Review Article

Pathogenetic significance of ecotropic viral integration site-1 in hematological malignancies

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The ecotropic viral integration site-1 (*Evi-1*) gene was first identified as a common locus of retroviral integration in murine leukemia models. In humans, *EVI-1* is located on chromosome 3q26, and rearrangements on chromosome 3q26 often activate EVI-1 expression in hematological malignancies. Overexpression of EVI-1 also occurs with high frequency in leukemia patients without 3q26 abnormalities, and importantly, high EVI-1 expression is an independent negative prognostic indicator irrespective of the presence of 3q26 rearrangements. Recent gene targeting studies in mice revealed that Evi-1 is preferentially expressed in hematopoietic stem cells and plays an essential role in proliferation and maintenance of hematopoietic stem cells. In addition, intense attention has been focused on the *EVI-1* gene complex as retrovirus integration sites because transcription-activating integrations into the *EVI-1* locus confer survival and self-renewing ability to hematopoietic cells. The experimental results using animal models suggest that activation of Evi-1 in hematopoietic cells leads to clonal expansion or dysplastic hematopoiesis, whereas onset of full-blown leukemia requires cooperative genetic events. *EVI-1* possesses diverse functions as an oncoprotein, including suppression of transforming growth factor-β-mediated growth inhibition, upregulation of GATA2, inhibition of the Jun kinase pathway, and stimulation of cell growth via activator protein-1. In this article, we summarize current knowledge regarding the biochemical properties and biological functions of EVI-1 in normal and malignant hematopoiesis, with specific focus on its pathogenetic significance in hematological malignancies. (*Cancer Sci* 2009; 100: 990–995)

Despite the development of multiple new agents, relapse continues to be the most common cause of death for hematological malignancies. In order to improve the rate of cure, it is necessary to clarify the molecular mechanisms underlying therapy-resistant leukemia. The ecotropic viral integration site-1 (*EVI-1*) is an oncogene that confers a poor prognosis in human hematological malignancies, including acute myeloid leukemia (AML), chronic myeloid leukemia, and myelodysplastic syndrome (MDS). Evi-1 was first identified as the integration site of the ecotropic retrovirus leading to myeloid leukemia in murine model systems.[1,2] Since its identification, numerous studies have shown that EVI-1 is associated with particularly aggressive forms of human myeloid malignancies. EVI-1 is a nuclear transcription factor and contains DNA-binding zinc finger motifs. In addition to its DNA-binding activity, Evi-1 has the potential to recruit diverse proteins, such as Smad[3] and C-terminal Binding Protein (CtBP), thus generating regulatory complexes for transcriptional regulation. Recently, gene targeting studies in mice showed that Evi-1 is essential for proliferation and maintenance of hematopoietic stem cells (HSC). Furthermore, the *EVI-1* genomic locus attracts much attention as a 'hotspot' of retroviral integration sites after gene therapy. The significant function of Evi-1 in HSC regulation implies that Evi-1 might participate in the generation of leukemia stem cells (LSC), which has been receiving particular attention as a cause of therapeutic resistance of leukemia. This review summarizes the biological roles and biochemical properties of EVI-1 in normal and malignant hematopoiesis, and describes future prospects for molecular therapy targeting EVI-1 in hematological malignancies.

EVI-1 genomic locus and its gene products

Human *EVI-1* is localized to chromosome 3 band q26,[3] spans 60 kb, and contains 16 exons, with multiple alternative 5′ mRNA variants and several alternative spliced transcripts[4] (Fig. 1a,b). The major EVI-1 form is a 1051 amino acid protein with an apparent molecular weight of 145 kDa.[5,6] EVI-1 has multiple zinc finger domains that are organized into two sets of seven and three zinc finger domains, respectively. Between the two sets of zinc finger domains, a repression domain has been identified as well as an acidic region at the C-terminus (Fig. 1c). The Δ324 transcript is an alternative splice variant of EVI-1 encoding an 88-kDa protein lacking zinc fingers 6 and 7, and is found at low levels in human and mouse cells.[7] The -Rp9 variant lacks nine amino acids in the repression domain and is quite common in human and mouse cells (Fig. 1b,c). EVI-1 also exists as a longer form, called MDS1–EVI-1, generated from the in-frame splicing of the small gene *MDS1* to the second exon of *EVI-1*.[8] The human *MDS1* gene was first identified because it is rearranged in 3;21 translocation in myeloid leukemias.[9] Human *MDS1* spans 500 kb, contains three exons, and is located 3 kb telomeric to the first exon of *EVI-1* (Fig. 1a). The MDS1–EVI-1 protein contains 188 additional amino acids at its N-terminus, encoding a so-called 'PR' domain (Positive Regulatory-Domain 1-Binding Factor 1 (PRD1-BF1)/Retinoblastoma-Interacting Zinc-finger protein 1 (RIZ1) homology), in addition to the entire EVI-1 sequence[8] (Fig. 1c). The PR domain has homology with the Su(var)3-9, Enhancer-of-zest, Trithorax (SET) domain, which is associated with histone methyltransferase activity. However, to date there is no indication that this activity is functional in MDS1–EVI-1. Rather, the PR domain in MDS1–EVI-1 prevents oligomerization, which affects its biochemical functions.[10] Several experimental and clinical observations indicate that EVI-1 (the PR-absent form) promotes tumor growth, whereas MDS1–EVI-1 (the PR-containing form) contributes to tumor suppression. However, EVI-1 and MDS1–EVI-1 cause similar effects in some biological settings (described in more detail below). Therefore, the precise roles of these proteins remain to be elucidated.

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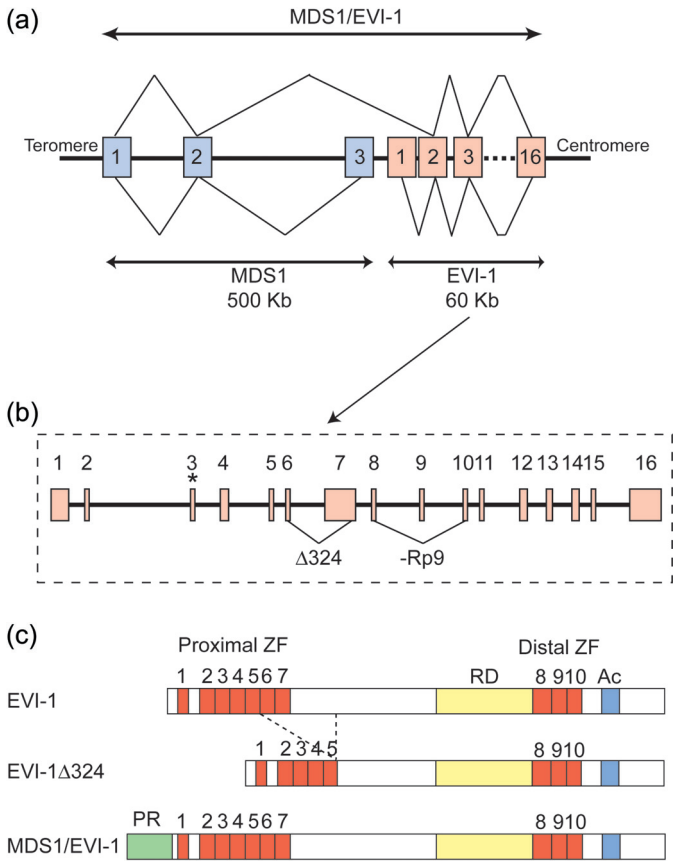

Fig. 1. Structure of ecotropic viral integration site-1 (*EVI-1*) and myelodysplastic syndrome (MDS) 1–EVI-1. (a) Genomic structure of human *EVI-1*, *MDS1*, and *MDS1–EVI-1*. Exons are represented by boxes and numbered. Z-lines represent splicing to produce mRNA. The *MDS1* gene has three exons and spans 500 kb. The *EVI-1* gene spans only 60 kb but has 16 exons. MDS1–EVI-1 is produced by splicing of the second exon of *MDS1* and the second exon of *EVI-1*. (b) Detailed genomic structure of human *EVI-1*. The translation start codon of *EVI-1* in exon 3 is indicated by an asterisk. The alternative splice variants are indicated by triangular lines. (c) Diagrams of EVI-1, EVI-1Δ324, and MDS1–EVI-1 proteins. EVI-1 has two sets of the zinc finger domains (ZF). Between the two sets of zinc finger domains, a repression domain (RD) has been identified as well as an acidic region (Ac) at the C-terminus. Structurally in MDS1–EVI-1, the PR domain is located at the N-terminus of EVI-1. Another naturally occurring splice variant, EVI-1Δ324, has been described that lacks 324 internal amino acids including zinc fingers 6 and 7 of the proximal zinc finger domain.

EVI-1 and transcriptional regulation

The EVI-1 protein is located in the nucleus and can bind to specific DNA sequences through both of its zinc finger domains independently.[11–13] The proximal zinc finger domain recognizes a consensus sequence of 15 nucleotides consisting of GA(C/T)AAGA(T/C)AAGATAA, and Evi-1 was shown to bind directly to the Gata2 promoter through this domain.[14,15] In addition, the binding site for this domain has a Gata1 consensus motif, and could potentially compete with Gata1 for DNA binding.[16] Although *in vitro* studies showed that the distal zinc finger domain recognizes the consensus GAAGATGAG, so far there are no reports of genes that are directly regulated by EVI-1 through the distal zinc finger domain. EVI-1 also interacts with several transcription regulators. In particular, interaction with the corepressor CtBP is important for EVI-1 function.[17,18] This interaction relies on amino acids 544–607 on the EVI-1 protein, a stretch that contains CtBP binding consensus motifs. CtBP increases the transcriptional repression of a reporter gene by EVI-1, and point mutations in *EVI-1* that abolish the interaction significantly decrease EVI-1-mediated transcriptional repression, growth inhibition of Mv1Lu cells in response to transforming growth factor (TGF)-β, and transformation of Rat-1 fibroblasts. EVI-1 also interacts with histone deacetylases directly or through CtBP, and the histone deacetylase inhibitor partially relieves transcriptional repression by EVI-1.[17,19] It was also shown that EVI-1 binds to the coactivators CREB binding protein (CBP) and P300/CBP-associated factor (P/CAF), and coexpression of CBP could turn a repressive effect of EVI-1 on a reporter gene into a moderately activating effect.[20] Furthermore, it was recently shown that EVI-1 associates with the histone H3 lysine 9-specific histone methyltransferases SUV39H1 and G9a.[21,22] Thus, EVI-1 forms higher-order complexes with various transcriptional regulators, and these associations are important for transcriptional regulation by EVI-1 (Fig. 2).

EVI-1 and signaling pathways

It has been shown that EVI-1 affects various signaling pathways. Among these, TGF-β is the best-characterized pathway that is interfered by EVI-1. TGF-β controls proliferation and cellular differentiation of most cell types, and plays an important role in tumor development. EVI-1 significantly represses TGF-β-mediated activation of the p3TP-Lux reporter plasmid in HepG2 cells, and suppresses TGF-β-mediated growth inhibition in Mv1Lu and 32D cells.[23,24] Furthermore, EVI-1 interferes with the induction of endogenous genes by TGF-β and other TGF-β family members in *Xenopus* animal cap explants as well as in C2C12 cells.[25] EVI-1 inhibits TGF-β signaling through at least two possible mechanisms: reduction of Smad3 activity by physical interaction and recruitment of the corepressor CtBP.[17,23] In contrast to EVI-1, MDS1–EVI-1 enhances TGF-β-induced growth inhibition in 32D cells[24] and cannot efficiently repress TGF-β-mediated activation of p3TP-Lux in HepG2 cells.[10] The lower repressive activity correlates with a reduced ability of MDS1–EVI-1, as compared to EVI-1, to bind to the corepressor CtBP[10] (Fig. 2).

The c-Jun N-terminal kinases (JNK) are mitogen-activated protein kinases that are responsive to various stress stimuli and play an important role in triggering apoptosis. EVI-1 significantly suppresses the JNK1-mediated phosphorylation of c-Jun. Conversely, reduction of EVI-1 expression using antisense oligonucleotide recovers endogenous JNK1 activity in MOLM-1 and HEC1B cells. EVI-1 physically interacts with JNK through the proximal zinc finger domain, and an EVI-1 mutant lacking this domain fails to suppress JNK1 activity. EVI-1 also protects cells from stress-induced cell death with dependence on the ability to inhibit JNK[26] (Fig. 2). In addition to JNK, several mechanisms have been proposed to play a role in the survival function of EVI-1. EVI-1 protects murine bone marrow progenitors from apoptosis by activating the Promyelocytic leukemia (*Pml*) gene.[27] It was also reported that EVI-1 suppresses TGF-β or taxol-mediated apoptosis through a Phosphoinositide 3-kinase (PI3K)-Akt dependent mechanism in RIE cells.[28]

Activator protein (AP)-1 is a transcription factor complex consisting of a Fos–Jun heterodimer or Jun–Jun homodimer. It regulates gene expression in response to a variety of stimuli, and controls a number of cellular processes including differentiation, proliferation, and apoptosis. EVI-1 raises AP-1 activity and stimulates c-fos promoter activation with dependence on its distal zinc finger domain in NIH3T3 and P19 cells.[29] Because the distal zinc finger domain is required for EVI-1-mediated transformation of Rat-1 cells, the enhanced AP-1 activity probably contributes to cell transformation by EVI-1 (Fig. 2).

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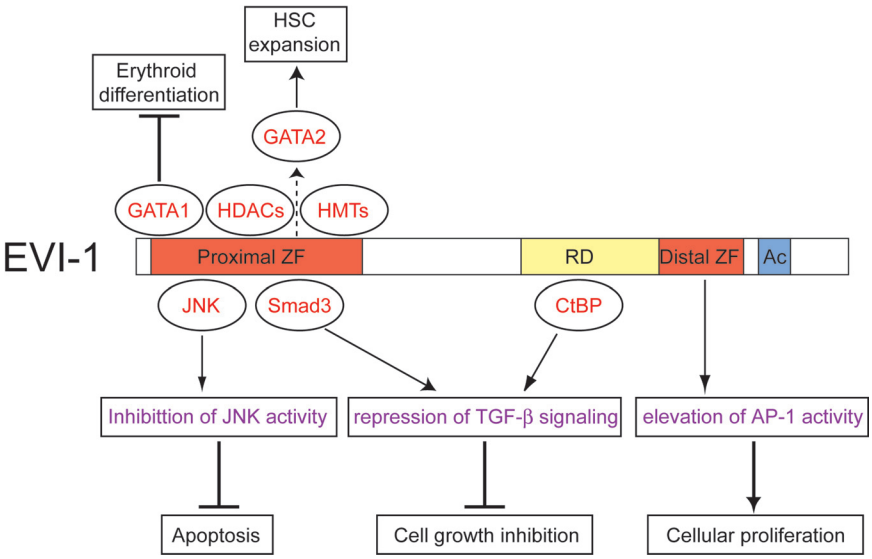

Fig. 2. Biochemical properties of ecotropic viral integration site-1 (*EVI-1*). EVI-1 inhibits c-Jun N-terminal kinase (JNK) activity and prevents apoptosis. EVI-1 interacts with Smad3 and C-terminal Binding Protein (CtBP), and blocks the growth inhibitory effect of transforming growth factor (TGF)-β. EVI-1 raises activator protein (AP)-1 activity and promotes cell proliferation. EVI-1 interacts with GATA1 and inhibits erythroid differentiation. EVI-1 upregulates GATA2 expression and promotes hematopoietic stem cell (HSC) expansion. Interactions with histone deacetylases (HDAC) and histone methyltransferases (HMT) contribute to EVI-1-mediated transcriptional regulation. Through these molecular mechanisms, EVI-1 acts both as a HSC regulator and an oncogenic protein. Proteins that interact with specific regions of EVI-1 are depicted in corresponding positions. Ac, acidic region; RD, repression domain; ZF, zinc finger domain.

Roles of Evi-1 in HSC regulation

Recently, several studies using gene targeting mice revealed an essential role for EVI-1 in HSC regulation. First, Yuasa *et al.* showed that Evi-1 is preferentially expressed in HSC and is downregulated upon differentiation.[14] Furthermore, development of HSC in the para-aortic splanchnopleural region, from which definitive hematopoiesis originates, was severely impaired in Evi-1-knockout embryos.[14] Interestingly, Evi-1-mediated para-aortic splanchnopleural hematopoiesis seems to depend on Gata2 activation and repression of TGF-β signaling.[14,30] These findings suggest that Evi-1 has a role in the early expansion of HSC during embryogenesis. However, the Evi-1-knockout mice used in the study carry a targeted deletion of exon 7, resulting in the expression of a truncated Evi-1 transcript (Evi-1Δ324). In addition, the role for Evi-1 in fetal liver (FL) and bone marrow hematopoiesis was not clarified because these mice die around embryonic day 10.[31] Our group created new Evi-1 mutant mice in which exon 4 of the *Evi-1* gene can be deleted by the expression of Cre recombinase (Evi-1-flox), as well as mice in which the same region was completely deleted (Evi-1-KO).[32] Because exon 4 of *Evi-1* exists in all known Evi-1 transcripts, this strategy resulted in complete deletion of Evi-1. Interestingly, the new Evi-1-KO mice survived slightly longer (they died between embryonic days 13.5 and 16.5) than the prior Evi-1 mutant mice, which enabled the analysis of FL hematopoiesis. Although the Evi-1-KO FL was morphologically indistinguishable from the wild-type FL, the population of lineage$^-$, c-Kit$^+$, Sca-1$^+$ cells (LSK cells), which enriches hematopoietic stem and progenitor cells, was severely reduced in Evi-1-KO mice. The total number of colony-forming cells, especially the number of mixed colonies, in Evi-1-KO FL cells was also severely decreased. Furthermore, Evi-1-KO FL cells did not reconstitute hematopoiesis of the irradiated recipient mice. Thus, Evi-1 deletion causes severe reduction in HSC in FL hematopoiesis.[32] The roles of Evi-1 in adult hematopoiesis were assessed by crossing Evi-1-flox mice with Mx-Cre transgenic mice, in which a high level of Cre recombinase is produced by treatment with the interferon inducer pI-pC, leading to recombination in hematopoietic cells of in all lineages. By 4 weeks after pI-pC injection, Evi-1-excised mice exhibited a significant decrease in the frequency of HSC compared with control mice. Moreover, by 12 weeks after pI-pC injection, Evi-1-deleted hematopoietic cells were outcompeted by the cells that escaped Cre-mediated

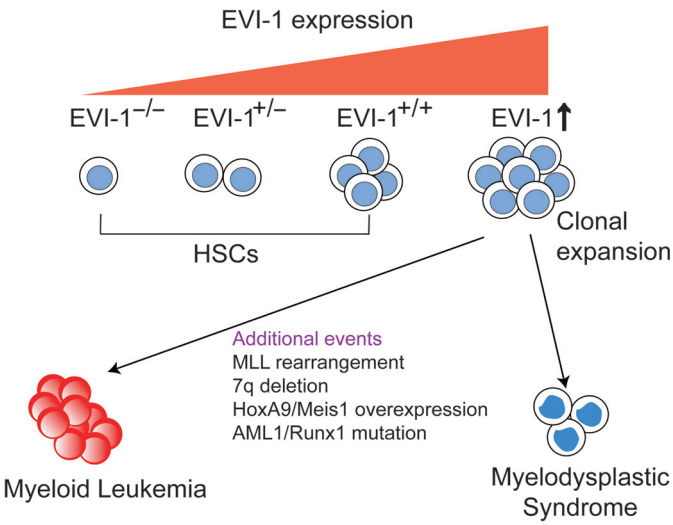

Fig. 3. Gene dosage effects of ecotropic viral integration site-1 (*EVI-1*) in hematopoietic stem cells (HSC) and hematological malignancies. EVI-1 regulates proliferation and maintenance of HSC in a dose-dependent manner. Activation of EVI-1 in hematopoietic cells leads to clonal expansion or dysplastic hematopoiesis, whereas onset of full-blown leukemia requires cooperative genetic events. Hox, Homeobox; Meis, Myeloid ecotropic viral integration site; MLL, Mixed-Lineage leukemia; Runx, Runt-related transcription factor.

Evi-1 excision. Thus, Evi-1 is also required for proliferation and maintenance of adult HSC.[32] In addition, Evi-1$^{+/-}$ mice exhibited the intermediate phenotype as for the number of HSC, as well as hematopoietic reconstitution activity of the bone marrow cells, suggesting a gene dosage requirement for Evi-1 in the regulation of HSC[32] (Fig. 3).

Effects of EVI-1 overexpression on hematopoietic differentiation

In Evi-1-excised mice, the frequencies of mature myeloid cells, lymphoid cells, and erythroid cells were not affected in spite of the efficient excision of Evi-1 alleles.[32] Therefore, it is likely that Evi-1 is physiologically dispensable for lineage commitment in normal hematopoiesis. However, a large body of evidence

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suggests that aberrant expression of EVI-1 affects hematopoietic differentiation in various lineages. Ectopic expression of EVI-1 blocks granulocytic differentiation of interleukin-3-dependent 32D cells when stimulated with granulocyte-colony stimulating factor (G-CSF)[33] whereas MDS1–EVI-1 had no such effect.[24] In addition, retrovirally expressed EVI-1 delayed myeloid differentiation of murine bone marrow cells and induce the enhanced replating of myeloid progenitors in methylcellulose medium.[34] These results indicate that EVI-1 overexpression interferes with myeloid differentiation. EVI-1 might also block erythroid differentiation. Ectopic expression of EVI-1 into murine bone marrow cells drastically reduced the capacity to produce erythroid colonies or $Ter119^+$ immature erythroid cells both *in vitro* and *in vivo*.[16,35,36] The EVI-1-mediated block of erythroid differentiation may be due to the physical interaction and competition for DNA binding with GATA1, a crucial transcription factor required for erythropoiesis[16,36] (Fig. 2). Finally, several experiments suggest that EVI-1 promotes megakaryopoiesis. Using an experimental protocol to differentiate embryonic stem (ES) cells to hematopoietic cells, Sitailo *et al*. showed that Evi-1, but not Mds1–Evi-1, promotes megakaryocytic differentiation.[37] Similarly, EVI-1 induced megakaryocytic differentiation in UT7-GM cells.[38] These findings are interesting because leukemias with EVI-1 rearrangements typically show elevated platelet counts.

Effects of retroviral insertions into the *EVI-1* and *MDS1–EVI-1* loci

The *EVI-1* and *MDS1–EVI-1* genomic loci have been defined as 'hotspots' for retroviral integration in both clinical and experimental models. The vast majority of the insertions are located in the introns of MDS1, between the last MDS1 and the first EVI-1 exons, or in the first two introns of EVI-1.[39] In a gene therapy trial for chronic granulomatous disease (CGD), activating insertions of the therapeutic vector into the EVI-1 locus lead to preferential expansion of the affected cells.[40] Two young adult CGD patients were reinfused with autologous $CD34^+$ cells transduced with a retroviral vector containing an intact copy of the *gp91phox* gene, whose mutation causes CGD. Both patients showed significant clinical improvement, and analysis of vector insertion sites revealed that cell clones containing insertions in the MDS1–EVI-1 locus increasingly dominated hematopoiesis after transplantation. Despite their marked clonal dominance, no evidence for the development of leukemia was found in either patient at up to 2 years. Similar to the findings in the human CGD trial, a study using rhesus macaques showed significant overrepresentation of vector insertions in the *MDS1–EVI-1* locus in granulocytes long-term after transplantation.[41] These MDS1–EVI-1 clones became apparent 3–6 months after transplantation and then contributed continuously with no signs of leukemia. Furthermore, the *in vivo* analysis of mice using serial transplantation showed that retroviral integrations at the *Evi-1* or *Mds1–Evi-1* locus can be related to long-term non-malignant clonal expansion in healthy C57Bl/6 recipient mice.[42] In this experiment, a relatively small number of clones always became dominant in recipient mice, and the dominant clones always had insertions near genes with a role in self-renewal or survival of HSC. Thus, transcription-activating retroviral integrations into the *EVI-1* and *MDS1–EVI-1* loci confer survival and self-renewing ability to hematopoietic cells.

Experimental models of EVI-1-related hematological malignancies

Several mouse models of EVI-1-related hematological malignancies have been generated. Transgenic mice expressing Evi-1 under the Sca-1 promoter, which is active in HSC and progenitor cells, developed normally and did not show clear hematological abnormalities. However, these mice were more susceptible to retrovirally induced leukemia than controls, supporting an oncogenic role for Evi-1.[43] Other groups used bone marrow infection and transplantation to establish the mouse model for Evi-1 overexpression. Cuenco and Ren used Balb/c mice as bone marrow donors and recipients, and found that some Evi-1-positive mice developed lymphoma with a block in early B-cell development. In contrast, Mds1–Evi-1-positive cells did not develop any disease.[44] Buonamici *et al*. used C57BL/6 mice and found that all Evi-1-positive mice displayed a lethal condition resembling human MDS, including hyperproliferation of bone marrow and progressive pancytopenia that resulted in their death.[35] Jin *et al*. also reported the emergence of a MDS-like disease in C57BL/6 mice transplanted with Evi-1-transduced bone marrow cells. In addition, they showed that coexpression of Evi-1 with Homeobox A9 (HoxA9) and Myeloid ecotropic viral integration site 1 (Meis1) significantly accelerated the onset of HoxA9/Meis1-induced AML.[45] It was also shown that Evi-1 collaborated with AML1/RUNX1 mutants to induce AML in recipient mice.[46] These results suggest that activation of Evi-1 in hematopoietic cells primarily promotes clonal expansion or myeloid dysplasia, whereas leukemogenesis requires additional genetic events. The extent of Evi-1 expression, genetic background of the host, and the cooperative genetic events will determine the fate of Evi-1-positive hematopoietic cells (Fig. 3).

Recently, our group found that complete loss of Evi-1 attenuates proliferative activity in a wide variety of leukemic cells.[32] Disruption of Evi-1 in MLL/ENL-transformed or E2A/HLF-transformed progenitors in methylcellulose medium caused a significant reduction in colony numbers to approximately 30 or 60% of the control, respectively. Furthermore, Cre-mediated Evi-1 deletion in cMyc-bcl2-transduced bone marrow cells delayed the onset of leukemia *in vivo*. In particular, Evi-1 deletion led to a large decrease in colony numbers in MLL/ENL-transformed cells, indicating a crucial role for Evi-1 in MLL leukemias.

Clinical aspects of EVI-1-related hematological malignancies

The EVI-1 overexpression often occurs as a consequence of chromosomal rearrangements involving 3q26, where EVI-1 is mapped. Of these, the translocations t(3;21)(q26;q22) and t(3;12)(q26;p13) lead to the formation of the AML1–MDS1–EVI-1 and Translocation Ets Leukemia (TEL)–(MDS1)–EVI-1 fusion transcripts, respectively.[9,47,48] Meanwhile, inv(3)(q21q26), t(3;3)(q21;q26), and other 3q26 rearrangements lead to overexpression of intact EVI-1 mRNA.[49,50] These cases have elevated platelet counts, marked hyperplasia with dysplastic megakaryocytes, and poor prognosis, which are characterized as 3q21q26 syndrome.[51] EVI-1 is also highly expressed in a subgroup of AML without 3q26 rearrangements, indicating the presence of other mechanisms for EVI-1 activation. A study using gene expression profiles of 285 patients with AML revealed that EVI-1 expression could define a new subtype of AML. These samples have overall expression patterns closest to normal $CD34^+$ cells, suggesting a stem cell phenotype for EVI-1-positive leukemias, and these patients have a very poor prognosis.[52] Although previous studies could not distinguish between EVI-1 and MDS1–EVI-1 expression, recently, several groups developed real-time quantitative PCR assays to measure specific expression of both EVI-1 and MDS1–EVI-1 transcripts. Interestingly, studies involving a large number of *de novo* AML patients without 3q26 rearrangements found a prognostic significance of EVI-1, but not of MDS1–EVI-1, overexpression in this patient group.[53,54] However, another group reported that

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MDS1–EVI-1 overexpression also predicts short remission duration in AML patients.[55] Therefore, further study will be necessary to clarify the significance of EVI-1 versus MDS1–EVI-1 expression in AML. The clinical studies also revealed that high EVI-1 expression correlates with the presence of 7q-deletions and translocations involving 11q23 (MLL-rearrangements).[54] The relationship between EVI-1 and these genetic abnormalities should be investigated in the future.

Conclusions and future directions

Recently, *EVI-1* has received much attention as a crucial gene for HSC regulation as well as an oncogene whose expression predicts poor patient survival in hematological malignancies. Current evidence suggests that leukemias are maintained by rare stem cells (LSC), and elimination of LSC is necessary and potentially sufficient for cure of the leukemia. It is tempting to speculate that activation of EVI-1 enhances proliferation and/or survival of LSC and, thus, confers drug resistance on various types of leukemia. Therefore, therapies designed to target EVI-1 will be an attractive option in treatment for hematopoietic malignancies. Significant in this regard is the report that arsenic trioxide (ATO) selectively degrades EVI-1 protein.[56] Furthermore, ATO and thalidomide combination therapy produces multilineage hematological responses in MDS patients, particularly in those with high EVI-1 expression.[57] Thus, ATO may be used as part of a target therapy for patients with EVI-1-positive MDS. Further elucidation of the mechanisms regulating EVI-1 expression (i.e. upstream signaling pathways, regulation of mRNA translation, and regulation of protein stability of EVI-1) will be helpful to develop novel therapies targeting EVI-1. In order to determine the 'true' therapeutic target, the precise roles of EVI-1 variants should be clarified. In particular, the relationship between EVI-1 and MDS1–EVI-1 in normal and malignant hematopoiesis is important because they act either in a similar or opposite manner depending on the context. Furthermore, identification of downstream target genes as well as interacting proteins of EVI-1 will provide essential information on EVI-1-mediated hematopoiesis and leukemogenesis. Because EVI-1 is preferentially expressed in the stem cell compartment, marking of EVI-1 expression might enable the *in vivo* visualization of HSC and LSC. Thus, future investigations regarding EVI-1 will shed light on the basic mechanisms underlying stem cell behavior and leukemia development, and then improve the current treatment strategies for hematological malignancies.

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