研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
原田浩徳 今川　潤 原田結花 木村昭郎	C/EBPα変異パターンによる急性骨髄性白血病(AML)および骨髄異形成症候群(MDS)病型の解析	広島医学	63	311-313	2010
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IV. 研究成果の刊行物・別刷

（主なもの）

Evi-1 is a transcriptional target of MLL oncoproteins

in hematopoietic stem cells

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Running title: MLL oncoproteins activate Evi-1 transcription

Abstract

Ecotropic viral integration site-1 (Evi-1) is a nuclear transcription factor that plays an essential role in the regulation of hematopoietic stem cells (HSCs). Aberrant expression of Evi-1 has been reported in up to 10% of acute myeloid leukemia (AML) patients, and is a diagnostic marker that predicts a poor outcome. Although chromosomal rearrangement involving the Evi-1 gene is one of the major causes of Evi-1 activation, overexpression of Evi-1 is detected in a subgroup of AML without any chromosomal abnormalities, indicating the presence of other mechanisms for Evi-1 activation. In this study, we found that Evi-1 is frequently up-regulated in bone marrow cells transformed by MLL-ENL or MLL-AF9. Analysis of the Evi-1 gene promoter region revealed that MLL-ENL activates transcription of Evi-1. MLL-ENL-mediated up-regulation of Evi-1 occurs exclusively in the undifferentiated hematopoietic population, in which Evi-1 particularly contributes to the propagation of MLL-ENL-immortalized cells. Furthermore, gene expression analysis of human AML cases demonstrated the stem cell-like gene expression signature of Evi-1 high MLL-rearranged leukemia. Our findings indicate that Evi-1 is one of the targets of MLL oncoproteins, and is selectively activated in HSC-derived MLL leukemic cells.

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Introduction

The ecotropic viral integration site-1 (Evi-1) is a nuclear transcription factor and plays an essential role in proliferation and maintenance of hematopoietic stem cells (HSCs) [1-3]. There are two major alternative forms generated from Evi-1 gene, Evi-1a and Mds1-Evi-1 (also called Evi-1c). Mds1-Evi-1 is a fusion variant of Evi-1 generated through intergenic splicing with Mds1 [4], a gene located approximately 140 kb and 500 kb upstream of Evi-1 in human and mouse genome, respectively. In contrast to Evi-1a, Mds1-Evi-1 possesses the PRDI-BF1-RIZ1 homologous (PR) domain in the N-terminus, which regulates oligomerization of the Evi-1 proteins [5]. Both Evi-1a and Mds1-Evi-1 are normally co-expressed in several developing and adult tissues [6], and differences in the normal function between these proteins remain to be elucidated. Like all other PR domain proteins, Evi-1 contains several zinc finger motifs. They are grouped into N-terminal seven and C-terminal three clusters, which are called the first and second zinc finger domain, respectively [7,8]. Between these two zinc finger domains lie the C-terminal binding protein (CtBP)-binding domain and the repression domain. The first zinc finger, the repression, and CtBP-binding domains exhibit growth promoting effect by blocking transforming growth factor-β (TGF-β) signaling [9]. The first zinc finger domain also exhibits anti-apoptotic effect by repressing c-Jun N-terminal kinase signaling [10]. The second zinc finger domain stimulates proliferation by increasing AP-1 activity [11]. Thus, Evi-1 possesses diverse functions as an oncoprotein.

Aberrant expression of EVI-1 has been frequently found in myeloid leukemia as well as in several solid tumors, and is associated with a poor prognosis of patients with leukemia [12-15]. Rearrangements of chromosome 3q26, which contains the EVI-1 gene, lead to overexpression of EVI-1 and are implicated in the development or progression of high-risk acute myeloid leukemia (AML) [16]. Importantly, EVI-1 is also highly expressed in a subgroup of AML without 3q26 rearrangements [12], indicating the presence of other mechanisms for EVI-1 activation. Recently, several clinical studies revealed a positive correlation between EVI-1 (both EVI-1a and

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MDS1-EVI-1) overexpression and rearrangements of the mixed lineage leukemia (MLL) gene located on chromosome 11q23 [14,15]. Furthermore, we have previously shown that Evi-1 deletion in transformed cells by MLL-ENL, a chimeric gene generated in t(11;19) leukemia, caused distinct reduction of their proliferative activity [3]. These results raise a possibility of functional interaction between Evi-1 and MLL oncoproteins.

The MLL gene encodes a DNA-binding protein that involves SET [Su(var)3–9, Enhancer of zeste, and trithorax] domain with histone H3 lysine 4 (H3K4) methyltransferase activity, regulates gene expression including multiple Hox genes [17,18]. Chromosome translocations involving the MLL gene are associated with aggressive forms of acute leukemia [19]. Generation of MLL fusion proteins in leukemia deletes the SET domain which mediates H3K4 methylation, and fuses the amino portion of MLL in frame with up to fifty different fusion partners, including ENL, AF9, and AF4 [19]. It has been shown that several Hox genes are consistently expressed at high levels in MLL leukemias, suggesting that MLL oncoproteins inappropriately maintain their expression [20]. Hox proteins form hetero-oligomers with TALE (3-amino-acid loop extension) homeobox-proteins of the Pbx and Meis families, and Meis1 is also highly expressed in MLL leukemias [21,22]. A large body of evidence suggests that Hox/Meis genes are crucial targets of MLL oncoproteins in almost all the cases of MLL leukemias [22,23]. However, MLL leukemias are biologically and clinically diverse, and additional factors that underlie these differences have been characterized incompletely.

In this study, we found that Evi-1 is frequently up-regulated by MLL-ENL or MLL-AF9 in retroviral transduction assay. The reporter assay and chromatin immunoprecipitation analysis (ChIP) revealed that MLL-ENL binds to and activates the promoter of Evi-1. Retroviral transduction assay with defined populations of bone marrow (BM) progenitors revealed that MLL-ENL-mediated Evi-1 up-regulation occurs exclusively in HSCs but not in committed myeloid progenitors. These results suggest that up-regulation and maintenance of Evi-1 expression are the features of MLL oncoproteins, which work specifically in undifferentiated hematopoietic stem/progenitor cells.

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Material and Method

Plasmid construction

The plasmids pMSCV-neo-Flag-MLL-ENL, pMSCV-neo-MLL-AF9, pMSCV-neo-AML1-ETO, pMXs-neo-E2A-HLF, and pMYs-HoxA9-ires-Meis1, have been described previously [3,24,25,26]. The procedure of construction was described in Supplemental Methods.

Mice

C57BL/6 mice were purchased from Sankyo Labo Service (Tokyo, Japan). For the experiments of Evi-1 deletion, BM progenitors were harvested from wild-type (Evi-1^{+}), loxP-flanked (Evi-1^{f}), and Evi-1-deleted mutant mice (Evi-1$^{+/-}$ and Evi-1$^{f/-}$) [3]. Mice were kept at the Center for Disease Biology and Integrative Medicine, University of Tokyo, according to institutional guidelines, and all animal experiments were approved by the University of Tokyo Institutional Animal Care and Use Committee.

Retrovirus transduction

To obtain retrovirus supernatants, Plat-E packaging cells [27] were transfected with retrovirus vectors by the use of FuGENE 6 (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. Viral Supernatants were collected after forty-eight hours of culture and used immediately for infection. To produce GFP- or Cre-GFP- expressing retrovirus, we used ψMP34 packaging cells (Takara) stably transduced with pGCDNsam-eGFP or pGCDNsam-eGFP-iCre. Methods to isolate hematopoietic stem/progenitor cells from mice were described in Supplemental Methods [28].

Myeloid progenitor transformation assay

Myeloid progenitor transformation assay was performed as described previously [26] with minor modifications. In brief, retrovirus-infected cells were cultured in Methocult M3434 (Stem Cell Technologies) and 1 mg/ml G418 at a density of 1 × 10^5 cells per 35-mm dish. Colonies were

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counted weekly, and cells were cultured again at 1×10^4 per plate in M3434 without G418. Colony counts scoring and replating were repeated every 7 days. For the experiments of Evi-1 deletion, BM progenitors from Evi-1$^{+/-}$ or Evi-1$^{f/-}$ mice were used.

in vivo **leukemogenesis assay**

Bone marrow mononuclear cells (BM MNCs) harvested from 5-FU injected mice were transduced with MLL-ENL or cMyc/bcl2 [29] under identical conditions to those for myeloid progenitor transformation assay. Retrovirally transduced BM progenitors (1×10^6) were injected into sublethally irradiated (6.5 Gy) recipients. When transplanted mice became moribund, they were euthanized and their BM MNCs were isolated.

Luciferase reporter assay

For analysis of luciferase activities, Jurkat cells were seeded in 12-well culture plates at a density of 0.5×10^5 per well. The cells were transfected with 100 ng of pGL4-Luc or equimolar amount of each reporter construct, together with 100 ng of pME18S or equimolar amount of each expression plasmid and 5 ng of PSS-LacZ by the use of FuGENE6. After forty-eight hours of culture, cells were harvested, and luciferase activities were measured in a Lumat LB9507 luminometer (Berthold Technologies) using Picagene luminescence kit (Toyo Ink). Each luciferase activity was normalized to that of β-galactosidase, which was measured using Galacton-Plus (Roche). The data were expressed as mean +/- s.d. from two or more separate experiments.

Chromatin immunoprecipitation (ChIP)

ChIP analysis was performed as described previously [30], with minor modifications. The procedures were described in Supplemental Methods.

Bioinformatics Analyses

The gene expression pattern in MLL-rearranged leukemia cells was assessed by using data of 285 individuals with AML published by Valk et al. from Gene Expression Omnibus (GEO) (GSE1159) [15]. We used 13 cases with MLL rearrangement (except three cases with MLL-partial tandem

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duplication), and divided them into two groups according to the level of EVI-1 expression; five EVI-1 high (GSM20760, 20794, 20838, 20959, and 20966) and eight EVI-1 low (GSM20757, 20844, 20879, 20891, 20934, 20936, 20938, and 20961) cases. Gene set enrichment analyses (GSEA) were performed using GSEA v2.0 software available from the Broad Institute (http://www.broad.mit.edu/gsea) [31] with a Signal2Noise metric for ranking genes and 1000 data permutations. Functional 1892 gene sets (C2) or eight selected gene sets representing hematopoietic stem and progenitor clusters [32] were evaluated (Supplemental Methods).

Additionally, gene expression data of murine c-Kit$^+$, Sca-1$^+$, Lin$^-$ (KSL) cells and granulocyte–macrophage progenitors (GMPs) were obtained from GEO (GSE3725) [33]. Data set comparisons of 13 cases of EVI-1 high or low leukemias were performed using dChip (http://www.hsph.harvard.edu/~cli/complab/dchip/) [34]. The expression value was calculated using a perfect match/mismatch (PM/MM) model, after transformed into log2 scale. Differently regulated probe sets in EVI-1 high and low leukemias were determined using fold change > 1.2, $p < 0.05$, and 90% lower confidence bound criteria. In this way, 120 probes enriched in EVI-1 high leukemias and 192 probes enriched in EVI-1 low leukemias were extracted to make gene sets representing EVI-1 high and low MLL-rearranged leukemia, respectively (Supplemental Table S2). GSEA were performed using these gene sets and the gene expression data of murine KSL cells and GMPs.

Statistics

Data were analyzed by Student's t-test. $P < 0.05$ was considered significant.

Results

Evi-1 is upregulated in myeloid progenitors immortalized by MLL oncoproteins

We first assessed Evi-1 expression in myeloid progenitors immortalized by various oncogenes. They include two MLL chimeric genes (MLL-ENL and MLL-AF9), E2A-HLF, and co-expression of HoxA9 and Meis1. MLL-ENL and MLL-AF9 are major forms of MLL oncoproteins generated in t(11;19) and t(9;11) leukemias respectively, which contain nuclear proteins as a fusion partner. E2A-HLF is a chimeric gene generated in t(17;19) leukemia, and it transforms myeloid progenitors through Hox-independent mechanisms in mice [35]. HoxA9 and Meis1 are crucial downstream targets of MLL oncoproteins, and co-expression of HoxA9 and Meis1 is sufficient for myeloid transformation [36]. Primary murine hematopoietic progenitors (c-Kit$^+$ cells) transduced with these oncogenes, but not those with the empty vector, formed colonies in methylcellulose medium that can be replated through at least three rounds of culture (data not shown). After establishment of sustained clonogenic activity following more than three rounds of replating, the cells were harvested and the expression level of Evi-1 was assessed using real-time quantitative PCR analysis. As shown in Figure 1A, Evi-1 was highly expressed in myeloid progenitors transformed by MLL-ENL or MLL-AF9 oncoproteins compared with those transformed by HoxA9 and Meis1, which are critical transcriptional targets of MLL oncoproteins. Therefore, HoxA9 and Meis1 seemed unable to complement the transcriptional effect of MLL oncoproteins on Evi-1. On the other hand, Evi-1 was not activated in E2A-HLF-immortalized cells.

Next, we assessed immediate changes in expression level of Evi-1 in hematopoietic cells induced by transduction of myeloid leukemia genes. We employed two chimeric genes frequently found in AML, AML1-ETO and PML-RARA. The former is generated in t(8;21) leukemia, whereas the latter is generated in t(15;17) leukemia. We retrovirally transduced these myeloid leukemia genes into BM myeloid progenitors. Transduced cells were isolated forty-eight hours later, and the expression level of Evi-1 was assessed. As shown in Figure 1B, Evi-1 expression was higher in

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MLL-ENL-transduced BM cells than in GFP-transduced cells, while it was neither enhanced in PML-RARA nor AML1-ETO transduced cells, which belong to the most common forms of myeloid leukemia. Evi-1 in MLL-AF9-transduced BM cells was not significantly up-regulated, either.

To see the dependency of the activation of Evi-1 on MLL-ENL, we constructed MLL-ENL fused to the estrogen receptor (MLL-ENL-ER), which rendered the transcriptional and transforming properties of MLL-ENL strictly dependent on the presence of 4-hydroxy-tamoxifen (4-OHT). Consistent with the earlier report [23], MLL-ENL-ER-transduced hematopoietic progenitors required 4-OHT for myeloid transformation (Figure 1C). Using this system, we quantified the expression of Evi-1 and HoxA9 in MLL-ENL-ER–immortalized cells. By seventy-two hours after 4-OHT withdrawal, the expression of Evi-1 as well as HoxA9 was significantly reduced compared with that seen in 4-OHT (+) cells. Thus inactivation of MLL-ENL results in downregulation of Evi-1 (Figure 1D), again suggesting a potential relationship between a distinct expression of Evi-1 and MLL-ENL.

Expression of Evi-1 in MLL fusion-transformed leukemic cells *in vivo*

We next assessed Evi-1 expression in leukemic cells transformed by MLL-ENL using a mouse leukemia model. We employed cMyc/bcl2-induced biphenotypic leukemia as a control, whose BM infiltration consists of a large number of myeloblasts and a small number of lymphoblasts. We harvested BM MNCs from mice treated with 5-FU. These cells were transduced with MLL-ENL or cMyc/bcl2, and then intravenously injected into sublethally irradiated recipient mice. Mice transplanted with MLL-ENL- or cMyc/bcl2- transduced cells developed leukemia within eighty-five or twenty-six days, respectively, which is consistent with the previous reports (Figure 2A) [29,37]. Leukemic cells were isolated from BM of moribund mice, and the expression level of Evi-1 was determined along with HoxA9, a well-known target of MLL-ENL. As shown in Figure 2B, Evi-1 was distinctly up-regulated in MLL-transformed leukemic cells of the four mice out of eleven, but was never activated in cMyc/bcl2-transformed cells (p = 0.037). The expression level of Evi-1 varied

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considerably among individuals, while that of HoxA9 expression was almost comparable (Figure 2B). These results suggest that the regulation of Evi-1 is independent of HoxA9. Because Evi-1 gene gives rise to two major alternative forms, Evi-1a and Mds1-Evi-1 (Evi-1c), we then assessed the expression of those Evi-1 isoforms using specific primers to detect respective forms. Interestingly, in all four individuals with high Evi-1 expression, the expression of both isoforms, Evi-1a and Mds1-Evi-1, was up-regulated (Figure 2C).

MLL oncoproteins specifically up-regulate Evi-1 through 5 promoter regions

To determine whether MLL-ENL regulates the transcription of Evi-1, we performed luciferase reporter assay. Because the genomic region of 5.7 kb upstream of TSS of Evi-1a is highly conserved among species (Supplemental Figure S1), we divided the region into five fragments and inserted them upstream of luciferase cDNA in the pGL4-Basic vector (Figure 3A). Each reporter plasmid was transfected into Jurkat cells along with the MLL-ENL expression plasmid. MLL-ENL exhibited highest increase of luciferase activity with the pGL4-E2265 (Figure 3A). These data suggest that MLL-ENL up-regulates Evi-1a through the region between -2.3 and -1.3 kb of the TSS. We next cloned three fragments within the genomic region around the Mds1-Evi-1 TSS, which is also evolutionally conserved (Figure 3B, Supplemental Figure S1). The luciferase reporter assay revealed that MLL-ENL activated Mds1-Evi-1 transcription mainly through the region between -0.1 to +0.3 kb of the TSS (Figure 3B).

To further confirm the crucial region for Evi-1a activation by MLL-ENL, we generated a series of pGL4-E2265 deletions and performed luciferase reporter assay (Supplemental methods). We observed no significant changes of luciferase activity between pGL4-E2265 and E1661 (Figure 3C). In contrast, remarkable reduction of luciferase activity was observed by the deletion of N-terminal 146 bases from pGL4-E1661 (Figure 3C). These data suggested that the responsive elements for MLL-ENL are within 1.7 and 1.5 kb upstream of the Evi-1a TSS.

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To determine whether MLL-ENL binds to these genomic regions *in vivo*, we performed ChIP with lysates from MLL-ENL-transformed cells collected from the leukemia mice. The ChIP assay demonstrated that MLL-ENL bound to the 5' promoter regions of both Evi-1a and Mds1-Evi-1 that are responsible for activation in the reporter assay, but not to the irrelevant region (Figure 3D).

MLL oncoproteins, but not wild-type MLL activates the promoter of Evi-1

We then performed the luciferase assay using another MLL-associated gene, MLL-AF9, and other several leukemia-associated genes, PML-RARA, AML1-ETO, E2A-HLF, and the combination of HoxA9 and Meis1. MLL-AF9 exhibited a transcriptional activity comparable with MLL-ENL on Evi-1 promoter (Figure 4A). In contrast, PML-RARA, AML1-ETO, E2A-HLF, or HoxA9/Meis1 exhibited just minimal or no transcriptional activity on Evi-1 promoter (Figure 4A) in agreement with the results of the expression analysis of Evi-1 in BM cells (Figure 1A, 1B).

Because wild-type MLL (WT MLL) also works as a transcriptional activator for such as Hox genes, we tested its transcriptional activity on Evi-1 promoter. We observed no significant transcriptional activities of WT MLL on Evi-1 promoter, suggesting that wild-type MLL by itself is not sufficient for the activation of Evi-1 (Figure 4A).

Next, we determined domain contribution of MLL-ENL in the activation of Evi-1 using a series of MLL-ENL mutants (Figure 4B). The CXXC domain of MLL mediates binding to nonmethylated CpG DNA and is essential for myeloid transformation [38]. The AT hook motifs of MLL are thought to facilitate binding to AT-rich DNA in the minor grove but are dispensable for myeloid transformation [38]. Consistent with their contribution for transforming activity of MLL-ENL, CXXC domain was essential for Evi-1 activation (Figure 4C). Unexpectedly, deletion of the AT hook motifs affected the reporter activity although it is dispensable for MLL-ENL-mediated myeloid transformation and up-regulation of Hox genes, and the menin binding motif (MBM) was not required for Evi-1 activation (Figure 4C) [39].

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Evi-1 is up-regulated by MLL-ENL exclusively in HSC-derived transformed cells

Although clinical studies revealed a positive correlation between high Evi-1 expression and MLL rearrangements, there exists a subset of MLL-rearranged leukemia with normal Evi-1 expression level [14,15]. We have also observed that the expression level of Evi-1 in MLL leukemia cells considerably varied among the individual mice (Figure 2B). Because Evi-1 is preferentially expressed in HSCs and the expression level decreased upon differentiation [2], we hypothesized that Evi-1 expression in MLL leukemia cells depends on their cellular origin.

To test this hypothesis, we transduced MLL-ENL into the defined hematopoietic populations including KSL cells which contain HSCs, myeloid-restricted common myeloid progenitors (CMPs), and GMPs [28]. Consistent with the earlier report, MLL-ENL immortalized committed myeloid progenitors (CMPs and GMPs) as well as KSL cells (data not shown) [40]. Following three rounds of replating in semisolid medium, we compared expression levels of Evi-1 and HoxA9 in MLL-ENL-immortalized cells derived from KSL cells, CMPs and GMPs. Notably, Evi-1 expression was significantly high in KSL-derived MLL-ENL-immortalized cells compared to that in CMP- and GMP-derived cells immortalized by MLL-ENL (Figure 5A). In contrast, HoxA9 was similarly up-regulated in the three populations (Figure 5A).

We next evaluated the effect of HoxA9/Meis1 on the expression of Evi-1 in the three hematopoietic populations noted above. HoxA9/Meis1 transformed KSL cells (data not shown), while CMPs and GMPs were not transformed in our experiments. Consistent with our results in myeloid progenitor transformation assay, KSL cells transformed by HoxA9/Meis1 exhibited low expression level of Evi-1 (Figure 5A). These data indicate that Evi-1 is not a transcriptional target of HoxA9 or Meis1 even in KSL cells.

To identify whether Evi-1 transcription in KSL cells is activated, or maintained by MLL-ENL, we first assessed immediate changes in Evi-1 expression level induced by MLL-ENL. We

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retrovirally transduced MLL-ENL into KSL cells and GMPs. Transduced cells were isolated forty-eight hours later, and the expression level of Evi-1 was assessed. Remarkably, Evi-1 expression in MLL-ENL-transduced KSL cells was significantly higher than that in freshly isolated KSL cells (Figure 5B). On the other hand, MLL-ENL-transduced GMPs showed low expression of Evi-1, compared to freshly isolated GMPs (Figure 5B).

To further examine the transcriptional regulation of Evi-1 in undifferentiated hematopoietic cells, we retrovirally transduced MLL-ENL into BM MNCs from 5-FU treated mice, in which HSCs were propagated. Thirty-six hours later, transduced cells were isolated from KSL population. Consistent with our results shown in Figure 5B, MLL-ENL-transduced KSL population cells exhibited significantly higher expression of Evi-1 than freshly isolated KSL cells (Figure 5C).

We also examined the transcriptional regulation of Evi-1 by MLL-AF9, because MLL-AF9 exhibited a transcriptional activity comparable with MLL-ENL on Evi-1 promoter (Figure 4A). Transduction analysis using undifferentiated hematopoietic cells clearly demonstrated that MLL-AF9 activates Evi-1 expression in the same manner as MLL-ENL (Figure 5B, 5C). Collectively, our results show that Evi-1 transcription is not only maintained, but also activated by MLL-ENL or MLL-AF9 exclusively in undifferentiated hematopoietic population like KSL cells.

Propagation of MLL-ENL-immortalized HSCs is highly dependent on Evi-1

Previous studies showed that Evi-1 is required for efficient propagation of MLL-ENL-immortalized BM cells [3]. However, it had not been assessed whether requirement of Evi-1 differs depending on the cellular origin that is immortalized by MLL-ENL. To address this issue, BM progenitors from Evi-1$^{+/-}$ and Evi-1$^{f/-}$ mice were sorted into KSL cells, CMPs, and GMPs. Then, they were transduced with MLL-ENL, and immortalized through myeloid transformation assay. First, using Evi-1$^{+/-}$ cells, we revealed that deletion of one Evi-1 allele had no significant impact on clonogenic

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activity of each hematopoietic population (Figure 5D). Next, using Evi-1$^{f/-}$ cells and Cre-GFP

retrovirus, we completely disrupted Evi-1 alleles in MLL-ENL-immortalized cells derived from the

defined populations (Supplemental Figure S2). Then, GFP or Cre-GFP-infected cells were sorted

and cultured for another round in semisolid medium, to compare the effects of Evi-1 deletion on the

clonogenic activity among populations. Notably, colony formation of MLL-ENL-immortalized cells

derived from KSL cells was most severely attenuated by disruption of Evi-1, compared to those

cells derived from CMPs or GMPs (Figure 5E). On subsequent replating of Evi-1 deleted cells,

however, we observed no significant difference in colony counts among populations (Supplemental

Figure S2). These results indicate that MLL-ENL-immortalized cells are heterogenous in regard to

the dependency on Evi-1 for proliferation, even if they are derived from KSL cells. The frequency of

Evi-1 dependent cells should be highest in HSCs-derived cells, and low in progenitors-derived cells.

Thus, if colony counts decrease immediately after Evi-1 deletion in HSCs-derived cells, the residual

cells, most of which are no longer dependent on Evi-1, would show almost the same clonogenic

activity as progenitors-derived cells in the next round.

HSC genes are enriched in EVI-1 high cases of MLL-rearranged leukemia

Based on the finding that up-regulation of Evi-1 in MLL fusion-transformed cells is related to their

origin, we hypothesized that the gene expression pattern in human cases of MLL-rearranged

leukemia would also reflect their origin. To address this issue, we extracted the gene expression

data of 13 MLL-rearranged AML patients by Valk et al. from GEO [14], and divided them into two

groups; Five EVI-1 high cases and eight EVI-1 low cases. We then applied GSEA to identify

functional gene sets (C2) correlated with EVI-1 expression, and found that two and 68 gene sets

were particularly enriched in the EVI-1 high and low groups, respectively (FDR<0.01; Gene sets

consisting of less than thirty genes were excluded; Supplemental Table S1). Of note, GSEA

revealed a strong correlation of genes up-regulated in EVI-1 high leukemias with the gene set that

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