeloid cells.

Lineage-Specific Fine-Tuning of Graded PU.1 Levels

Precise regulation of PU.1 protein concentration is important for the development and function of B cells and myeloid cells. Soon after its discovery, PU.1 was recognized to be expressed at significantly higher levels in macrophages than in B cells (Ross et al. 1994). The difference in PU.1 concentration between macrophages and B cells is approximately fivefold, and enforcement of this difference is critically required for both B cell and macrophage development (Dakic et al. 2005; DeKoter and Singh 2000; Houston et al. 2007b; Iwasaki et al. 2005; Spooner et al. 2009). PU.1 concentration plays a critical role in myeloid versus erythroid cell fate decisions (Galloway et al. 2005; Rhodes et al. 2005) and macrophage versus neutrophil cell fate decisions (Dahl et al. 2003; Laslo et al. 2006). Inappropriately increasing PU.1 concentration can have dramatic effects. In erythroid cells, ectopic PU.1 expression leads to immortalization and eventually erythroleukemia in mice (Houston et al. 2007a; Moreau-Gachelin et al. 1996; Schuetze et al. 1993). In developing lymphocytes, ectopic expression of PU.1 efficiently blocks both B cell and T cell development (Anderson et al. 2002; DeKoter and Singh 2000). Conversely, reduced PU.1 expression leads to leukemia, as will be discussed later in this review.

A variety of mechanisms exist for controlling PU.1 levels and/or activity, underscoring the importance of controlling PU.1 levels. First, PU.1 expression is controlled at the level of transcription. Several distal regulatory elements have been identified that are important for upregulation of myeloid-specific expression, or conversely silencing in the T cell lineage (Ebralidze et al. 2008; Hoogenkamp et al. 2007; Leddin et al. 2011). Second, PU.1 transcript levels are fine-tuned by microRNA-155 (miR-155). Reduced levels of miR-155 in B cells causes up-regulation of PU.1 transcript levels and protein expression (Vigorito et al. 2007). Conversely, forced expression of miR-155 reduces PU.1 transcript and protein levels, leading to a myeloproliferative disorder (O'Connell et al. 2008). Third, PU.1 levels and/or activity are regulated by protein–protein interaction. The best documented of such interactions is with the transcription factor GATA-1. PU.1 interacts directly with GATA-1 in the absence of DNA (Nerlov et al. 2000) as well as at GATA-1 binding sites on DNA (Stopka et al. 2005). As a consequence of this interaction, high PU.1:GATA-1 ratios favor developmental decisions of multipotent progenitor cells to generate myeloid lineages over erythroid lineages, and the converse is also true (Arinobu et al. 2007). PU.1 also interacts with the transcription factor growth factor independence-1 (Gfi-1) (Dahl et al. 2007). A high ratio of PU.1:Gfi-1 in myeloid progenitor cells favors developmental decisions of myeloid progenitors to generate macrophages instead of neutrophils (Laslo et al. 2006). High Gfi-1:PU.1 ratios favor the generation of neutrophils over macrophages, as well as the generation of B cells over macrophages (Spooner et al. 2009). Recently, a novel interaction of PU.1 with transcriptional intermediary factor 1 gamma (TIF-1γ) was reported (Kusy et al. 2011). TIF-1γ interacts with PU.1 to repress a subset of PU.1 target genes, resulting in impaired long-term hematopoiesis. PU.1 has been reported to interact with a number of other proteins that are beyond the scope of this review, but have been reviewed elsewhere

(Gupta et al. 2009). In summary, PU.1 activity must be kept under strict control during hematopoiesis. Mechanisms for controlling PU.1 levels and activity include transcriptional regulation, miRNA-mediated regulation of translation, and protein–protein interaction.

Lessons from the Identities of PU.1 Target Genes

PU.1 has been studied for over 20 years and a large number of target genes have been described. We set out to determine if aspects of PU.1 function can be inferred from the identity of target genes. This information was collected from the published literature. PU.1 has been primarily described as an activator of transcription. A list of genes transcriptionally activated by PU.1 was assembled based on the following criteria: (1) The genes were reported as activated by PU.1 because mRNA transcripts are decreased in cells lacking PU.1 and/or increased by ectopic expression of PU.1. (2) At least one binding site was identified for PU.1 that contains the core motif GGAA or AGAA that is required for interaction as shown by X-ray crystallographic studies (Escalante et al. 2002; Kodandapani et al. 1996). (3) Transcriptional activation by PU.1 was demonstrated by transient transfection analysis and/or site directed mutagenesis of predicted binding site(s). (4) Direct PU.1 binding was demonstrated at a minimum by electrophoretic mobility shift assay, chromatin immunoprecipitation (ChIP), or both. Based on these criteria, 110 genes were validated as direct targets of transcriptional activation by PU.1 (Fig. 1; supplementary Table 1). For this analysis, genes that are transcriptionally repressed by PU.1 were not considered further, since in most cases direct binding to DNA by PU.1 has not been demonstrated (Kamath et al. 2008; Stopka et al. 2005).

Next, Ingenuity Pathway Analysis (IPA) software was used to describe the function and subcellular location of the protein products of PU.1-activated genes (Ingenuity Systems, Redwood City CA). IPA analysis suggested that 22 gene products are located in the nucleus, 21 in the cytoplasm, 44 in the plasma membrane, and 23 in the extracellular space (Fig. 2). Therefore, 61% of proteins produced by PU.1-regulated genes are located in the plasma membrane or are secreted proteins. These results infer that PU.1 is an important regulator of cellular communication. Moreover, several gene products located in the cytoplasm also function as important mediators of intracellular signaling downstream of plasma membrane proteins (*BTK*, *FES*, *NF1*, *TEC*). Most nuclear proteins regulated by PU.1 are themselves important transcription factors (*CIITA*, *EBF1*, *GATA1*, *IRF4*, *JUNB*, *KLF4*, *LMO2*, *LYL1*, *MEF2C*, *MYB*, *REL*, *TAL1*, *TFEC*, *ZNF300*; Fig. 1), suggesting that PU.1 may function within one or more hierarchies that control downstream networks of genes.

Vignettes of PU.1 Function

It is beyond the scope of this review to describe the function of all 67 plasma membrane or extracellular gene products. However, the genes activated by PU.1 can be grouped into functional clusters or pathways. Specific examples of cellular communication pathways regulated by PU.1 include (1) antibodies and antibody receptors, (2) cytokines and cytokine receptors regulating leukocyte growth and development, and (3) cytokines and cytokine receptors regulating inflammation. In the remaining space the genes regulated by PU.1 in these three functional pathways are described in more detail. For the sake of simplicity and consistency, human gene names are used even though in many cases genes from other species were shown to be regulated (supplementary Table 1).

Regulation of Genes Encoding Antibodies and Antibody Receptors

The immunoglobulin (*Ig*) genes encode proteins that are assembled into cell-surface or secreted antibodies and are thus critical for humoral immune responses. Among the first target genes of PU.1 identified was the Igκ 3′ enhancer (*IGK@*, Fig. 3), where PU.1 was shown to bind cooperatively with a transcription factor later identified as interferon response factor 4 (*IRF4*) (Pongubala et al. 1992, 1993). A PU.1 binding site was also identified in the *IgH* intronic enhancer (*IGH@*) (Nelsen et al. 1993), itself the first transcriptional enhancer identified in a mammalian gene (Banerji et al. 1983). Since this time numerous other PU.1 binding sites were identified in the *Ig* loci including in an *IgH* 5′ enhancer (Pawlitzky et al. 2006), *Ig*κ variable region promoters (Schwarzenbach et al. 1995), the *Ig*λ 2–4 enhancer (*IGL@*) (Eisenbeis et al. 1993), and the *IgJ* chain promoter (*IGJ*) (Shin and Koshland 1993). Despite these numerous reports, the role of PU.1 in regulating *Ig* transcription and in turn controlling antibody production is not yet fully understood. The *IgH* locus is transcribed efficiently, perhaps even upregulated in B cells lacking PU.1 (Polli et al. 2005; Schweitzer and DeKoter 2004; Ye et al. 2005). More work will be needed to elucidate the exact role of PU.1 in *Ig* transcription, keeping in mind the additional complication that the highly related transcription factors Spi-B and Spi-C are expressed in B cells and have demonstrated functional redundancy with PU.1 (DeKoter et al. 2010).

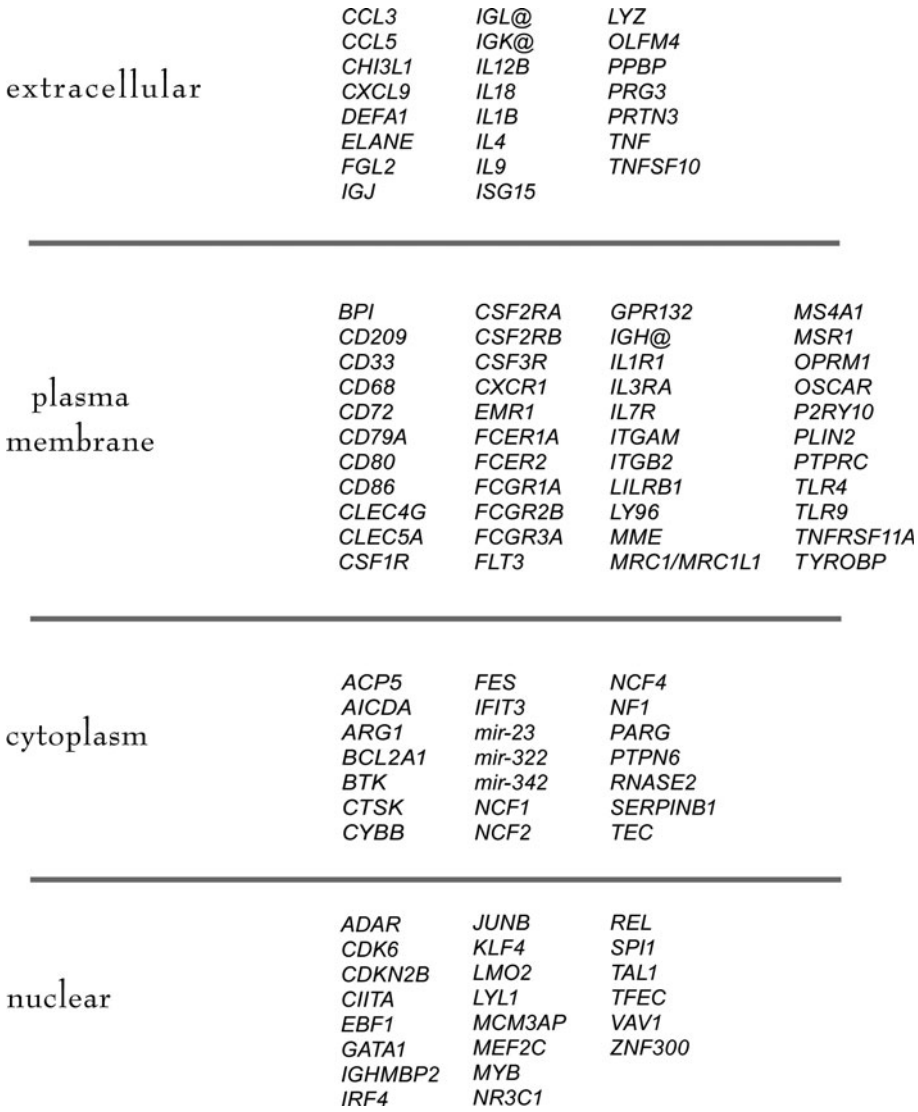

Fig. 1 The identities of genes directly activated by PU.1. Genes activated by the transcription factor PU.1 are indicated in alphabetical order using the human nomenclature. Genes are grouped according to the subcellular location of the proteins that are the products of the indicated genes. Subcellular location is indicated on the *left side*

In addition to regulating genes encoding components of antibodies, PU.1 also regulates genes encoding antibody receptors. Fc receptors are expressed in most cells of the immune system and interact with the constant region of antibodies. PU.1 regulates at least five genes encoding Fc receptors (Fig. 3). These are *FCER1A* encoding the high affinity IgE receptor Fcεr1a (Nishiyama et al. 2002); *FCER2* encoding the low affinity IgE receptor CD23 (DeKoter et al. 2010); *FCGR1A* encoding the high affinity IgG receptor CD64 (Eichbaum et al. 1994); *FCGR2B* encoding the low affinity IgG receptor CD32 (Houston et al. 2007b); and *FCGR3A* encoding the low affinity IgG receptor CD16 (Feinman et al. 1994). It has been shown that expression of these Fc receptors is decreased or absent in cells lacking PU.1 expression in vivo or ex vivo (Houston et al. 2007b; DeKoter et al. 1998, 2010).

In summary, PU.1 regulates genes encoding antibodies and antibody receptors. PU.1 may play a role in fine-tuning humoral immune responses by controlling the amount of antibody produced, as well as the antibody receptors that are important for negative feedback mechanisms. Consistent with this idea, B cells lacking miR-155 were shown to have impaired class switching and antibody production. Mir-155 targets PU.1 for repression, so B cells lacking miR-155 have increased PU.1 expression that is partially responsible for impairing antibody production (Vigorito et al. 2007).

Regulation of Genes Encoding Cytokines and Cytokine Receptors Involved in Leukocyte Growth and Development

PU.1 activates the transcription of a number of important cytokines and cytokine receptors in lymphocytes, macrophages, neutrophils, and dendritic cells, including the

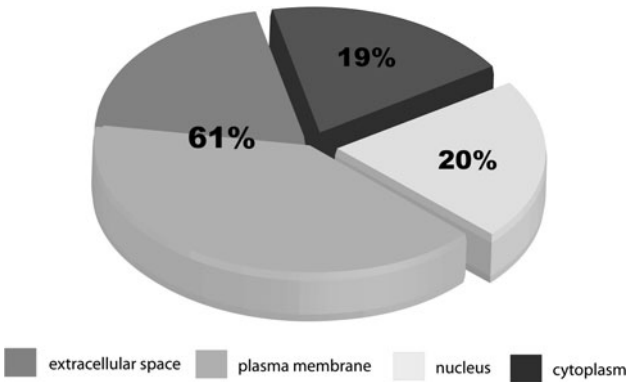

Fig. 2 Most genes activated by PU.1 encode proteins located in the extracellular space or plasma membrane. Of 110 gene products, 45 gene products are located in the plasma membrane, 22 gene products are located in the extracellular space, 21 gene products are located in the cytoplasm, and 22 gene products are located in the nucleus. Together, the gene products located in the plasma membrane and extracellular space make up 61% of all proteins encoded by PU.1-regulated genes. A legend is shown beneath the chart

receptors for macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), granulocyte–macrophage colony stimulating factor (GM-CSF), and interleukin 3 (IL-3). The receptors for these growth factors are, respectively M-CSF receptor (M-CSFR/c-Fms, encoded by *CSF1R*) (Zhang et al. 1994); G-CSFR, encoded by *CSF3R* (Smith et al. 1996); and the heterodimeric GM-CSFR, comprised of alpha (*CSF2RA*) and beta (*CSF2RB*) subunits (Hohaus et al. 1995; van Dijk et al. 1998). The IL-3 receptor shares the beta subunit with GM-CSFR, but the gene encoding the alpha subunit (*IL3RA*) has also been shown be regulated by PU.1 (Akagawa et al. 2003) (Fig. 4). Transcription of these genes is impaired or absent in *Sfpi1* mutant murine progenitor cells (DeKoter et al. 1998; Houston et al. 2007b; Iwasaki et al. 2005).

In the lymphoid lineage, PU.1 regulates several key cytokines and/or receptors. PU.1 activates transcription of the IL-7 receptor alpha gene (*IL7R*) (DeKoter et al. 2002) as well as the Flt3 ligand receptor *FLT3* (Carotta et al. 2010) in early lymphoid and myeloid progenitor cells. Because these two receptors are critical for the generation and survival of B cell progenitors from lymphoid precursors, this may partially explain why B cells are not generated in the absence of PU.1 (Houston et al. 2007b; Medina et al. 2004). PU.1 also activates transcription of the gene encoding IL-4 (*IL4*) in mast cells (Henkel and Brown 1994). IL-4 functions as an important survival factor for B lymphocytes.

Finally, PU.1 activates transcription of genes involved in cellular migration and homing, including chemokines, chemokine receptors, and integrins. Chemokine genes activated by PU.1 include *CCL3* (Grove and Plumb 1993), *CCL5* (Liu and Ma 2006), and *CXCL9* (Ellis et al. 2010). On the receptor side, PU.1 regulates the receptor for IL-8, *CXCR1* (Wilkinson and Navarro 1999) (Fig. 4). Integrin genes activated by PU.1 include *ITGAM*, encoding CD11b (Pahl et al. 1993) and *ITGB2*, encoding CD18 (Rosmarin et al. 1995). These observations suggest that PU.1 may play an important role in the process of leukocyte migration and homing. This has in fact been shown to be the case, as *Sfpi1* knockout hematopoietic stem cells fail to properly home to the bone marrow upon i.v. injection into recipient mice (Fisher et al. 1999).

In summary, PU.1 regulates genes encoding cytokines and cytokine receptors in both the lymphoid and myeloid lineages. Additional evidence for the biological importance of these observations are that deletion of the gene *Sfpi1* encoding PU.1 in vivo leads to cell-intrinsic defects in lymphoid and myeloid development. Importantly, lymphoid and myeloid development can be partially rescued in

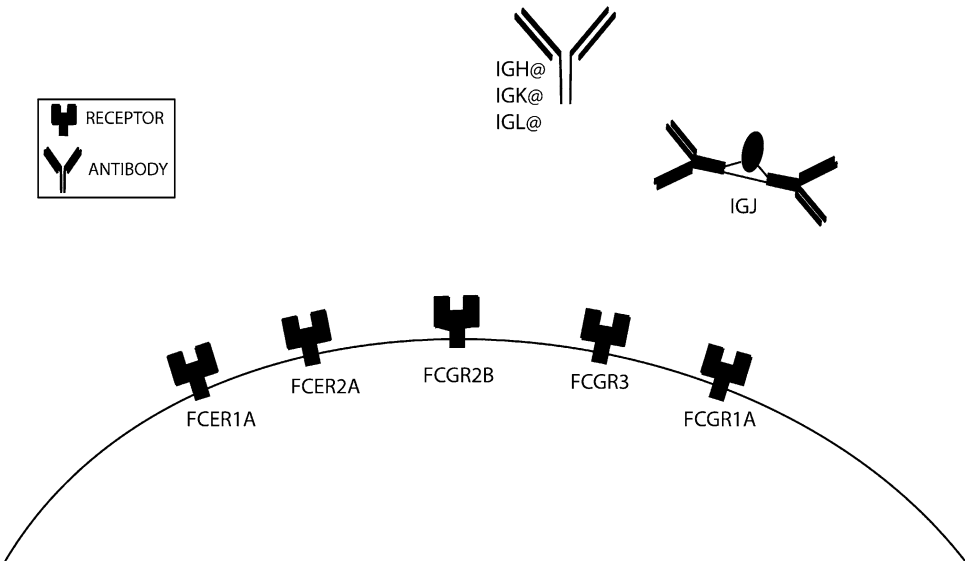

Fig. 3 PU.1 regulates genes encoding antibodies and antibody receptors. The names of genes encoding components of antibodies and antibody receptors are indicated. The *curved line* represents the plasma membrane. A legend is shown on the *left*

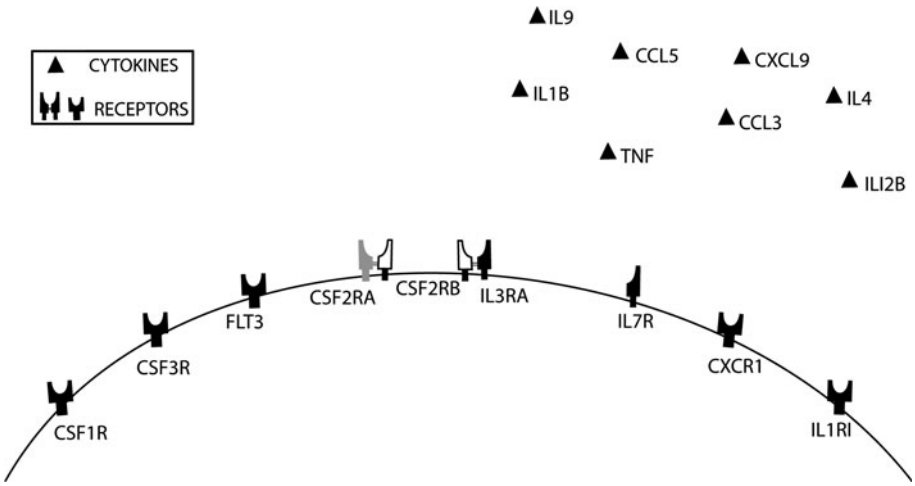

Fig. 4 PU.1 regulates genes encoding cytokines and cytokine receptors. The names of genes encoding cytokines or cytokine receptors are indicated on the figure. Soluble cytokines are indicated by *upright triangles*. Cytokine receptors are located in the plasma membrane, indicated by the *curved line*. CSF2RB (*open symbol*) is a cytokine receptor subunit shared by CSF2RA (*grey symbol*) and IL3RA (*black symbol*). The IL7R functions as a heterodimer with the common gamma chain, which is not a PU.1-regulated gene and is therefore not shown. A legend is shown on the *left*

Sfpi1 knockout progenitor cells by retroviral transduction with cDNA encoding cytokine receptors including IL-7Rα (DeKoter et al. 2002), GM-CSFRα (Singh et al. 1999), and M-CSFR (DeKoter et al. 1998). These observations suggest that in the absence of PU.1, lymphoid and myeloid progenitors are generated but have a "failure to thrive" due to impaired or absent cytokine signaling.

Regulation of Genes Encoding Cytokines and Cytokine Receptors Involved in Inflammation

PU.1 activates the promoter for the gene encoding IL-1β (*IL1B*) (Buras et al. 1995; Kominato et al. 1995). This member of the IL-1 family of cytokines was one of the first discovered growth factors and has recently returned to prominence as a central mediator of inflammation. Inflammatory pathways are activated in response to the sensing of pathogens and danger signals through pattern recognition receptors, trigger nuclear signaling through the nuclear factor κB pathway, and culminate by maturation of pro-IL-1β to IL-1β (Takeuchi and Akira 2010). In addition to IL-1β, PU.1 regulates several other genes important for inflammation including secretory IL-1 receptor antagonist (*IL1R1*) (Smith et al. 1998), IL-18 (*IL18*) (Kim et al. 1999), the IL-12 p40 subunit (*IL12B*) (Becker et al. 2001), and not least, tumor necrosis factor (*TNF*) (Fukai et al. 2009). Interestingly, PU.1 is implicated in transcriptional regulation of several pattern recognition molecules that trigger inflammation, including Toll-like receptor 4 (*TLR4*) (Rehli et al. 2000; Roger et al. 2005) and *TLR9* (Schroder et al. 2007). PU.1 activates the gene encoding a requisite co-receptor of TLR4, MD-2 (*LY96*) (Tissieres et al. 2009). PU.1 has also been implicated in production of the pro-inflammatory cytokine IL-9 in a subset of T cells (Chang et al. 2010) (Fig. 4). The identities of target genes suggest that PU.1 plays important roles at each step of the inflammatory response. Recently, several genome-wide studies were performed to identify PU.1 binding sites in B cells and macrophages (Ghisletti et al. 2010; Heinz et al. 2010). These studies showed that in macrophages, PU.1 occupies regulatory regions in a large proportion of genes involved in inflammatory pathways, suggesting that PU.1 may be a central regulator of inflammation (Natoli et al. 2011). In summary, PU.1 plays key roles in several steps of the inflammatory pathway, and genome-wide analysis suggests PU.1 may play a central role.

PU.1 Levels and Disease: a Unified Perspective

Heterozygous inactivating mutations of the gene encoding human PU.1 (*SPI1*) have been identified in acute myeloid leukemia (AML) patients (Bonadies et al. 2010; Link et al. 2007; Mueller et al. 2002). In addition, PU.1 expression is frequently suppressed in AML by the altered protein products of chromosomal translocations (Vangala et al. 2003). Restoring expression of PU.1 is sufficient to induce differentiation in cultured AML cells (Mueller et al. 2006). In mouse models, mutation of *Sfpi1* is sufficient to cause AML (Cook et al. 2004; Metcalf et al. 2006; Rosenbauer et al. 2005; Walter et al. 2005). There are several reports of mutation or reduced PU.1 levels in lymphoid leukemia and lymphoma (Jundt et al. 2002; Mullighan et al. 2011;

Nishii et al. 2000). Therefore, there is a significant literature showing that mutation or repression of PU.1 is involved in leukemia. Why does reduced PU.1 expression tip the balance from normal hematopoietic differentiation toward cancer? All cancers, including leukemia, share the hallmarks of sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis, resisting cell death, evading immune destruction, and reprogramming energy metabolism (Hanahan and Weinberg 2011). Leukemias acquire these characteristics through mutation, which is often accelerated by intrinsic genomic instability of the cancer. Leukemias and lymphomas typically become addicted to oncogenes, mutation of which result in the hallmarks described above (Weinstein and Joe 2008). It has been speculated that reduced PU.1 levels lead to genomic instability (Dakic et al. 2007). However, the analysis performed in this review indicates that PU.1 is a critical regulator of cellular communication. Therefore, reduced PU.1 levels inhibit the ability of hematopoietic cells to communicate with one another as well as with their microenvironment. For example, developing hematopoietic progenitors with *SPI1* mutations might fail to send or receive signals to and from the environmental stromal niche, leading to unchecked proliferation and impaired differentiation. We expect that mutation or repression of PU.1 (as opposed to activation or amplification) will be an emerging theme as the genomes of individual leukemias are sequenced.

Conclusion and Future Directions

In this review, we performed a retrospective analysis of the identity of genes activated by PU.1 to make inferences about its function. This analysis reveals that PU.1 plays a central role in cellular communication within the immune system. We highlighted three functional pathways regulated by PU.1: (1) antibodies and antibody receptors, (2) cytokines and cytokine receptors regulating leukocyte growth and development, and (3) cytokines and cytokine receptors regulating inflammation. We expect that the molecular underpinnings of how PU.1 regulates these and other pathways will be elucidated in the near future because of rapid advances in whole-genome analysis, such as ChIP coupled with massively parallel sequencing (ChIP-Seq). Repression and/or mutation of the gene encoding PU.1 may be an important initiating event in acute myeloid and lymphoblastic leukemia. A detailed understanding of the pathways controlled by PU.1 will ultimately lead to ideas for novel molecular targeted therapies for these diseases.

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References

Akagawa E, Muto A, Arai K et al (2003) Analysis of the 5′ promoters for human IL-3 and GM-CSF receptor alpha genes. Biochem Biophys Res Commun 300:600–608

Anderson MK, Weiss AH, Hernandez-Hoyos G et al (2002) Constitutive expression of PU.1 in fetal hematopoietic progenitors blocks T cell development at the pro-T cell stage. Immunity 16:285–296

Arinobu Y, Mizuno S, Chong Y et al (2007) Reciprocal activation of GATA-1 and PU.1 marks initial specification of hematopoietic stem cells into myeloerythroid and myelolymphoid lineages. Cell Stem Cell 1:416–427

Back J, Dierich A, Bronn C et al (2004) PU.1 determines the self-renewal capacity of erythroid progenitor cells. Blood 103:3615–3623

Back J, Allman D, Chan S et al (2005) Visualizing PU.1 activity during hematopoiesis. Exp Hematol 33:395–402

Banerji J, Olson L, Schaffner W (1983) A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. Cell 33:729–740

Becker C, Wirtz S, Ma X et al (2001) Regulation of IL-12 p40 promoter activity in primary human monocytes: roles of NF-kappaB, CCAAT/enhancer-binding protein, and PU.1 and identification of a novel repressor element (GA-12) that responds to IL-4 and prostaglandin E(2). J Immunol 167:2608–2618

Bonadies N, Pabst T, Mueller BU (2010) Heterozygous deletion of the PU.1 locus in human AML. Blood 115:331–334

Buras JA, Reenstra WR et al (1995) NF beta A, a factor required for maximal interleukin-1beta gene expression is identical to the ets family member PU.1. Mol Immunol 32:541–554

Carotta S, Dakic A, D'Amico A et al (2010) The transcription factor PU.1 controls dendritic cell development and Flt3 cytokine receptor expression in a dose-dependent manner. Immunity 32:628–641

Chang HC, Sehra S, Goswami R et al (2010) The transcription factor PU.1 is required for the development of IL-9-producing T cells and allergic inflammation. Nat Immunol 11:527–534

Colucci F, Samson SI, DeKoter RP et al (2001) Differential requirement for the transcription factor PU.1 in the generation of natural killer cells versus B and T cells. Blood 97:2625–2632

Cook WD, McCaw BJ, Herring C et al (2004) PU.1 is a suppressor of myeloid leukemia, inactivated in mice by gene deletion and mutation of its DNA binding domain. Blood 104:3437–3444

Dahl R, Walsh JC, Lancki D et al (2003) Regulation of macrophage and neutrophil cell fates by the PU.1:C/EBPalpha ratio and granulocyte colony-stimulating factor. Nat Immunol 4:1029–1036

Dahl R, Iyer SR, Owens KS et al (2007) The transcriptional repressor GFI-1 antagonizes PU.1 activity through protein–protein interaction. J Biol Chem 282:6473–6483

Dakic A, Metcalf D, Di Rago L et al (2005) PU.1 regulates the commitment of adult hematopoietic progenitors and restricts granulopoiesis. J Exp Med 201:1487–1502

Dakic A, Wu L, Nutt SL (2007) Is PU.1 a dosage-sensitive regulator of haemopoietic lineage commitment and leukaemogenesis? Trends Immunol 28:108–114

DeKoter RP, Singh H (2000) Regulation of B lymphocyte and macrophage development by graded expression of PU.1. Science 288:1439–1441

DeKoter RP, Walsh JC, Singh H (1998) PU.1 regulates both cytokine-dependent proliferation and differentiation of granulocyte/macrophage progenitors. EMBO J 17:4456–4468

DeKoter RP, Lee H-J, Singh H (2002) PU.1 regulates expression of the interleukin-7 receptor in lymphoid progenitors. Immunity 16:297–309

DeKoter RP, Geadah M, Khoosal S et al (2010) Regulation of follicular B cell differentiation by the related E26 transformation-specific transcription factors PU.1, Spi-B, and Spi-C. J Immunol 185:7374–7384

Ebralidze AK, Guibal FC, Steidl U et al (2008) PU.1 expression is modulated by the balance of functional sense and antisense RNAs regulated by a shared cis-regulatory element. Genes Dev 22:2085–2092

Eichbaum QG, Iyer R, Raveh DP et al (1994) Restriction of interferon gamma responsiveness and basal expression of the myeloid human Fc gamma R1b gene is mediated by a functional PU.1 site and a transcription initiator consensus. J Exp Med 179:1985–1996

Eisenbeis CF, Singh H, Storb U (1993) PU.1 is a component of a multiprotein complex which binds an essential site in the murine immunoglobulin lambda-2–4 enhancer. Mol Cell Biol 13:6452–6461

Ellis SL, Gysbers V, Manders PM et al (2010) The cell-specific induction of CXC chemokine ligand 9 mediated by IFN-gamma in microglia of the central nervous system is determined by the myeloid transcription factor PU.1. J Immunol 185:1864–1877

Escalante CR, Brass AL, Pongubala JM et al (2002) Crystal structure of PU.1/IRF-4/DNA ternary complex. Mol Cell 10:1097–1105

Feinman R, Qiu WQ, Pearse RN et al (1994) PU.1 and an HLH family member contribute to the myeloid-specific transcription of the Fc-gamma-RIIIa promoter. EMBO J 13:3852–3860

Fisher RC, Lovelock JD, Scott EW (1999) A critical role for PU.1 in homing and long-term engraftment by hematopoietic stem cells in the bone marrow. Blood 94:1283–1290

Fukai T, Nishiyama C, Kanada S et al (2009) Involvement of PU.1 in the transcriptional regulation of TNF-alpha. Biochem Biophys Res Commun 388:102–106

Galloway JL, Wingert RA, Thisse C et al (2005) Loss of gata1 but not gata2 converts erythropoiesis to myelopoiesis in zebrafish embryos. Dev Cell 8:109–116

Ghisletti S, Barozzi I, Mietton F et al (2010) Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. Immunity 32:317–328

Grove M, Plumb M (1993) C/EBP, NF-kappa B, and c-Ets family members and transcriptional regulation of the cell-specific and inducible macrophage inflammatory protein 1 alpha immediate-early gene. Mol Cell Biol 13:5276–5289

Gupta P, Gurudutta GU, Saluja D et al (2009) PU.1 and partners: regulation of haematopoietic stem cell fate in normal and malignant haematopoiesis. J Cell Mol Med 13:4349–4363

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674

Heinz S, Benner C, Spann N et al (2010) Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell 38:576–589

Henkel G, Brown MA (1994) PU.1 and GATA: components of a mast cell-specific interleukin 4 intronic enhancer. Proc Natl Acad Sci USA 91:7737–7741

Hohaus S, Petrovick MS, Voso MT et al (1995) PU.1 (Spi-1) and C/EBP alpha regulate expression of the granulocyte-macrophage colony-stimulating factor receptor alpha gene. Mol Cell Biol 15:5830–5845

Hoogenkamp M, Krysinska H, Ingram R et al (2007) The Pu.1 locus is differentially regulated at the level of chromatin structure and noncoding transcription by alternate mechanisms at distinct developmental stages of hematopoiesis. Mol Cell Biol 27:7425–7438

Houston IB, Huang KJ, Jennings SR et al (2007a) PU.1 immortalizes hematopoietic progenitors in a GM-CSF-dependent manner. Exp Hematol 35:374–384

Houston IB, Kamath MB, Schweitzer BL et al (2007b) Reduction in PU.1 activity results in a block to B cell development, abnormal myeloid proliferation, and neonatal lethality. Exp Hematol 35:1056–1068

Hromas R, Orazi A, Neiman RS et al (1993) Hematopoietic lineage- and stage-restricted expression of the ETS oncogene family member PU.1. Blood 82:2998–3004

Iwasaki H, Somoza C, Shigematsu H et al (2005) Distinctive and indispensable roles of PU.1 in maintenance of hematopoietic stem cells and their differentiation. Blood 106:1590–1600

Jundt F, Kley K, Anagnostopoulos I et al (2002) Loss of PU.1 expression is associated with defective immunoglobulin transcription in Hodgkin and Reed-Sternberg cells of classical Hodgkin disease. Blood 99:3060–3062

Kamath MB, Houston IB, Janovski AJ et al (2008) Dose-dependent repression of T-cell and natural killer cell genes by PU.1 enforces myeloid and B-cell identity. Leukemia 22:1214–1225

Kim YM, Kang HS, Paik SG et al (1999) Roles of IFN consensus sequence binding protein and PU.1 in regulating IL-18 gene expression. J Immunol 163:2000–2007

Klemsz MJ, Maki RA (1996) Activation of transcription by PU.1 requires both acidic and glutamine domains. Mol Cell Biol 16:390–397

Klemsz MJ, McKercher SR, Celada A et al (1990) The macrophage and B cell-specific transcription factor PU.1 is related to the ets oncogene. Cell 61:113–124

Kodandapani R, Pio F, Ni CZ et al (1996) A new pattern for helix-turn-helix recognition revealed by the PU.1 ETS-domain-DNA complex [published erratum appears in Nature 1998, 392:630]. Nature 380:456–460

Kominato Y, Galson PL, Waterman WR et al (1995) Monocyte expression of the human prointerleukin 1β gene (IL-1β) is dependent on promoter sequences which bind the hematopoietic transcription factor Spi-1/PU.1. Mol Cell Biol 15:58–68

Kusy S, Gault N, Ferri F et al (2011) Adult hematopoiesis is regulated by TIF1gamma, a repressor of TAL1 and PU.1 transcriptional activity. Cell Stem Cell 8:412–425

Laslo P, Spooner CJ, Warmflash A et al (2006) Multilineage transcriptional priming and determination of alternate hematopoietic cell fates. Cell 126:755–766

Leddin M, Perrod C, Hoogenkamp M et al (2011) Two distinct auto-regulatory loops operate at the PU.1 locus in B cells and myeloid cells. Blood 117:2827–2838

Link DC, Kunter G, Kasai Y et al (2007) Distinct patterns of mutations occurring in de novo AML versus AML arising in the setting of severe congenital neutropenia. Blood 110:1648–1655

Liu J, Ma X (2006) Interferon regulatory factor 8 regulates RANTES gene transcription in cooperation with interferon regulatory factor-1, NF-kappaB, and PU.1. J Biol Chem 281:19188–19195

McKercher SR, Torbett BE, Anderson KL et al (1996) Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. EMBO J 15:5647–5658

Medina KL, Pongubala JM, Reddy KL et al (2004) Assembling a gene regulatory network for specification of the B cell fate. Dev Cell 7:607–617

Metcalf D, Dakic A, Mifsud S et al (2006) Inactivation of PU.1 in adult mice leads to the development of myeloid leukemia. Proc Natl Acad Sci USA 103:1486–1491

Moreau-Gachelin F, Tavitian A, Tambourin P (1988) Spi-1 is a putative oncogene in virally induced murine erythroleukaemias. Nature 331:277–280

Moreau-Gachelin F, Wendling F, Molina T et al (1996) Spi-1/PU.1 transgenic mice develop multistep erythroleukemias. Mol Cell Biol 16:2453–2463

Mueller BU, Pabst T, Osato M et al (2002) Heterozygous PU.1 mutations are associated with acute myeloid leukemia. Blood 100:998–1007

Mueller BU, Pabst T, Fos J et al (2006) ATRA resolves the differentiation block in t(15;17) acute myeloid leukemia by restoring PU.1 expression. Blood 107:3330–3338

Mullighan CG, Zhang J, Kasper LH et al (2011) CREBBP mutations in relapsed acute lymphoblastic leukaemia. Nature 471:235–239

Natoli G, Ghisletti S, Barozzi I (2011) The genomic landscapes of inflammation. Genes Dev 25:101–106

Nelsen B, Tian G, Erman B et al (1993) Regulation of lymphoid-specific immunoglobulin μ heavy chain gene enhancer by ETS-domain proteins. Science 261:82–86

Nerlov C, Querfurth E, Kulessa H et al (2000) GATA-1 interacts with the myeloid PU.1 transcription factor and represses PU.1-dependent transcription. Blood 95:2543–2551

Nishii K, Kita K, Miwa H et al (2000) Expression of B cell-associated transcription factors in B-cell precursor acute lymphoblastic leukemia cells: association with PU.1 expression, phenotype, and immunogenotype. Int J Hematol 71:372–378

Nishiyama C, Hasegawa M, Nishiyama M et al (2002) Regulation of human Fc epsilon RI alpha-chain gene expression by multiple transcription factors. J Immunol 168:4546–4552

Nutt SL, Metcalf D, D'Amico A et al (2005) Dynamic regulation of PU.1 expression in multipotent hematopoietic progenitors. J Exp Med 201:221–231

O'Connell RM, Rao DS, Chaudhuri AA et al (2008) Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med 205:585–594

Pahl HL, Scheibe RJ, Zhang DE et al (1993) The proto-oncogene PU.1 regulates expression of the myeloid-specific CD11b promoter. J Biol Chem 268:5014–5020

Pawlitzky I, Angeles CV, Siegel AM et al (2006) Identification of a candidate regulatory element within the 5′ flanking region of the mouse Igh locus defined by pro-B cell-specific hypersensitivity associated with binding of PU.1, Pax5, and E2A. J Immunol 176:6839–6851

Polli M, Dakic A, Light A et al (2005) The development of functional B lymphocytes in conditional PU.1 knock-out mice. Blood 106:2083–2090

Pongubala JM, Nagulapalli S, Klemsz MJ et al (1992) PU.1 recruits a second nuclear factor to a site important for immunoglobulin kappa 3′ enhancer activity. Mol Cell Biol 12:368–378

Pongubala JM, van Beveren C, Nagulapalli S et al (1993) Effect of PU.1 phosphorylation on interaction with NF-EM5 and transcriptional activation. Science 259:1622–1625

Ray-Gallet D, Mao C, Tavitian A et al (1995) DNA-binding specificities of Spi-1/PU.1 and Spi-B transcription factors and identification of a Spi-1/Spi-B binding site in the c-fes/c/fps promoter. Oncogene 11:303–313

Rehli M, Poltorak A, Schwarzfischer L et al (2000) PU.1 and interferon consensus sequence-binding protein regulate the myeloid expression of the human Toll-like receptor 4 gene. J Biol Chem 275:9773–9781

Rhodes J, Hagen A, Hsu K et al (2005) Interplay of pu.1 and gata1 determines myelo-erythroid progenitor cell fate in zebrafish. Dev Cell 8:97–108

Roger T, Miconnet I, Schiesser AL et al (2005) Critical role for Ets, AP-1 and GATA-like transcription factors in regulating mouse Toll-like receptor 4 (Tlr4) gene expression. Biochem J 387(Pt 2):355–365

Rosenbauer F, Koschmieder S, Steidl U et al (2005) Effect of transcription-factor concentrations on leukemic stem cells. Blood 106:1519–1524

Rosmarin AG, Caprio DG, Kirsch DG et al (1995) GABP and PU.1 compete for binding, yet cooperate to increase CD18 (β2 leukocyte integrin) transcription. J Biol Chem 270:23627–23633

Ross IL, Dunn TL, Yue X et al (1994) Comparison of the expression and function of the transcription factor PU.1 (SPI-1 proto-oncogene) between murine macrophages and B lymphocytes. Oncogene 9:121–132

Schroder K, Lichtinger M, Irvine KM et al (2007) PU.1 and ICSBP control constitutive and IFN-gamma-regulated Tlr9 gene expression in mouse macrophages. J Leukoc Biol 81:1577–1590

Schuetze S, Stenberg PE, Kabat D (1993) The Ets-related transcription factor PU.1 immortalizes erythroblasts. Mol Cell Biol 13:5670–5678

Schwarzenbach H, Newell JW, Matthias P (1995) Involvement of the Ets family factor PU.1 in the activation of immunoglobulin promoters. J Biol Chem 270:898–907

Schweitzer BL, DeKoter RP (2004) Analysis of gene expression and Ig transcription in PU.1/Spi-B-deficient progenitor B cell lines. J Immunol 172:144–154

Scott EW, Simon MC, Anastasi J et al (1994) Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Science 265:1573–1577

Shin MK, Koshland ME (1993) Ets-related protein PU.1 regulates expression of the immunoglobulin J-chain gene through a novel Ets-binding element. Genes Dev 7:2006–2015

Singh H, DeKoter RP, Walsh JC (1999) PU.1, a shared transcriptional regulator of lymphoid and myeloid cell fates. Cold Spring Harb Symp Quant Biol 64:13–20

Smith LT, Hohaus S, Gonzalez DA et al (1996) PU.1 (Spi-1) and C/EBP alpha regulate the granulocyte colony-stimulating factor receptor promoter in myeloid cells. Blood 88:1234–1247

Smith MF Jr, Carl VS, Lodie T et al (1998) Secretory interleukin-1 receptor antagonist gene expression requires both a PU.1 and a novel composite NF-kappaB/PU.1/GA-binding protein binding site. J Biol Chem 273:24272–24279

Spooner CJ, Cheng JX, Pujadas E et al (2009) A recurrent network involving the transcription factors PU.1 and Gfi1 orchestrates innate and adaptive immune cell fates. Immunity 31:576–586

Stopka T, Amanatullah DF, Papetti M et al (2005) PU.1 inhibits the erythroid program by binding to GATA-1 on DNA and creating a repressive chromatin structure. EMBO J 24:3712–3723

Takeuchi O, Akira S (2010) Pattern recognition receptors and inflammation. Cell 140:805–820

Tissieres P, Araud T, Ochoda A et al (2009) Cooperation between PU.1 and CAAT/enhancer-binding protein beta is necessary to induce the expression of the MD-2 gene. J Biol Chem 284:26261–26272

van Dijk TB, Baltus B, Caldenhoven E et al (1998) Cloning and characterization of the human interleukin-3 (IL-3)/IL-5/granulocyte-macrophage colony-stimulating factor receptor betac gene: regulation by Ets family members. Blood 15:3636−3646

Vangala RK, Heiss-Neumann MS, Rangatia JS et al (2003) The myeloid master regulator transcription factor PU.1 is inactivated by AML1-ETO in t(8;21) myeloid leukemia. Blood 101:270–277

Vigorito E, Perks KL, Abreu-Goodger C et al (2007) MicroRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. Immunity 27:1–13

Walter MJ, Park JS, Ries RE et al (2005) Reduced PU.1 expression causes myeloid progenitor expansion and increased leukemia penetrance in mice expressing PML-RARalpha. Proc Natl Acad Sci USA 102:12513–12518

Weinstein IB, Joe A (2008) Oncogene addiction. Cancer Res 68:3077–3080 (discussion 3080)

Wilkinson NC, Navarro J (1999) PU.1 regulates the CXCR1 promoter. J Biol Chem 274:438–443

Ye M, Ermakova O, Graf T (2005) PU.1 is not strictly required for B cell development and its absence induces a B-2 to B-1 cell switch. J Exp Med 202:1411–1422

Zhang DE, Hetherington CJ, Chen HM et al (1994) The macrophage transcription factor PU.1 directs tissue-specific expression of the macrophage colony-stimulating factor receptor. Mol Cell Biol 14:373–381