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Negative regulatory elements are present in the human *LMO2* oncogene and may contribute to its expression in leukemia

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Abstract

Ectopic expression of *LMO2* occurs in approximately 45% of T-lineage acute lymphoblastic leukemias (T-ALL), sometimes in association with chromosomal translocations. Recently, a lymphoproliferative disorder developed in two participants in a gene therapy trial due to *LMO2* activation via integration of the retroviral vector. To investigate these regulatory disruptions, we analyzed the promoter region and identified a tissue-specific repressor. The fragment containing this element could also produce tissue-specific suppression of transcription from the SV40 promoter. This suppression involves histone acetylation which can be relieved with Trichostatin A (TSA). The negative element is in a region consistently removed from *LMO2* in the known chromosomal translocations.
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1. Introduction

The acute lymphoblastic leukemias (ALLs) comprise the most common type of childhood cancers [1]. Historically, the T-lineage ALL (T-ALL) was associated with a poorer prognosis, although current treatment regimens have substantially improved outcomes. Continued advancements in these therapies, particularly with the identification of additional targets, will be accelerated by a more detailed understanding of the molecular mechanisms involved in leukemogenesis. One step toward this goal has been the demonstration that ∼25% of T-ALL cases are associated with specific chromosomal translocations [2]. The molecular analysis of these breakpoint regions has repeatedly resulted in the discovery of genes encoding transcription factors, including *LMO2* [3], *HOX11* [4], *TAL1/SCL* [5], *TAL2* [6], *LMO1* [7], and *LYL1* [8]. These translocations generally involve the movement of these genes into a T cell receptor (TCR) locus, and it has been presumed that this leads to the ectopic or unregulated expression of the gene in T cells.

In addition to the association of such chromosomal rearrangements with leukemogenesis, two cases of a T cell lymphoproliferative disorder have recently been reported in children participating in a gene therapy trial to treat X-linked severe combined immunodeficiency (X-SCID) [9]. In each case, the leukemia-like disorder appears to have developed as a consequence of the aberrant activation of the *LMO2* gene via integration of the retroviral vector. A role for *LMO2* in leukemogenesis had been previously established in a mouse model [10,11] in which transgenic mice were produced with the *LMO2* cDNA under the control of a thymic-specific promoter. These animals developed lymphoblastic lymphomas with associated leukemias. An average latency of 9 months was noted for development of the lymphoma. This latency has been attributed to the need for a second genetic event. Many of the phenotypic aspects displayed by these *LMO2* transgenic mice correlate with the T cell lymphoproliferation that has developed secondary to the retroviral activation of *LMO2* in the two patients, including this latency period which for the children was approximately 3 years. A role for the ectopic expression of *LMO2* in the oncogenic pathway in T cell leukemia is further suggested by microarray analysis of patient samples demonstrating *LMO2* expression in 45% of cases, even in the absence of chromosomal changes [12].

The function of LMO2 appears to be as a mediator of protein–protein interactions in the nucleus [13,14]. The two LIM domains that comprise the protein are cysteine- and

Abbreviations: T-ALL, T cell acute lymphoblastic leukemia; TCR, T cell receptor locus; X-SCID, X-linked severe combined immunodeficiency; HDAC, histone deacetylase; TSA, trichostatin A

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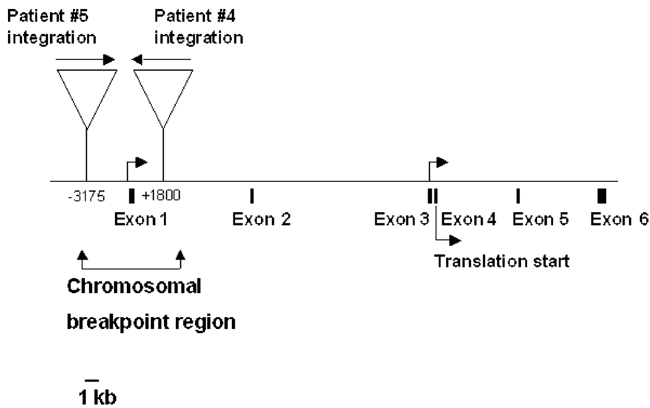

Fig. 1. T-ALL chromosomal breakpoints and retroviral insertions cluster near the *LMO2* distal promoter. The genomic structure of the human *LMO2* gene is shown with the distal and proximal promoters indicated by arrows. The translation start site in exon 4 is also indicated. The chromosomal breakpoint region from patients with *t*(11;14)(p13;q11) T-ALL is shown below the gene. The retroviral insertion sites from the two X-SCID patients that developed a lymphoproliferative disorder are shown above.

histidine-rich motifs that are structurally similar to zinc finger DNA binding domains [15,16]. No evidence has ever been found, however, for direct DNA binding by LMO proteins. Rather, LMO2 has been shown to physically associate with a number of other nuclear factors [17–23], and in erythroid cells, can be found in a pentameric complex composed of LMO2, GATA-1, TAL1/SCL, LDB, and E2A [17,18,24]. Such a complex has been implicated in the transcriptional regulation of several genes including c-kit [19], erythroid Kruppel-like factor (EKLF) [25], and, in a T-ALL cell line, retinaldehyde dehydrogenase (RALDH) [26]. Consistent with this proposed activity in a multiprotein complex, is the finding that the knockout of either the *Tal1/Scl* or the *Lmo2* gene in mice produced a similar null phenotype [27,28]. It is thus of interest that two members of the *LMO* gene family (*LMO1* and *LMO2*) and three member of the *TAL/SCL* family of transcription factors (*TAL1*, *TAL2*, and *LYL*) are all involved in chromosomal translocations associated with T-ALL. These findings raise the possibility that an alternation in the expression of proteins from either family triggers an oncogenic pathway, perhaps with common steps.

As part of our studies to determine how the regulatory mechanism for *LMO2* is disrupted in leukemia, we have carried out an extensive analysis of the *LMO2* distal promoter region. Although two promoters have been identified in the *LMO2* gene [29], our studies have focused on the distal promoter because of the inferred involvement in translocation-induced leukemia and now, retroviral insertional mutatgenesis. Of the approximately two dozen chromosomal breakpoints from T-ALL patients that have been mapped, 90% occur within a 6.5 kb region containing the distal promoter (Fig. 1) [29]. In addition, insertion site analyses on samples from the two patients in the XSCID trial with lymphoproliferative disorders have revealed both insertions are within approximately 3 kb of the distal *LMO2* promoter, one 5′ of the transcription start site and one in the first intron [9]. We have demonstrated this distal promoter directs hematopoietic-specific expression in fetal liver and adult bone marrow with no activity in thymus [30], while Royer-Pokora et al. [29] have detected specific transcription from this promoter in several T-ALL derived cell lines. Our previous work identified an enhancer element at the boundary of exon 1 and the first intron of the *LMO2* gene that is required for expression from this distal promoter [30]. We have now identified a tissue-specific repressor element upstream of the promoter and near one of the retroviral insertion sites. The element mediates transcriptional repression in T cells through a mechanism involving histone acetylation.

2. Materials and methods

2.1. Constructs

LMO2 fragments are designated by their relative distance from the distal promoter transcription start site. The −3190, −2468, −512, and −125 luciferase vectors have been previously described [30]. These *LMO2* genomic fragments were also moved into a pGEM7 plasmid (Promega, Madison, WI) containing a CAT reporter gene in the *Hin*dIII/*Bam*HI sites. The intermediate constructs with truncations at −1405, −1072, and −338 were produced from the −2468 vector by digesting at convenient restriction sites (*Eco*RV, *Nhe*I, and *Nsi*I, respectively).

For the heterologous promoter constructs, we utilized the pGL3 promoter vector (Promega, Madison, WI). −2468/−60 represents the fragment from the upstream *Cla*I restriction site to the *Sac*I site at −60. Constructs −1405/−60, −1072/−60, −338/−60, and −125/−60 were produced from the −2468/−60 construct by cutting at the *Nhe*I, *Eco*RV, *Nsi*I, and *Pst*I restriction sites respectively, and religating. −2468/−1915 represents the fragment from *Cla*I to *Nhe*I, while −1915/−1405 is an internal *Nhe*I fragment. The −2468/−1915 fragment was subsequently subdivided by digesting with *Hin*cII to produce −2468/−2187 and −2187/−1915. These were subcloned utilizing *Kpn*I and *Bgl*II sites.

The Gfi5′ (−2187/−2120) and Gfi3′ (−2131/−1915) constructs were derived from the −2187/−1915 construct by PCR. The Gfi5′ fragment was generated with primers RV3 and LMO GFI RV (5′-CTA GAT CTG GCG CGG AGA TTT CGC-3′), and Gfi3′ with primers GL2 and LMO GFI FW (5′-ACT GAG CTC AGC GAA ATC TCC GCG CC-3′). The RV3 and GL2 primers were purchased from Promega. A 4–5 bp replacement mutation in the GFI primers yielded constructs with disruptions of the GFI consensus binding site. MutGfi5′ (2187/2120) was produced with the RV3 and LMO GFI mut Rv (5′-CTA GAT CTG GCG CGG A**CG CGT** CGC-3′) primer set, and MutGfi3′ (2131/1915) from the primer set GL2 and LMO GFI mut Fw (5′-ACT GAG CTC AGC GA**C GCG** TCC GCG CC-3′).

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