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Novel transcriptional targets of *ETV6*, a transcription factor frequently altered in childhood pre-B acute lymphoblastic leukemia

Camille Malouf[1,2], Karine Lagacé[1,2], Claire Drullion[1], Sylvie Langlois[1], Josiane Larose[1], Ekat A. Kritikou[1], Christian Sirard[1], Simon Drouin[1] and Daniel Sinnett*[1-3]

[1]*Division of Hematology-Oncology, Sainte-Justine University Health Center Research Center, Montreal, Canada H3T 1C5*

[2]*Department of Biochemistry*

[3]*Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Canada, H3T 1C5*

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***Corresponding author:** Daniel Sinnett, Sainte-Justine University Health Center Research Center, 3175 chemin de la Côte-Ste-Catherine, Montreal (Quebec) Canada, H3T 1C5, Tel:+1-514-345-4931 ext. 2990; E-mail: daniel.sinnett@umontreal.ca*

Abstract

Pre-B Acute Lymphoblastic Leukemia (ALL), the most common hematological malignancy in children, represents ~25% of all pediatric cancer cases. The most frequent genetic alteration associated with pre-B ALL is the t(12;21) translocation, which results in the expression of the ETV6-RUNX1 chimera. The frequent deletion of the residual ETV6 allele leads to the complete loss of wildtype *ETV6*, a ubiquitously expressed Ets family transcriptional repressor with few known target genes. We had previously identified candidate ETV6 transcriptional targets using microarray gene expression profiling. Here we show, using chromatin immunoprecipitation experiments and reporter gene assays, that the sphingosine kinase 1 (*SPHK1*) and prostaglandin E2 receptor EP4 subtype (*PTGER4*) genes are direct ETV6 transcriptional targets. Furthermore, ETV6-mediated transcriptional repression of both genes requiresboth ETV6's pointed (PNT) and Erythroblast Transformation Specific (ETS) functional domains, and depends on Ets-Binding Sites (EBS) in the proximal promoter region of the target genes. Functional studies in leukemic cells implicated SPHK1 and PTGER4 in cell survival, proliferation, clonogenic capacity and migration. This study is one of the first to elucidate the functional role of ETV6 transcriptional targets and to suggest their role in childhood leukemogenesis.

Introduction

Acute Lymphoblastic Leukemia (ALL) is the most frequent pediatric cancer in children and accounts for ~25% of all pediatric cancers [1]. Precursor B cell ALL (pre-B ALL), the predominant form of childhood ALL, has been associated with many genetic abnormalities including chromosomal translocations [2]. The t(12;21) translocation is the most common genetic aberration in childhood pre-B ALL, occurring in 25% of pre-B ALL cases [2]. This translocation leads to the formation of the ETV6-RUNX1 chimera, an in-frame fusion of an *ETV6* (erythroblast transformation-specific variant 6) allele with an allele of *RUNX1* (runt-related transcription factor 1) [3, 4]. The expression of the ETV6-RUNX1chimera is under the control of the *ETV6* promoter, which leads to the expression of a chimeric transcription factor composed of the N-terminal part of ETV6 fused to the near complete RUNX1 protein.

ETV6 is a ubiquitously expressed transcription factor of the Ets family [5]. Unlike most Ets transcription factors that function as transcriptional activators, ETV6 has been shown to act as a transcriptional repressor [6]. ETV6 is essential for embryonic development as *ETV6* knockout mice succumb to severe defects in the vascular network of the yolk sac and to increased apoptosis of mesenchymal and neural tissues [7]. Using inducible gene disruption and *ETV6*$^{-/-}$ chimeric mice, ETV6 was shown to be involved in the survival/homing of the hematopoietic stem cells and in the differentiation of the megakaryocytic precursors within the bone marrow microenvironment [8, 9].

ETV6 has a C-terminal ETS DNA-binding domain and a pointed (PNT) helix-loop-helix domain required for protein-protein interactions [6]. The ETS domain has been shown to recognize a consensus Ets-binding site (EBS), which consists of a core GGAA/T sequence with adjacent purine-rich sequences [10]. The PNT domain is involved in the interactions of Ets transcription factors with other proteins [10]. Unlike other Ets proteins, ETV6 has been shown to homodimerize *in vivo.* This interaction requiresits PNT domain, which is also required for its transcriptional repression activity [6]. The central domain of ETV6 is also implicated in protein-protein interactions with members of the SMRT/N-CoR/mSin3A/HDAC corepressor complexes [11-13].

Several lines of evidence indicate that the inactivation of ETV6 is one of the early events in leukemogenesis of ALL [14]. Studies in transgenic mice [15, 16], zebrafish [17], and humanized NOD/SCIDγ mice transplanted with cord blood [18] have clearly shown that the *ETV6-RUNX1* translocation is not sufficient for pre-B ALL development, indicating that additional steps are required for leukemic transformation [16]. The non-translocated *ETV6* allele is inactivated through deletion (loss of heterozygosity (LOH)) in up to 90% of pre-B ALL cases carrying t(12;21) [19, 20]. Interestingly, *ETV6* expression is also absent in patients