

Molecular mechanisms of leukemogenesis by AML1/EVI-1

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The AML1/EVI-1 chimeric gene is generated by the t(3;21)(q26;q22) translocation and plays a pivotal role in progression of hematopoietic stem cell malignancies such as chronic myelocytic leukemia and myelodysplastic syndrome. In AML1/EVI-1, an N-terminal half of AML1 including a runt homology domain is fused to the entire zinc-finger EVI-1 protein. AML1 is essential for hematopoietic cell development in fetal liver and its lineage-specific differentiation in adult. In contrast, EVI-1 is barely expressed in normal hematopoietic cells, but it is overexpressed in chronic myelocytic leukemia in blastic crisis and myelodysplastic syndrome-derived leukemia. There are at least four mechanisms identified in AML1/EVI-1 fusion protein that possibly lead into malignant transformation of hematopoietic stem cells. Firstly, AML1/EVI-1 exerts dominant-negative effects over AML1-induced transcriptional activation. Although target genes repressed by AML1/EVI-1 are still not known, binding competition to a specific DNA sequence and histone deacetylase recruitment through a co-repressor CtBP in EVI-1 part are conceivable underlying mechanisms for the dominant-negative effects. Secondly, AML1/EVI-1 interferes with TGF β signaling and antagonizes the growth-inhibitory effects of TGF β . The first zinc-finger domain of EVI-1 associates with Smad3, a TGF β signal transducer, and represses its transcriptional activity by recruiting histone deacetylase through CtBP that interacts with EVI-1. Thirdly, AML1/EVI-1 blocks JNK activity and prevents stress-induced apoptosis. AML1/EVI-1 associates with JNK through the first zinc-finger domain of EVI-1 and disturbs the association between JNK and its substrates. Lastly, AML1/EVI-1 enhances AP-1 activity by activating the c-Fos promoter depending on the second zinc-finger domain of EVI-1, and promotes cell proliferation. All these functions cooperatively contribute to the malignant transformation of the hematopoietic stem cells by AML1/EVI-1.

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Introduction

In more than the past 10 years, most of the recurrent chromosomal abnormalities associated with specific subtypes of human leukemia have been molecularly characterized. These studies advanced our understanding in molecular mechanisms of leukemogenesis and provided deep insights for hematopoietic cell development. In particular, reciprocal translocations involving transcription factor-encoding genes play an important role in leukemogenesis through either inappropriate expression of a transcription factor or pathogenic generation of a chimeric transcription molecule. Hirai and his colleagues at the University of Tokyo have cloned several chimeric genes, including the AML1/EVI-1 gene generated by the t(3;21)(q26;q22) translocation (Mitani *et al.*, 1994), and established a number of unique molecular models for leukemogenesis induced by this molecule. In this short review, I will focus on the molecular mechanisms of leukemogenesis by AML1/EVI-1 chimeric protein, which have been clarified in his laboratory. I dedicate this review to late Dr Hisamaru Hirai who had been my reliable supervisor as well as helpful mentor, but regrettably passed away on August 23, 2003.

Cloning of the AML1/EVI-1 gene

We first established SKH1 leukemia cell line from a patient with chronic myelocytic leukemia (CML) in megakaryoblastic crisis acquiring the t(3;21)(q26;q22) translocation in addition to the t(9;22)(q34;q11) translocation. The AML1/EVI-1 fusion gene generated by the t(3;21)(q26;q22) translocation was cloned from this cell line 10 years ago. The t(3;21)(q26;q22) translocation fuses the AML1 gene on 21q22 and the EVI-1 gene on 3q26. The t(3;21)(q26;q22) translocation is occasionally observed in CML in blastic crisis or myelodysplastic (MDS)-derived leukemia. Since it is very rare that *de novo* acute leukemia carries the t(3;21)(q26;q22) translocation, the appearance of this chromosomal abnormality may trigger the transformation of chronic hematopoietic stem cell disorder into acute leukemic phase. Actually, treatment with antisense oligonucleotide complementary to the coding sequence of the AML1/EVI-1 junction markedly inhibits the growth of SKH1 cells that carry both the t(3;21)(q26;q22) and the t(9;22)(q34;q11) translocations, but does not inhibit the

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growth of K562 cells that carry only the t(9;22) (q34;q11) translocation (Mitani *et al.*, 1995). Thus, the AML1/EVI-1 fusion protein could maintain proliferation capacity of the t(3;21)-carrying clones arising after blastic transformation of CML.

Structure of AML1/EVI-1

The AML1 (now referred to RUNX1) gene was originally isolated as a gene that is located at the translocation breakpoint of chromosome 21 in the t(8;21)(q22;q22) translocation specifically found in acute myeloblastic leukemia (AML) (M2 according to the French–American–British classification) (Miyoshi *et al.*, 1991). AML1 has a runt homology domain (RHD) at the N-terminus that is highly homologous to the product of *Drosophila* segmentation gene *runt* and α subunit of polyomavirus enhancer-binding protein 2 (PEBP2 or PAE2) (Daga *et al.*, 1992; Bae *et al.*, 1993; Ogawa *et al.*, 1993b) (Figure 1). The *AML1* gene is thought to be ubiquitously expressed in multiple hematopoietic lineages because human leukemia cell lines derived from myeloid, B and T lymphoid cells show normal AML1 transcripts (Miyoshi *et al.*, 1991). AML1 heterodimerizes with non-DNA-binding subunit PEBP2 β to form an active DNA-binding complex and binds to a specific DNA consensus sequence named the PEBP2 site that contains R/TACCRAC through RHD (Ogawa *et al.*, 1993a, b). PEBP2 β enhances the DNA-binding activity of AML1 and protects AML1 from ubiquitin-proteasome-mediated degradation (Huang *et al.*, 2001). The proline–serine–threonine (PST) domain at the C-terminus is required for transcriptional activation. AML1-mediated transcription depends on direct binding of transcriptional co-activators p300, CBP and P/CAF that have intrinsic histone acetyltransferase (HAT) activity (Kitabayashi *et al.*, 1998). AML1 is indispensable for expression of a number of hematopoietic lineage-specific genes, including the *myeloperoxidase* (MPO), *macrophage colony-stimulating factor* (M-CSF) *receptor*, *granulocyte-macrophage colony-stimulat-*

ing factor (GM-CSF), *interleukin-3* (IL-3), *T-cell receptor* (TCR) and *NP-3* genes. Gene knockout experiments show that null mutation in either AML1 (Okuda *et al.*, 1996) or PEBP2 β (Sasaki *et al.*, 1996; Wang *et al.*, 1996a, b) is embryonic lethal at E12.5 due to complete lack of fetal liver hematopoiesis and lethal central nervous hemorrhages, although primitive yolk sac erythropoiesis appears normal. Using conditional gene inactivation strategy, AML1 is shown to play a role in T-cell development (Taniuchi *et al.*, 2002). Thus, AML1 is a key transcription factor for the hematopoietic cell development, proliferation and differentiation.

The EVI-1 (ecotropic viral integration site) gene was initially identified as a common locus of retrovirus integration in myeloid tumors in AKXD mouse (Morishita *et al.*, 1988). EVI-1 is a transcriptional regulator that possesses two Cys2His2-type zinc-finger domains and acidic domain (Morishita *et al.*, 1990). Expression of EVI-1 is barely detectable in healthy murine or human hematopoietic cells. However, EVI-1 is highly expressed in MDS or AML patients showing 3q26 abnormalities such as inv(3)(q21q26) or t(3;3)(q21;q26) (Morishita *et al.*, 1992). Even in the absence of evident 3q36 abnormalities, elevated expression of EVI-1 is reported in CML in blastic crisis and MDS-derived leukemia (Ogawa *et al.*, 1996a). These findings suggest a critical role for EVI-1 in malignant transformation of hematopoietic cells as a dominant oncogene.

In AML1/EVI-1 chimeric cDNA, an open reading frame of 4185 nucleotides encodes a 1395 amino-acid protein (Mitani *et al.*, 1994). The N-terminal portion of AML1 is abruptly interrupted at the end of RHD, followed by a 5' non-coding sequence of EVI-1 cDNA that is translated through the entire coding region. Therefore, AML1/EVI-1 fusion protein is a chimeric transcription molecule that consists of RHD of AML1 and two zinc-finger domains of EVI-1. In SKH1 cells, two major transcripts in sizes of 8.2 and 7.0 kb are transcribed from the *AML1/EVI-1* chimeric gene, and Western analysis shows that AML1/EVI-1 is a 180 kDa chimeric molecule. AML1/EVI-1 promotes pre-existing stem cell disorder into acute leukemia through multiple

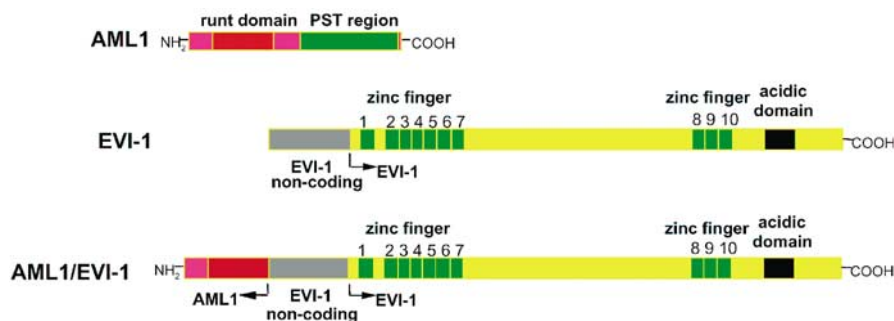


Figure 1 Schematic structure of wild-type AML1, EVI-1 and AML1/EVI-1. Wild-type AML1 possesses RHD at the N-terminus and PST region at the C-terminus. In AML1/EVI-1, N-terminal AML1 sequences are abruptly interrupted at the end of the RHD and followed by almost the entire coding region of EVI-1

mechanisms. I will describe the unique functions of each DNA-binding domain of AML1/EVI-1 fusion molecule in detail below.

Dominant-negative effects over AML1

AML1 transactivates Tww-tk-Luc reporter that contains PEBP2 sites derived from the TCR β promoter (Tanaka *et al.*, 1995). However, AML1/EVI-1 does not have such transactivation ability, presumably because it does not contain the PST domain that is necessary for the reporter transactivation. Notably, AML1/EVI-1 reduces the reporter transactivation by AML1 in a dose-dependent manner. The gel shift assay shows that AML1/EVI-1 binds to the PEBP2 site more tightly than AML1 does. Therefore, AML1/EVI-1 could dominantly interfere with the AML1-induced transactivation by competing for the specific DNA binding. Deletion mutation analysis of AML1/EVI-1 shows that RHD is responsible for the dominant-negative effect. To exert transcription activation, AML1 needs to heterodimerize with PEBP2 β through its RHD. AML1 mainly locates in the nucleus, while PEBP2 β locates in the cytoplasm (Tanaka *et al.*, 1998). Immunofluorescence analysis shows that AML1/EVI-1 locates in the nucleus with or without RHD. AML1/EVI-1 seems to drag PEBP2 β into the nucleus because co-expression of the full-length AML1/EVI-1 relocates PEBP2 β from the cytoplasm to the nucleus. This effect is RHD-dependent because AML1/EVI-1 lacking RHD does not have such effect, which is consistent with the fact that AML1 associates with PEBP2 β through RHD. Since PEBP2 β more effectively associates with AML1/EVI-1 than AML1, PEBP2 β more efficiently translocates into the nucleus by AML1/EVI-1 than by AML1. This differential effect between AML1/EVI-1 and AML1 presumably causes the dominant-negative effect of AML1/EVI-1 over AML1 and accounts for one of the mechanisms through which this chimeric protein contributes to leukemogenesis.

Recently, we found that AML1/EVI-1 interacts with C-terminal binding protein (CtBP), which is essential for repressing the AML1-induced transactivation (Izutsu *et al.*, 2002) (Figure 2). CtBP was originally identified as a protein that interacts with a C-terminal portion of adenovirus E1A protein. To date, two highly related proteins, termed CtBP1 and CtBP2, have been identified in both mice and humans. CtBP belongs to a member of co-repressor proteins that mediates repression by associating with several transcription factors, including Krüppel-like factor (BKLf), friend of GATA (FOG) and T-cell factor (TCF). Although it is not clear how CtBP mediates transcriptional repression, it is supposed that histone deacetylase (HDAC)-1 that interacts with CtBP is involved in the process. We previously determined that EVI-1 interacts with CtBP exclusively through one of the two potential CtBP-binding amino-acid sequences (N-terminal PFDLT and C-terminal PLDLS) that locate next to the second zinc-finger

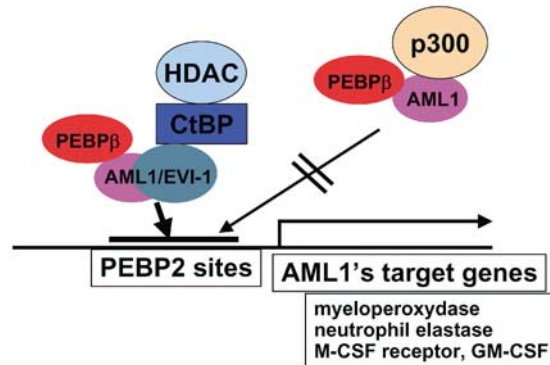


Figure 2 AML1/EVI-1 exerts dominant-negative effects over wild-type- AML1. AML1 becomes an active transcription factor by heterodimerizing with PEBP2 β through RHD. PEBP2 β increases the DNA-binding ability of AML1 and protects AML1 from the ubiquitin system-mediated degradation. As AML1/EVI-1 binds to PEBP2 β more tightly than AML1, AML1/EVI-1 competes AML1 out from the PEBP2 sites. Moreover, AML1/EVI-1 associates with a co-repressor CtBP and thereby recruits HDAC. AML1/EVI-1 actively represses the transcription of potential AML1 target genes. CtBP, C-terminal binding protein; HDAC, histone deacetylase; PEBP2, polyomavirus enhancer binding protein 2

domain (Izutsu *et al.*, 2001). These two sequences are conserved in AML1/EVI-1 and one of them mediates interaction with CtBP. AML1/EVI-1 interferes with the AML1-induced transactivation, while AML1/EVI-1 with mutations in PLDLS does not. Therefore, AML1/EVI-1 requires CtBP as a co-repressor to dominantly inhibit the AML1-induced transactivation. An HDAC inhibitor trichostatin A relieves this dominant-negative effect of AML1/EVI-1. Therefore, it is conceivable that AML1/EVI-1 positively prevents the AML1-induced transcription by recruiting HDAC through CtBP.

AML1/EVI-1 blocks granulocytic differentiation of a murine myeloid cell line 32Dcl3 induced by G-CSF (Tanaka *et al.*, 1995). Parental 32Dcl3 cells acquire mature granulocytic phenotype characterized by cytoplasmic granules and segmented/circular nucleus when cultured with G-CSF for several days. A robust induction of MPO mRNA is also observed by Northern analysis. 32Dcl3 cells overexpressing AML1/EVI-1 maintain immature morphological phenotypes characterized by a large unsegmented nucleus even in the presence of G-CSF (Izutsu *et al.*, 2002). In parallel, there is poor induction of MPO mRNA in these cells. Thus, overexpression of AML1/EVI-1 blocks G-CSF-induced differentiation of 32Dcl3 cells into mature granulocytic phenotype. On the contrary, in 32Dcl3 cells overexpressing mutant AML1/EVI-1 that is unable to interact with CtBP, the morphological changes and induction of MPO mRNA are indistinguishable from those of parental 32Dcl3 cells. These results suggest that AML1/EVI-1 blocks G-CSF-induced granulocytic maturation by interacting with CtBP. Taken together, CtBP-mediated repression of gene transcription could be one of the mechanisms for AML1/EVI-1-mediated block in granulocyte differentiation.

Repression of transforming growth factor β (TGF β)-mediated growth inhibition

Cell growth and differentiation are tightly regulated by delicate balance of growth factors and growth-inhibitory factors. TGF β inhibits proliferation of a wide range of cell types including epithelial, endothelial and hematopoietic cells. Binding of TGF β to the heteromeric serine/threonine kinase receptor complex leads to direct phosphorylation of intracellular signal transducers Smad2 and Smad3, followed by the formation of heteromeric complexes with a common signal transducer Smad4, and subsequent their translocation into the nucleus. Once in the nucleus, Smad complexes are thought to act as a transcriptional activator to inhibit cellular proliferation. We demonstrated that EVI-1 perturbs TGF β signaling and inhibits proliferation of a wide variety of cells. EVI-1 represses transcriptional activation of TGF β -responsive reporters such as the *plasminogen-activator inhibitor-1* (PAI-1) or *p15* promoter-Luciferase construct (Kurokawa *et al.*, 1998a). There is no effect of EVI-1 on the basal activity of these promoters, indicating that this inhibitory effect works specifically toward TGF β -induced transcriptional activation. In growth inhibition experiment, EVI-1 antagonizes the anti-proliferative effects of TGF β against TGF β -sensitive Mv1Lu cells. Biochemically, most of retinoblastoma (Rb) proteins in the EVI-1-expressing cells remain in the hyperphosphorylated forms even in the presence of TGF β , whereas the control cells treated with TGF β accumulate hypophosphorylated Rb and show a decreased level of Rb protein. The first zinc-finger domain of EVI-1 mediates interaction with Smad3, and the small region next to the second zinc-finger domain (repression domain) is required to repress TGF β signaling. The association between EVI-1 and CtBP is essential for the inhibition of *PAI-1* reporter

construct. Trichostatin A treatment alleviates EVI-1-mediated repression of TGF β -responsive reporters, suggesting that HDAC is involved in this repression. Consistent with this, Mv1Lu cells overexpressing EVI-1 with a mutated PLDLS show normal growth inhibition as parental Mv1Lu cells when treated with TGF β .

Similar to EVI-1, AML1/EVI-1 inhibits transactivation of TGF β -responsive promoters (Kurokawa *et al.*, 1998b). The constitutive expression of AML1/EVI-1 or EVI-1 in 32Dcl3 cells overcomes TGF β -mediated inhibition of cell growth. From these data, AML1/EVI-1 and EVI-1 can potentially block growth inhibition of hematopoietic cells mediated by TGF β . MOLM-1 is a human megakaryoblastoid cell line carrying the *inv(3)(q21q26)* and endogenously expressing a truncated form of EVI-1 proteins in which the C-terminal amino acids of wild-type EVI-1 are replaced by five amino acids (Ogawa *et al.*, 1996b). EVI-1 in MOLM-1 cells retain both the first zinc-finger and the repression domains that are required for repression of TGF β signaling. Thus, MOLM-1 cells do not respond to TGF β -mediated growth inhibition. However, it becomes TGF β sensitive when treated with antisense oligonucleotides complementary to the sequence encoding the N-terminus of the first zinc-finger domain of EVI-1. Just like wild-type-EVI-1, AML1/EVI-1 physically interacts with Smad3 and inhibits the Smad3 activity (Figure 3). AML1/EVI-1 also associates with CtBP through the CtBP-binding consensus sequence PLDLS. As endogenous CtBP proteins are detected in SKH1 cells, it could be possible that TGF β signaling is repressed in this leukemia cell line. While the interaction of CtBP and HDAC1 is clearly demonstrated, association between AML1/EVI-1 and HDAC1 is not yet determined. However, it is speculated that AML1/EVI-1 recruits HDACs through CtBP to repress TGF β -responsive transcription. Thus, in leukemia with the

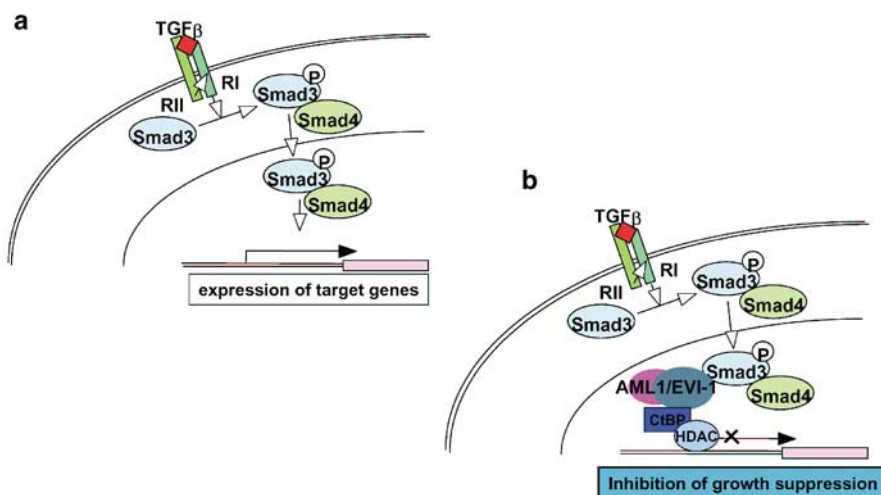


Figure 3 AML1/EVI-1 represses TGF β -mediated growth inhibitory signal. (a) Upon TGF β binding to the heteromeric serine/threonine kinase receptor complex, type II receptor activates type I receptor and thereby phosphorylates Smad3. Phosphorylated Smad3 forms a complex with Smad4 and translocates into the nucleus, where they work as a transcription factor to inhibit cellular growth. (b) AML1/EVI-1 binds to Smad3 through the first zinc-finger domain in EVI-1 and actively interferes with Smad3 transcriptional activity. Recruitment of HDAC by EVI-1 via CtBP is an underlying mechanism. TGF β transforming growth factor β ; R I, type I receptor; R II, type II receptor; CtBP, C-terminal binding protein; HDAC, histone deacetylase

t(3;21) translocation, AML1/EVI-1 may contribute to leukemogenesis by blocking the growth-inhibitory effect of TGF β .

Inhibition of c-Jun N-terminal kinase

Mitogen-activated protein (MAP) kinase cascades are important signaling pathways that are involved in a wide variety of biological response mechanisms. In vertebrates, at least three pathways have been identified: ERK, c-Jun N-terminal kinase (JNK) and p38. Generally, ERK signaling is involved in controlling cell proliferation and differentiation, while JNK or p38 signaling plays an important role in triggering apoptosis in response to cellular stresses such as UV light, γ -radiation, osmotic shock, protein synthesis inhibitors, tumor necrosis factor- α (TNF α) and interleukin-1. EVI-1 acts as an inhibitor of JNK, either when overexpressed JNK is unstimulated or stimulated by UV light, anisomycin, sorbitol and TNF α (Kurokawa *et al.*, 2000). However, EVI-1 does not affect the kinase activities of overexpressed ERK and p38. Furthermore, Rat1 cells overexpressing EVI-1 show downregulated endogenous JNK activity. In MOLM-1 cells, the endogenous JNK activity is reduced. However, when treated with EVI-1 antisense oligonucleotide, the JNK activity is restored. The same results are obtained in the experiment using human endometrial carcinoma cell line HEC1B that expresses EVI-1 at a high level. Consistently, the presence of EVI-1 actually reduces phosphorylated substrates of c-Jun. JNK activation requires phosphorylation on two specific amino-acid residues Thr183 and Tyr185 by MMK4 or MMK7. Although EVI-1 physically interacts with JNK, it does not affect its phosphorylation status. However, the binding

between JNK and c-Jun is significantly reduced by concomitant expression of EVI-1. This suggests that EVI-1 inhibits c-Jun phosphorylation by inhibiting the association between JNK and c-Jun (Figure 4). EVI-1 associates JNK through the first zinc-finger domain. This interaction is essential for inhibition of JNK activity. EVI-1 constitutively associates with JNK in HEC1B cells, and this interaction is increased by the treatment with UV light.

We also demonstrated that EVI-1 inhibits apoptotic cell death by interfering with JNK signaling pathway. 293 cells undergo apoptosis via JNK signaling induced by UV light. Overexpression of EVI-1 in 293 cells significantly represses apoptosis induction by UV light. On the other hand, HEC1B cells are resistant to apoptosis, presumably because they express endogenous EVI-1. However, HEC1B cells treated with EVI-1-specific antisense oligonucleotide easily undergo apoptosis. Stress activation of JNK promotes upregulation of FasL expression in T lymphocytes, which is one of the mechanisms potentially causing apoptosis. FasL expression induced by UV stimulation in Jurkat cells is prevented by EVI-1 depending on the first zinc-finger domain. EVI-1 actually blocks apoptosis induction by UV light, and represses activation of FasL reporter stimulated by UV light and anisomycin in Jurkat cells. Furthermore, EVI-1 inhibits apoptosis induction by TNF α in U937 cells by repressing JNK activity. In conclusion, EVI-1 blocks both the molecular and biological activities of JNK.

It has not been determined whether AML1/EVI-1 possesses the similar anti-apoptotic effect as EVI-1 does. However, because this function is dependent on the first zinc-finger domain in EVI-1, it is reasonable to speculate that AML1/EVI-1 also prevents apoptosis induction by inhibiting JNK.

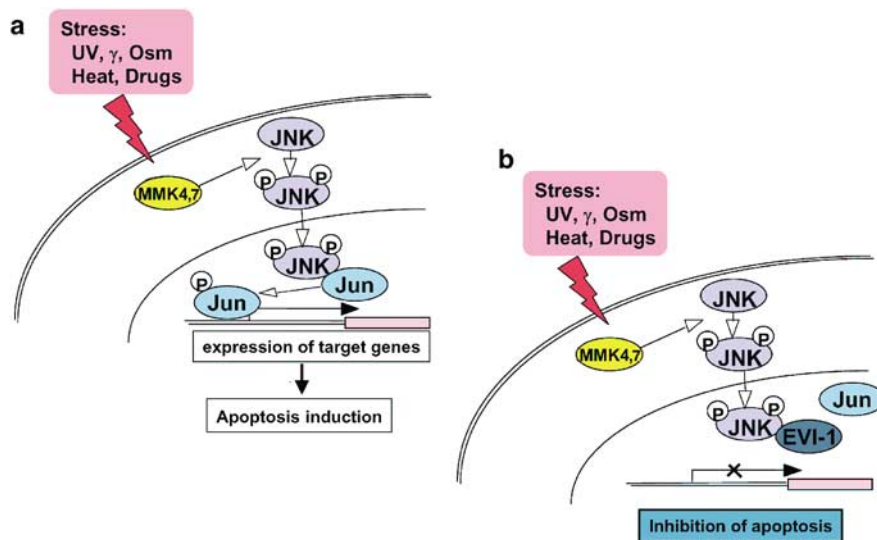


Figure 4 EVI-1 inhibits JNK activity. (a) Various kinds of stresses such as UV light, γ -radiation, osmotic shock, heat shock and protein synthesis inhibitor activate JNK by phosphorylation. Activated JNK translocates into the nucleus, phosphorylates transcription factors such as Jun and triggers apoptosis induction. (b) EVI-1 blocks JNK association between JNK and its substrates and thereby represses JNK activity to induce apoptosis. JNK, c-Jun N-terminal kinase

Stimulation of proliferation

AP-1 (Fos/Jun heterodimer or Jun/Jun homodimer) is activated by growth stimuli, including growth factors, phorbol esters such as 12-*o*-tetradecanoylphorbol 13-acetate (TPA) and various oncogene products, and stimulates transactivation through TPA responsive site (TRE). AP-1 functions as a positive or negative regulator in a variety of cellular differentiation and proliferation processes. EVI-1 raises AP-1 activity in NIH3T3 cells and embryonal carcinoma (EC) cell line P19 (Tanaka *et al.*, 1994). EVI-1-transfected P19 cells show differentiated phenotypes, characterized by flattened and enlarged morphology. These changes are indistinguishable from the morphological changes seen in P19 cells treated with retinoic acid or transfected with c-Jun. The stage-specific embryonic antigen SSEA-1, known as a stem cell marker in EC cells, is detected in parental P19 cells, but not in EVI-1-transfected P19 cells. In contrast to SSEA-1, the heat shock protein Hsp47, known as a differentiation marker, is detected in EVI-1-transfected P19 cells, but not in parental cells. In addition, expression of c-Jun and c-Fos is increased in EVI-1-transfected cells. EVI-1 enhances the activity of the *c-Fos* promoter in NIH3T3 and P19 cells, depending on the second zinc-finger domain (Figure 5). It is shown that the second zinc-finger domain is essential for both the activation of AP-1 and transactivation of the *c-Fos* promoter.

In order to evaluate the transforming activity of AML1/EVI-1, the fusion cDNA is introduced into Rat1 fibroblasts (Kurokawa *et al.*, 1995). Rat1 cells expressing AML1/EVI-1 form macroscopic colonies in soft agar, while the mock-transfected cells produce tiny, barely macroscopic ones. This indicates that AML1/EVI-1 is a transforming gene. Introduction of AML1/EVI-1 into the Rat1 clones harboring BCR/ABL confers enhanced capacity for anchorage-independent growth. AML1/EVI-1 also stimulates AP-1 activity. As the

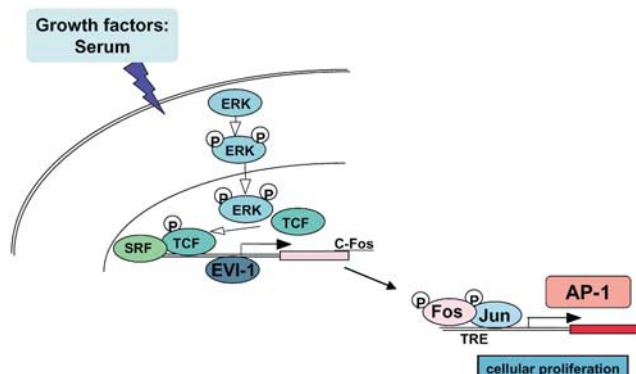


Figure 5 EVI-1 stimulates AP-1 activity. EVI-1 activates *c-Fos* transcription. Fos associates with Jun to form a heterodimer and stimulates TRE-mediated transcription. Increased AP-1 activity by EVI-1 promotes cellular proliferation. ERK, extracellular-regulated kinase; TCF, ternary complex factor; SRF, serum response factor; TRE, TPA responsive element; AP-1, activation protein 1

second zinc-finger domain is required for both transformation of Rat1 cells and an increase in AP-1 activity, it is conceivable that AML1/EVI-1 transforms Rat1 cells by AP-1 activation. Notably, AML1/EVI-1-transformed colonies express c-Jun mRNA. Since AML1/EVI-1 exaggerates the transforming activity of BCR/ABL, AML1/EVI-1 could perform a critical role in leukemic progression of CML.

Conclusion

Similar to other AML1-related chimeras, AML1/EVI-1 exerts dominant-negative effects over AML1-induced transcription. However, target genes that may have a key role in leukemogenesis are still not elucidated. On the other hand, almost the entire coding region of EVI-1 is expressed under the AML1 promoter in leukemic cells carrying AML1/EVI-1 chimeric gene. Since ectopic expression of EVI-1 is thought to be involved in leukemic transformation of CML and MDS, EVI-1 portion in AML1/EVI-1 fusion molecule may also play a critical role in leukemogenesis. Notably, AML1/EVI-1 and EVI-1 share similar functions; the first zinc-finger and the repression domains exhibit anti-growth repression effect by blocking TGF β signaling, the first zinc-finger domain exhibits anti-apoptotic effect by repressing JNK signaling, and the second zinc-finger domain exhibits proliferation stimulation effect by increasing AP-1 activity. All these effects are related to leukemic cell proliferation, directly or indirectly. The molecular mechanisms of leukemogenesis by AML1/EVI-1 are summarized in Figure 6. The multi-functions of AML1/EVI-1 fusion molecule are mostly demonstrated by *in vitro* experiments. Our next step is to generate AML1/EVI-1 leukemia model mice and demonstrate these functions *in vivo*. It is plausible that AML1/EVI-1 knock-in mice are embryonic lethal in the mid-gestation because of the dominant-negative effects of AML1/EVI-1 over normal AML1, as is the case with AML1/ETO knock-in mice. Thus, we are planning to make conditional AML1/EVI-1 knock-in mice to enable temporal and special expression control of the chimeric molecule.

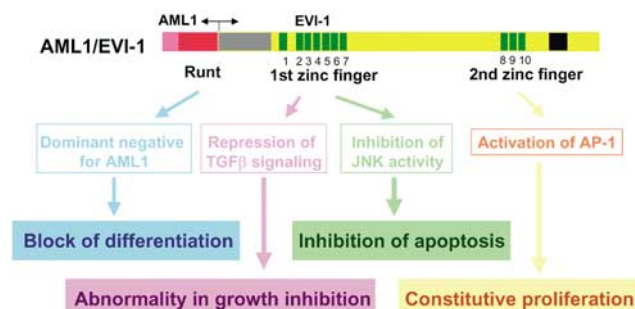


Figure 6 AML1/EVI-1 is a multi-functional oncoprotein. AML1/EVI-1 develops hematopoietic stem cell disorders to acute leukemia by at least four unique functions: dominant-negative effect over AML1, repression of TGF β signaling, inhibition of JNK activity and activation of AP-1

References

- Bae SC, Yamaguchi-Iwai Y, Ogawa E, Maruyama M, Inuzuka M, Kagoshima H, Shigesada K, Satake M and Ito Y. (1993). *Oncogene*, **8**, 809–814.
- Daga A, Tighe JE and Calabi F. (1992). *Nature*, **356**, 484.
- Huang G, Shigesada K, Ito K, Wee H-J, Yokomozo T and Ito Y. (2001). *EMBO J.*, **20**, 723–733.
- Izutsu K, Kurokawa M, Imai Y, Ichikawa M, Asai T, Maki K, Mitani K and Hirai H. (2002). *Oncogene*, **21**, 2695–2703.
- Izutsu K, Kurokawa M, Imai Y, Maki K, Mitani K and Hirai H. (2001). *Blood*, **97**, 2815–2822.
- Kitabayashi I, Yokoyama A, Shimizu K and Ohki M. (1998). *EMBO J.*, **17**, 2994–3004.
- Kurokawa M, Mitani K, Imai Y, Ogawa S, Yazaki Y and Hirai H. (1998a). *Blood*, **92**, 4003–4012.
- Kurokawa M, Mitani K, Irie K, Matsuyama T, Takahashi T, Chiba S, Yazaki Y, Matsumoto K and Hirai H. (1998b). *Nature*, **394**, 92–94.
- Kurokawa M, Mitani K, Yamagata T, Takahashi T, Izutsu K, Ogawa S, Moriguchi T, Nishida E, Yazaki Y and Hirai H. (2000). *EMBO J.*, **19**, 2958–2968.
- Kurokawa M, Ogawa S, Tanaka T, Mitani K, Yazaki Y, Witte ON and Hirai H. (1995). *Oncogene*, **11**, 833–840.
- Mitani K, Ogawa S, Tanaka T, Kurokawa M, Yazaki Y and Hirai H. (1995). *Br. J. Haematol.*, **90**, 711–714.
- Mitani K, Ogawa S, Tanaka T, Miyoshi H, Kurokawa M, Mano H, Yazaki Y, Misao O and Hirai H. (1994). *EMBO J.*, **13**, 504–510.
- Miyoshi H, Shimizu K, Kozu T, Maseki N, Kaneko Y and Ohki M. (1991). *Proc. Natl. Acad. Sci. USA*, **88**, 10431–10434.
- Morishita K, Parganas E, Douglass EC and Ihle JN. (1990). *Oncogene*, **5**, 963–971.
- Morishita K, Parganas E, Willman CL, Whittaker MH, Drabkin H, Oval J, Taetle R, Valentine MB and Ihle JN. (1992). *Proc. Natl. Acad. Sci. USA*, **89**, 3937–3941.
- Morishita K, Parker DS, Mucenski ML, Jenkins NA, Copeland NG and Ihle JN. (1988). *Cell*, **54**, 831–840.
- Ogawa E, Inuzuka M, Maruyama M, Satake M, Naito-Fujimoto M, Ito Y and Shigesada K. (1993a). *Virology*, **194**, 314–331.
- Ogawa E, Maruyama M, Kagoshima H, Inuzuka M, Lu J, Satake M, Shigesada K and Ito Y. (1993b). *Proc. Natl. Acad. Sci. USA*, **90**, 6859–6863.
- Ogawa S, Kurokawa M, Tanaka T, Tanaka K, Hangaishi A, Mitani K, Kamada N, Yazaki Y and Hirai H. (1996a). *Leukemia*, **10**, 788–794.
- Ogawa S, Kurokawa M, Tanaka T, Mitani K, Inazawa J, Hanagaishi A, Tanaka K, Matsuo Y, Minowada J, Tsubota T, Yazaki Y and Hirai H. (1996b). *Oncogene*, **13**, 183–191.
- Okuda T, van Deursen J, Hiebert SW, Grosveld G and Downing JR. (1996). *Cell*, **84**, 321–330.
- Sasaki K, Yagi H, Bronson RT, Tomonaga K, Matsunashi T, Deguchi K, Tani Y, Kishimoto T and Komori T. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 12359–12363.
- Tanaka K, Tanaka T, Kurokawa M, Imai Y, Ogawa S, Mitani K, Yazaki Y and Hirai H. (1998). *Blood*, **91**, 1688–1699.
- Tanaka T, Mitani K, Kurokawa M, Ogawa S, Tanaka K, Nishida J, Yazaki Y, Shibata Y and Hirai H. (1995). *Mol. Cell. Biol.*, **15**, 2383–2392.
- Tanaka T, Nishida J, Mitani K, Ogawa S, Yazaki Y and Hirai H. (1994). *J. Biol. Chem.*, **269**, 24020–24026.
- Taniuchi I, Osato M, Egawa T, Sunshine MJ, Bae S-C, Komori T, Ito Y and Littman D. (2002). *Cell*, **111**, 621–633.
- Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH and Speck NA. (1996a). *Proc. Natl. Acad. Sci. USA*, **93**, 3444–3449.
- Wang Q, Stacy T, Miller JD, Lewis AF, Gu TL, Huang X, Bushweller JH, Borie JC, Bories JC, Alt EW, Ryan G, Liu PP, Wynsaw-Boris A, Binder M, Marin-Padilla M, Sharpe AH and Speck NA. (1996b). *Cell*, **87**, 697–708.