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PU.1/Spi-1 Binds to the Human *TAL-1* Silencer to Mediate its Activity

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The *TAL-1/SCL* gene encodes a basic helix-loop-helix (bHLH) transcription factor essential for primitive hematopoiesis and for adult erythroid and megakaryocytic development. Activated transcription of *TAL-1* as a consequence of chromosomal rearrangements is associated with a high proportion of human T cell acute leukemias, showing that appropriate control of *TAL-1* is crucial for the formation and subsequent fate of hematopoietic cells. Hence, the knowledge of the mechanisms, which govern the pattern of *TAL-1* expression in hematopoiesis, is of great interest. We previously described a silencer in the 3′-untranslated region of human *TAL-1*, the activity of which is mediated through binding of a tissue-specific 40 kDa nuclear protein to a new DNA recognition motif, named *tal-RE*. Here, we show that *tal-RE*-binding activity, high in immature human hematopoietic progenitors is down regulated upon erythroid and megakaryocytic differentiation. This expression profile helped us to identify that PU.1/Spi-1 binds to the *tal-RE* sequences *in vitro* and occupies the *TAL-1* silencer *in vivo*. By expressing a mutant protein containing only the ETS domain of PU.1 in human erythroleukemic HEL cells, we demonstrated that PU.1 mediates the transcriptional repression activity of the silencer. We found that ectopic PU.1 is not able to induce silencing activity in PU.1-negative Jurkat T cells, indicating that PU.1 activity, although necessary, is not sufficient to confer transcriptional repression activity to the *TAL-1* silencer. Finally, we showed that the silencer is also active in TAL-1-negative myeloid HL60 cells that express PU.1 at high levels. In summary, our study shows that PU.1, in addition to its positive role in *TAL-1* expression in early hematopoietic progenitors, may also act as a mediator of *TAL-1* silencing in some hematopoietic lineages.

Keywords: transcriptional regulation; TAL-1; PU.1; hematopoiesis; differentiation

Introduction

Hematopoiesis involves the complex regulation of proliferation and differentiation of many cell types, from the immature stem cell to the functional components of bone marrow and peripheral blood. This process relies on the activation of precise combinations of cell-type specific and non-specific transcription factors. Many of the genes that encode crucial hematopoietic transcription factors were found to be mutated in leukemias,[1] strengthening the view that a strict regulation of transcription networks is pivotal for proper blood cell formation. One such factor is TAL-1/SCL (hereinafter named TAL-1), encoded by a member of the basic

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Abbreviations used: bHLH, basic helix-loop-helix; MK, megakaryocytic; EMSA, electrophoretic mobility shift assay; TF, transcription factor; siRNA, small interfering double-stranded RNA; ChIP, chromatin immunoprecipitation; RT-PCR, real time PCR; UTR, untranslated region.

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helix-loop-helix gene family (bHLH) initially identified at the breakpoint of a chromosomal translocation associated with human T cell leukemias.[2] *TAL-1* is expressed in hematopoietic stem cells as well as in multi-potent progenitors, then maintained in erythroid and megakaryocytic committed progenitors and in mast cells, but repressed in terminally differentiated granulocytes, macrophages, T cells and B cells.[3–5] Gene targeting and chimera analysis in mice have shown that *Tal-1* is required for the generation of primitive and definitive hematopoietic lineages.[6–9] More recently, conditional knockout experiments, which by-pass the early lethality of *Tal-1*$^{-/-}$ embryos, demonstrated that, in adult hematopoiesis, *TAL-1* is only required for erythroid and megakaryocytic cell formation.[10,11] *TAL-1* function is also required in the vascular system,[12–14] and more particularly for angiogenic remodeling processes.[12,15]

TAL-1 heterodimerizes with the ubiquitous bHLHs E47 and HEB, and binds to a specific E-box.[2] TAL-1 can either activate or repress transcription, depending on its integration within complexes containing other essential hematopoietic transcription factors such as GATA-1 and LMO2.[16–20] TAL-1 also interacts with co-activators (p300 and p/CAF) and co-repressors (mSin3A), the function of which is linked to histone acetyltransferases or deacetylases.[21,22]

Erythroid and megakaryocytic (MK) lineages are derived from a common progenitor and share several tissue-specific factors such as GATA-1, TAL-1, FOG and NF-E2. The role of GATA-1 in the maturation of both lineages is well established and several erythroid and MK-specific GATA-1 target genes have been identified.[23–25] TAL-1 acts as a promoter of erythroid differentiation of immature progenitors,[26–29] and controls several erythroid-specific genes.[19,20] Although *TAL-1* is required for the formation of MK lineage,[10,11] its role in MK cell-maturation is still unclear.[30]

Biological activity of *TAL-1* critically depends upon appropriate control of its expression. Both human and murine *TAL-1* are transcribed from two lineage-specific promoters. Promoter Ia, restricted to the erythroid, megakaryocytic and mast cell lineages, is controlled by GATA-1 together with SP1.[31,32] Promoter Ib, which directs *TAL-1* expression in CD34 (+) progenitors as well as in mast cells, is regulated by PU.1 in association with SP1 and SP3.[33,34] Finally, a third human promoter was characterized within exon IV (promoter IV), the activity of which is restricted to human acute T cell leukemias and T cell lines.[35,36] Transgenic experiments have also identified spatially distinct enhancers that mimic components of normal *Tal-1* expression in endothelium, mid-brain, hind-brain/spinal cord and hematopoietic progenitors.[37–40]

Accumulating evidence indicates that gene-specific repression is as important as transcriptional activation in normal development. Inappropriate or untimely transcriptional activation or repression in immature progenitors is likely to be responsible for the block of differentiation associated with several types of hematological malignancies. We recently described a silencer in the human 3′-UTR of *TAL-1*, the activity of which is mediated through binding of a tissue-specific 40 kDa nuclear protein to a new DNA recognition motif, named *tal-RE*.[36] Mutation of a single residue within the *tal-RE* element resulted in a loss of both protein binding and silencer activity, demonstrating that the *tal-RE*-binding protein plays a central role in regulating *TAL-1* silencer function in hematopoietic cells.

Here, we show that *tal-RE*-binding activity, high in immature human hematopoietic progenitors is down-regulated upon erythroid and megakaryocytic differentiation. This expression profile helped us to identify PU.1/Spi-1 (hereinafter named PU.1) as the silencer-binding protein and established its crucial role in mediating *TAL-1* silencer activity.

Results

We previously identified that the fully active human 3-UTR silencer was contained within a 750 bp BamHI DNA fragment that corresponds to nucleotides 319–1089 following the stop codon.[36] Several putative GATA and Ik2-LyF sites were present in this DNA fragment; however, none of these sites was found to be occupied in human immature erythroid HEL and K562 cells that display silencer activity. On the other hand, the activity of the *TAL-1* silencer was mediated through binding of a tissue-specific nuclear protein to a new DNA recognition motif **GTT**NN**GC**N**TTC** (at position 661–671) that we designated *tal-RE*, for *tal-1*-repressive element.[36] *tal-RE* binding as well as silencer activity was detected in all cell lines tested of the erythro-megakaryocytic lineages but not in leukemic T cells and non-hematopoietic cells. Given its tissue-restricted distribution, we investigated whether *tal-RE*-binding activity was present in primary primitive hematopoietic progenitors and modulated during erythroid and/or megakaryocytic differentiation.

tal-RE-binding activity is down-regulated during erythroid and megakaryocytic differentiation

Human hematopoietic early progenitors CD34(+) were induced to erythroid and megakaryocytic differentiation. Nuclear extracts, prepared at different time points, were tested for their ability to bind the *tal-RE* sequences by electrophoretic mobility shift assays (EMSA). The formation of the NF-Y/*CAAT* ubiquitous complex was used in these experiments to control the quality of nuclear extracts. Nuclear extracts prepared from the human immature erythroid cells HEL were used to identify the *tal-RE*-binding and the NF-Y/*CAAT* complexes. As shown in Figure 1(a) (left panel), the *tal-RE* probe induced a major specific complex, the formation of which was abolished by an excess of cold oligonucleotides