Biochemistry and Biophysics Reports 38 (2024) 101667

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

The ETV6-MECOM fusion protein promotes EMT-related properties by repressing the transactivation activity of E-cadherin promoter in K562 leukemia cells

Qian Li [a,b], Furong Wang [b,c], Xuehong Zhang [d], Shuqing Liu [e,***], Ming-Zhong Sun [a,f,**], Jinsong Yan [b,c,*]

[a] Department of Biotechnology, College of Basic Medical Sciences, Dalian Medical University, Dalian, 116044, China
[b] Department of Hematology, Liaoning Medical Center for Hematopoietic Stem Cell Transplantation, Liaoning Key Laboratory of Hematopoietic Stem Cell Transplantation and Translational Medicine, Blood Stem Cell Transplantation Institute, Dalian Key Laboratory of Hematology, Diamond Bay Institute of Hematology, The Second Hospital of Dalian Medical University, Dalian, 116027, China
[c] Department of Pediatric, Pediatric Oncology and Hematology Center, The Second Hospital of Dalian Medical University, Dalian, 116027, China
[d] Center of Genome and Personalized Medicine, Institute of Cancer Stem Cell, Dalian Medical University, Liaoning, 116044, China
[e] Department of Biochemistry, College of Basic Medical Sciences, Dalian Medical University, Dalian, 116044, China
[f] Liaoning Key Laboratory of Cancer Stem Cell Research, Dalian Medical University, Dalian, 116044, China

ARTICLE INFO

Keywords:
ETV6-MECOM
E-cadherin promoter
EMT
Transactivation
Leukemia

ABSTRACT

The ETV6-MECOM fusion gene, produced by the rare and recurrent chromosomal translocation t(3; 12) (q26; p13), is associated with high mortality and short survival in myeloid leukemia. However, its function and underlying mechanisms in leukemia progression remain unknown. In this study, leukemia-stable K562 cells expressing the ETV6-MECOM fusion protein were used to investigate the effects of the ETV6-MECOM oncoprotein. K562-ETV6-MECOM cells were undifferentiated and had reduced colony formation, increased cell migration and invasion, and increased sphere number and diameter in a spheroid formation assay, presenting epithelial-to-mesenchymal transition (EMT) traits. The expression of E-cadherin, a hallmark of EMT, was significantly downregulated at the transcriptional and translational level in K562-ETV6-MECOM cells to explore the mechanistic basis of EMT. Stepwise truncation, DNA sequence deletion, mutation analysis for E-cadherin promoter transactivation, and a dual luciferase assay indicated that the regulatory region of ETV6-MECOM is located in the DNA motif $^{-1116}$ TTAAAA^{-1111} of E-cadherin promoter. Moreover, a chromatin immunoprecipitation assay showed that this oncoprotein binds to the DNA motif $^{-1116}$ TTAAAA^{-1111} with the anti-EVI1 antibody. Although ETV6-MECOM upregulated the expressions of EMT master regulators, including SNAIL, SLUG, ZEB2, and TWIST2, their knockdown had no effect on EMT-related properties. However, overexpression of E-cadherin eliminated EMT traits in the presence of the ETV6-MECOM oncoprotein. These data confirmed that the ETV6-MECOM oncoprotein, not SNAIL, SLUG, ZEB2, or TWIST2, plays a critical role in inducing EMT traits in leukemia K562 cells. ETV6-MECOM induces EMT-related properties by downregulating the transcriptional expression of E-cadherin and repressing its transactivation activity by binding to its core motif $^{-1116}$TTAAAA^{-1111} in leukemia K562 cells. These findings could contribute to the development of a therapeutic target for patients with myeloid leukemia characterized by ETV6-MECOM.

* Corresponding author. Department of Hematology, Liaoning Medical Center for Hematopoietic Stem Cell Transplantation, Liaoning Key Laboratory of Hematopoietic Stem Cell Transplantation and Translational Medicine, Blood Stem Cell Transplantation Institute, Dalian Key Laboratory of Hematology, Diamond Bay Institute of Hematology, the Second Hospital of Dalian Medical University, Dalian, 116027, China.
** Corresponding author. Department of Biotechnology, College of Basic Medical Sciences, Dalian Medical University, Dalian, 116044, China.
*** Corresponding author. Department of Biochemistry, College of Basic Medical Sciences, Dalian Medical University, Dalian, 116044, China.
E-mail addresses: lsqsmz@163.com (S. Liu), smzlsq@163.com (M.-Z. Sun), yanjsdmu@dmu.edu.cn (J. Yan).

https://doi.org/10.1016/j.bbrep.2024.101667
Received 8 December 2023; Received in revised form 1 February 2024; Accepted 14 February 2024

1. Introduction

Approximately 4% of patients with acute myeloid leukemia (AML) carry rearrangements of the myelodysplasia syndrome 1 (MDS1) and ecotropic viral integration site 1 (EVI1) complex locus (MECOM) [1]. AML that is characterized by MECOM rearrangement has poor outcomes and a poor response to chemotherapy, with a dismal median overall survival of <1 year after diagnosis [2]. The 5th edition of the World Health Organization (WHO) classification of hematolymphoid tumors recognizes AML with MECOM rearrangement as a distinct entity, regardless of blast counts [3]. MECOM rearrangement is driven by the classic inv(3)/t(3; 3)(q21; q26.2) or inv(3)/t(3; 3) and atypical 3q26-rearrangement, such as t(2; 3)(p15-22; q26), t(3; 4)(q26; p15), t(3; 8)(q26; p23), t(3; 11)(q26; q24), t(3; 12)(q26; p13), and t(3; 21)(q26; q22) [4,5]. Among these, the ETV6-MECOM fusion protein, an oncoprotein produced by t(3; 12)(q26; p13), is a rare but recurrent aberration in AML, myelodysplastic syndromes (MDS), and chronic myelogenous leukemia (CML) [4]. Recent studies have reported over 70 cases of MDS and myeloid leukemia that carried t(3; 12)(q26; p13). Among these, eight patients with CML carried both t(9; 22)(q34; q11) and t(3; 12)(q26; p13), producing the BCR-ABL1 fusion gene coexisting with the ETV6-MECOM fusion gene, resulted in blasts or accelerated phases and a dismal outcome [4,6]. The ETV6-MECOM oncoprotein is associated with refractory/relapsed disease, high mortality, and short OS, with a median survival of 6.3 months after the detection of t(3; 12) [4]. However, its functions and underlying mechanisms remain unclear.

MECOM is a transcriptional regulator and an oncoprotein associated with an aggressive phenotype in human leukemia. It encodes different isoforms, including EVI1with two zinc finger domains (ZFD) at the N- and C-terminus, and MDS1-EVI1 contains a PR domain and is otherwise identical to the EVI1 protein (Fig. S1A) [7]. EVI1 is indispensable for hematopoietic stemness, which triggers myeloid differentiation arrest, retains self-renewal, and enhances cell survival in hematopoietic progenitors. In addition, it induces epithelial-to-mesenchymal transition (EMT) in cancers, including leukemia. This results in tumorigenesis and invasion [8–10]. The hallmark of EMT is the downregulation of E-cadherin expression to reinforce the destabilization of adherent junctions [11]. E-cadherin expression is directly repressed by several EMT transcription factors (EMT-TFs), including SNAIL, ZEB, and TWIST family proteins [12]. Some EMT-TFs, such as ZEB1, ZEB2, SNAIL1, and SNAIL2, contribute to the development and pathogenesis of acute leukemia [13–15].

As ETV6-MECOM retains the entire functional domain of MECOM (MDS1-EVI1), we hypothesized that it may be involved in regulating EMT traits in hematological malignancies. Therefore, this study aimed to evaluate its effects on EMT traits in K562 cells. It provides a new therapeutic target for patients with leukemia harboring the ETV6-MECOM oncoprotein.

2. Materials and methods

2.1. Cell culture

Human myeloid leukemia K562 and human embryonic kidney 293T cells were purchased from the Chinese Academy of Sciences (Shanghai, China). K562 cells were transduced with lentivirus expressing pLVX-Puro-V5-APEX2-ETV6-MECOM (pLVX-ETV6-MECOM) or pLVX-Puro-V5-APEX2 (pLVX-vector) plasmid, and cultured in RPMI-1640 (Gibco, Grand Island, NY, USA) supplemented with 15% fetal bovine serum (FBS; ExCell Bio, Shanghai, China), with 1.5 μg/ml puromycin added to screen stable cell lines (K562-ETV6-MECOM). The 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% FBS. All cells were cultured in an incubator with 5% CO_2 at 37 °C.

2.2. RNA extraction, quantitative real-time PCR (qPCR), and Western blot analysis

RNA extraction, qPCR, and Western blot analysis were performed as described in the Supplemental file. The corresponding primer sequences and antibodies are shown in Tables S1 and S2, respectively.

2.3. Spheroid formation, colony formation, migration and invasion, and cell morphology assays

The protocols for the spheroid formation, colony formation, migration and invasion, and cell morphology assays were described in the supplemental file.

2.4. Plasmid construction

Different lengths of E-cadherin truncations were cloned into the pGL3.0-basic luciferase reporter gene plasmid and named pGL3-E-cadherin to investigate the transactivation activity of the E-cadherin promoter. The corresponding primers are listed in Table S3 in the Supplemental file. E-cadherin promoter truncations included −2100 to +150, −1200 to +150, −997 to +150, −672 to +150, −488 to +150, and −236 to +150. They were named pGL3-E-cadherin −2100, pGL3-E-cadherin −1200, pGL3-E-cadherin −997, pGL3-E-cadherin −672, pGL3-E-cadherin −488, and pGL3-E-cadherin −236, respectively (Fig. 4B). DNA bases in the pGL3-E-cadherin-1200 plasmid were point-mutated or deleted according to the manufacturer's instructions (Supplemental file). These included the mutations of $^{-1125}$AAGACTAGATTAAAA^{-1111}, $^{-1123}$GACTAGA^{-1117}, and $^{-1110}$AAT^{-1108} in $^{-1125}$TGGC GTCAGTC CGCA^{-1111} (pGL3-E-cadherin-1200-MUT), $^{-1123}$CCTGCCG^{-1117} (pGL3-E-cadherin-MUT-1123∼-1117), and $^{-1110}$CCG^{-1108} (pGL3-E-cadherin-MUT-1110∼-1108), respectively, and the deletion of $^{-1116}$TTA AAA^{-1111} (pGL3-E-cadherin-Δ-1116∼-1111) (Fig. 4F). DNA sequences of $^{-1116}$TTAAAA^{-1111} in the pGL3-E-cadherin-1200 plasmid were point-mutated to gTAAAA, TgAAAA, TTgAAA, TTAgAA, TTAAgA, and TTAAAg and named pGL3-E-cadherin-MUT-1116, pGL3-E-cadherin-1115, pGL3-E-cadherin-1114, pGL3-E-cadherin-1113, pGL3-E-cadherin-1112, and pGL3-E-cadherin-1111, respectively (Fig. 4H).

Full-length E-cadherin cDNA was cloned into the eukaryotic expression vector PCDH-EF1-MCS-T2A-Puro (PCDH vector) and named PCDH-E-cadherin. The PCR primer sequences for E-cadherin transcripts were shown in Table S4 in the Supplemental file. The plasmid construction protocols were described in the supplemental file.

2.5. Transient transfection

The PCDH-E-cadherin plasmid was transiently transfected into K562-ETV6-MECOM cells. Target sequences of the siRNAs used to separately knockdown the expressions of SNAIL, SLUG, ZEB2, and TWIST2 in K562-ETV6-MECOM cells were shown in Table S5. The relative transfection protocols were described in the supplemental file.

2.6. Luciferase reporter gene activity assay

Plasmids pLVX-ETV6-MECOM, pGL3-E-cadherin truncations or mutations, and simian virus 40 (SV40) plasmid as an internal control were co-transfected into 293T cells. Protocols for the dual-luciferase reporter assay (DLR) are described in the supplemental file.

2.7. Chromatin immunoprecipitation assay (ChIP)

Plasmids pLVX-ETV6-MECOM, pGL3-E-cadherin-1200 or pGL3-E-cadherin-Δ-1116∼-1111, and SV40 were co-transfected into 293T cells. ChIP was performed 48 h after transfection using an anti-EVI1 antibody

(#2593, Cell Signaling Technology, Danvers, MA, USA) according to the manufacturer's protocol (Supplemental file). The PCR primers for ChIP are listed in Table S6 in the Supplemental file.

2.8. Statistical analysis

Each experiment was performed at least triplicate. Data were analyzed using the GraphPad Prism software (Version 6.01, La Jolla, CA, USA, www.graphpad.com). Data are presented as the mean ± standard error of the mean (SEM). Statistical differences between experimental groups were calculated using Student's t-test or one-way analysis of variance. $P < 0.05$ was considered statistically significant.

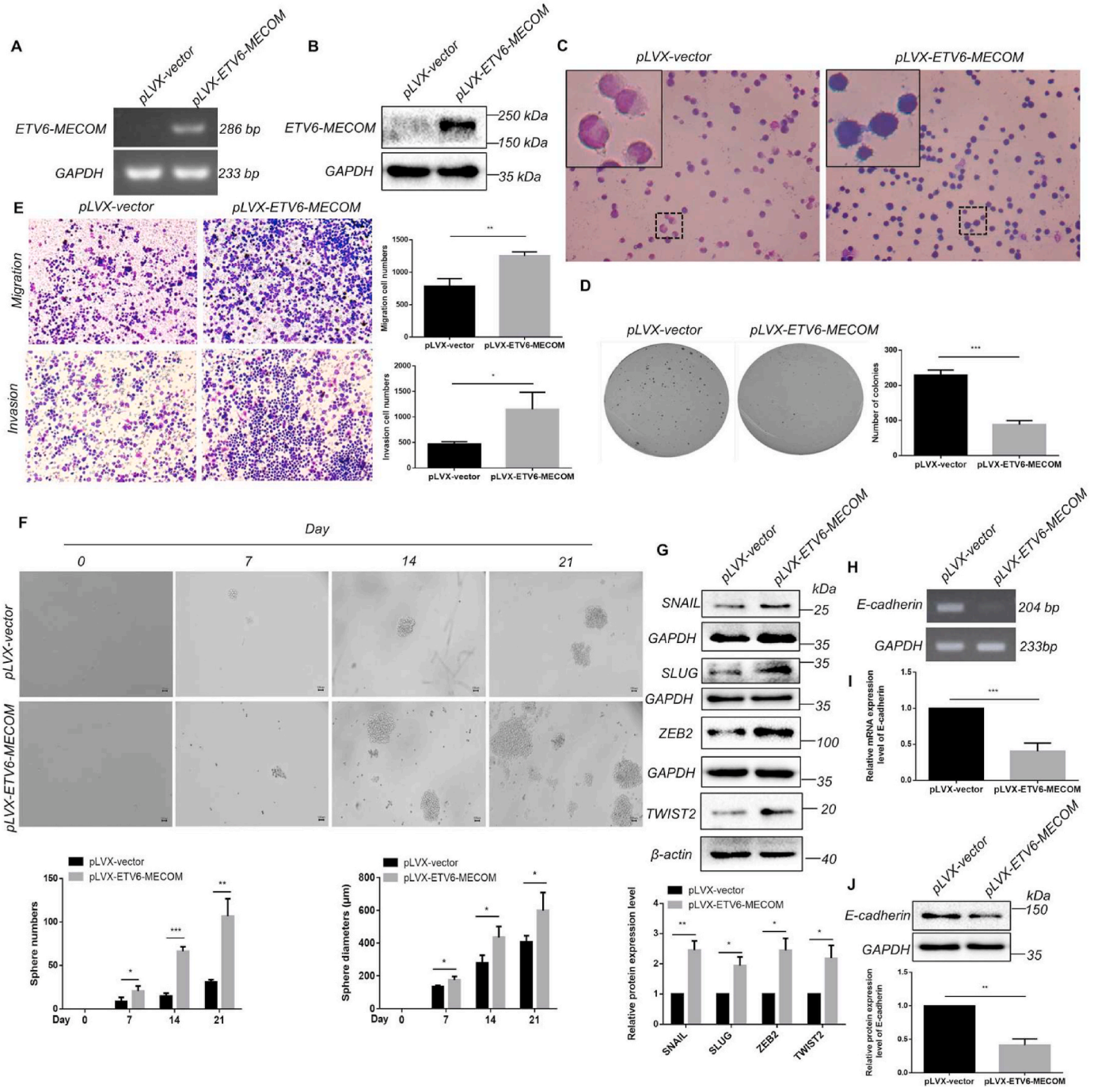

Fig. 1. ETV6-MECOM fusion protein induced EMT characteristics in K562 cells. RT-PCR **(A)** and Western blot **(B)** results of K562 cells stably transduced with the pLVX-ETV6-MECOM and the pLVX-vector lentivirus. Representative images showing inhibited cell differentiation (× 200 and × 400) **(C)**, reduced colony formation ability **(D)**, increased migration and invasion abilities (× 100) **(E)**, and increased self-renewal ability (× 100) **(F)** in K562-ETV6-MECOM cells. **(G)** Western blot results showing SNAIL, SLUG, ZEB2, and TWIST2 overexpression in K562-ETV6-MECOM cells. RT-PCR **(H)**, qPCR**(I),** and Western blot **(J)** results showing the downregulation of E-cadherin expression in K562-ETV6-MECOM cells. Data were presented as mean ± SEM. n = 3, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

3. Results

3.1. The ETV6-MECOM fusion protein induced stem cell-like properties and increased invasion in K562 cells

The ETV6-MECOM fusion gene encodes a 214 kDa fusion protein. Its ETV6-derived N-terminus contains no functional domain, whereas the C-terminal domain of MECOM (MDS1-EVI1) preserves the entire functional domain. A schematic of the structure of the ETV6-MECOM fusion protein is shown in Fig. S1B.

To determine the role of the ETV6-MECOM fusion protein in the regulation of EMT characteristics in leukemia, we established a stable cell line, namely K562-ETV6-MECOM. As shown in Fig. 1A–B, ETV6-MECOM expression remarkably increased at the transcriptional and translational levels. K562-ETV6-MECOM cells presented striking morphological changes, including a reduced cytoplasm-to-nucleus ratio, and round or elliptical nucleus with finer chromatin, with a deeply basophilic cytoplasm observed during staining. However, K562-pLVX-vector cells showed contrasting results (Fig. 1C). In addition, K562-ETV6-MECOM cells exhibited reduced colony numbers (Fig. 1D) and increased migration and invasion (Fig. 1E). The *in vitro* spheroid formation assay showed that sphere numbers and diameters were markedly increased in K562-ETV6-MECOM cells compared to those in K562-pLVX cells on Days 7, 14, and 21 (Fig. 1F). Furthermore, the ETV6-MECOM fusion protein enabled the K562 leukemia cells to exhibit EMT characteristics.

3.2. SNAIL, SLUG, ZEB2, and TWIST2 expression upregulated by the ETV6-MECOM oncoprotein did not induce EMT properties in K562-ETV6-MECOM cells

In the context of the ETV6-MECOM oncoprotein, K562-ETV6-MECOM stable cells presented with EMT characteristics. Therefore, EMT master regulators, namely EMT-TFs, were detected to determine the regulatory relationship between EMT-TFs and ETV6-MECOM. As shown in Fig. 1G and Fig. S2A–D, the ETV6-MECOM oncoprotein upregulated the expression of SNAIL, SLUG, ZEB2, and TWIST2 at the transcriptional and translational levels. However, the expression of SMUC, ZEB1, and TWIST1 remained stable in K562-ETV6-MECOM cells (Fig. S2A-D and F). Furthermore, E-cadherin was downregulated (Fig. 1H–J), N-cadherin was overexpressed, and Vimentin remained stable at the transcriptional and translational levels (Fig. S2A, E and G) in the presence of the ETV6-MECOM oncoprotein.

EMT master regulators generally downregulate the expression of E-cadherin, a hallmark of EMT, to induce EMT. In the present study, we investigated whether the upregulated SNAIL, SLUG, ZEB2, and TWIST2 expression can induce EMT-related properties in the context of the ETV6-MECOM oncoprotein in K562 stable cells. Therefore, SNAIL, SLUG, ZEB2, and TWIST2 were separately knocked down in K562-ETV6-MECOM cells and observed E-cadherin expression remained stable (Fig. 2A–D). Additionally, cell morphological features, migration and invasion abilities, and spheroid formation ability (Fig. S3) were similar to those in K562-ETV6-MECOM cells. Thus, EMT-related properties was not induced by the upregulation of SNAIL, SLUG, ZEB2, or TWIST2 expression in K562-ETV6-MECOM cells.

3.3. ETV6-MECOM downregulated E-cadherin expression induced EMT-related properties in K562 leukemia cells

In presence of the ETV6-MECOM oncoprotein, K562-ETV6-MECOM stable cells downregulated E-cadherin expression and EMT properties. However, E-cadherin overexpression in K562-ETV6-MECOM cells (Fig. 3A–C) eliminated their EMT-related features, including cell morphology changes (Fig. 3D), colony formation (Fig. 3E), migration and invasion (Fig. 3F), and spheroid formation ability (Fig. 3G). This suggests that this protein downregulated E-cadherin expression to induce EMT-related properties in K562 cells.

3.4. The ETV6-MECOM oncoprotein repressed E-cadherin transactivation by binding to its DNA sequence $^{-1116}TTAAAA^{-1111}$

The ETV6-MECOM oncoprotein downregulated E-cadherin expression at the transcriptional and translational levels. Here, the plasmids such as pGL3-E-cadherin-2100, pLVX-ETV6-MECOM, and SV40 were

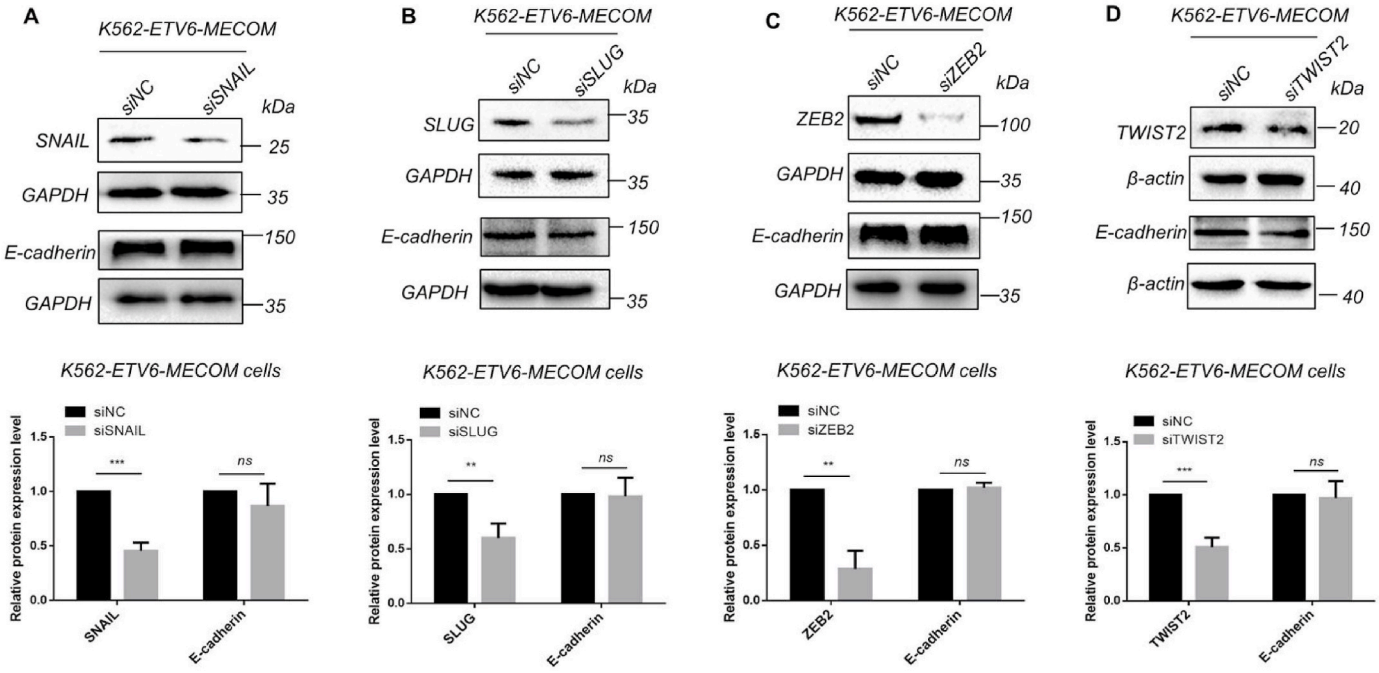

Fig. 2. Knockdown SNAIL, SLUG, ZEB2 or TWIST2 had no impact on E-cadherin in K562-ETV6-MECOM cells. Western blots demonstrating the detection of knockdown SNAIL **(A)**, SLUG **(B)**, ZEB2 **(C)**, or TWIST2 **(D)** by siRNAs in K562-ETV6-MECOM cells. Data were presented as mean ± SEM. n = 3, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns: not significant.

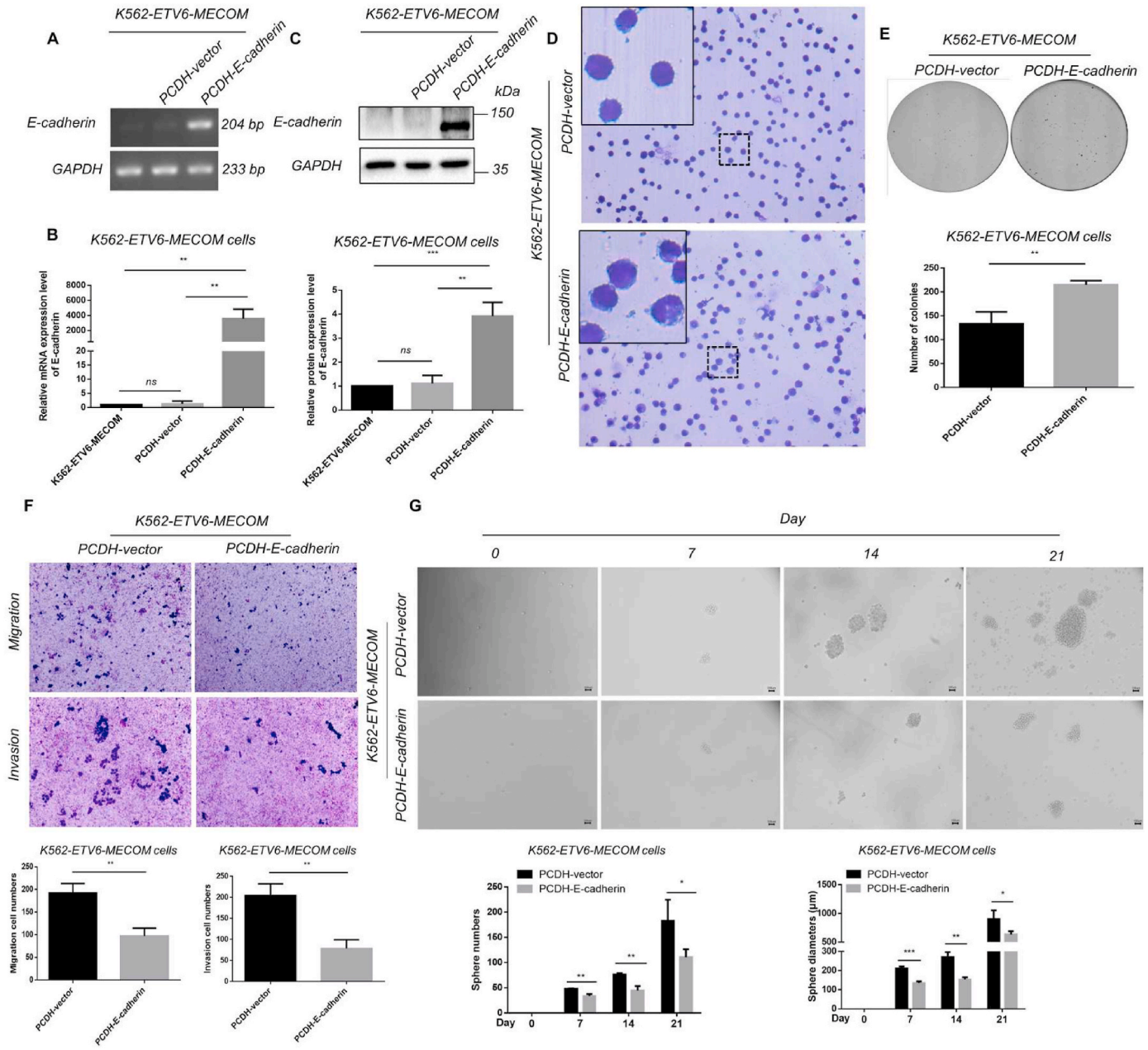

Fig. 3. ETV6-MECOM affected the EMT-related properties of K562 cells by downregulating E-cadherin expression. RT-PCR **(A)**, qPCR **(B)** and Western blot **(C)** results showing E-cadherin expression levels in K562-ETV6-MECOM cells. Representative images showing promoted cell differentiation (× 200 and × 400) **(D)** and colony formation **(E)**, reduced migration and invasion (× 100) **(F)**, and self-renewal abilities (× 100) **(G)** in K562-ETV6-MECOM cells after E-cadherin overexpression. Data were presented as mean ± SEM. n = 3. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns: not significant.

transiently co-transfected into 293T cells. The dual-luciferase activity significantly decreased, suggesting that ETV6-MECOM inhibited the transactivation of the E-cadherin promoter (Fig. 4A).

Using stepwise truncation analysis for E-cadherin transactivation, dual-luciferase activity revealed that the regulatory sequence was located at −1200 to −997 of E-cadherin from the transcription start site (Fig. 4B–C). Bioinformatic analysis (https://jaspar.genereg.net/) was further utilized to predict the putative DNA motif in the E-cadherin promoter. The 5′-AAGACTAGATTAAAA-3′ sequence was identified as a putative responsive DNA element regulated by ETV6-MECOM (Fig. 4D). Replacing the wild-type 5′-AAGACTAGATTAAAA-3′ sequence with the mutated 5′-TGGCGTCAGTCCGCA-3′ sequence eliminated dual-luciferase activity (Fig. 4E), indicating that 5′-AAGACTAGATTAAAA-3′ was the regulatory region of the ETV6-MECOM oncoprotein.

To precisely identify the core motif within the putative sequence $^{-1125}$AAGACTAGATTAAAA^{-1111}, we mutated $^{-1123}$GACTAGA^{-1117} into $^{-1123}$CCTGCCG^{-1117} (MUT-1123∼-1117), deleted $^{-1116}$TTAAAA^{-1111} (Δ-1116∼-1111), and mutated the neighbor region $^{-1110}$AAT^{-1108} into $^{-1110}$CCG^{-1108} (MUT-1110∼-1108) (Fig. 4F). Here, $^{-1116}$TTAAAA^{-1111} was the core DNA element that regulated the luciferase activity of the E-cadherin promoter by ETV6-MECOM (Fig. 4G). As shown in Fig. 4H, the DNA sequence $^{-1116}$TTAAAA^{-1111} in pGL3-E-cadherin-1200 was separately point-mutated into a series of mutants containing MUT-1116 (T > G), MUT-1115 (T > G), MUT-1114 (A > G), MUT-1113 (A > G), MUT-1112 (A > G), and MUT-1111 (A > G). These pGL3-E-cadherin mutants had increased luciferase activity (Fig. 4I). This indicated that each DNA base, particularly the bases $^{-1112}$A and A^{-1111} in $^{-1116}$TTAAAA^{-1111}, played a key role in repressing E-cadherin transactivation.

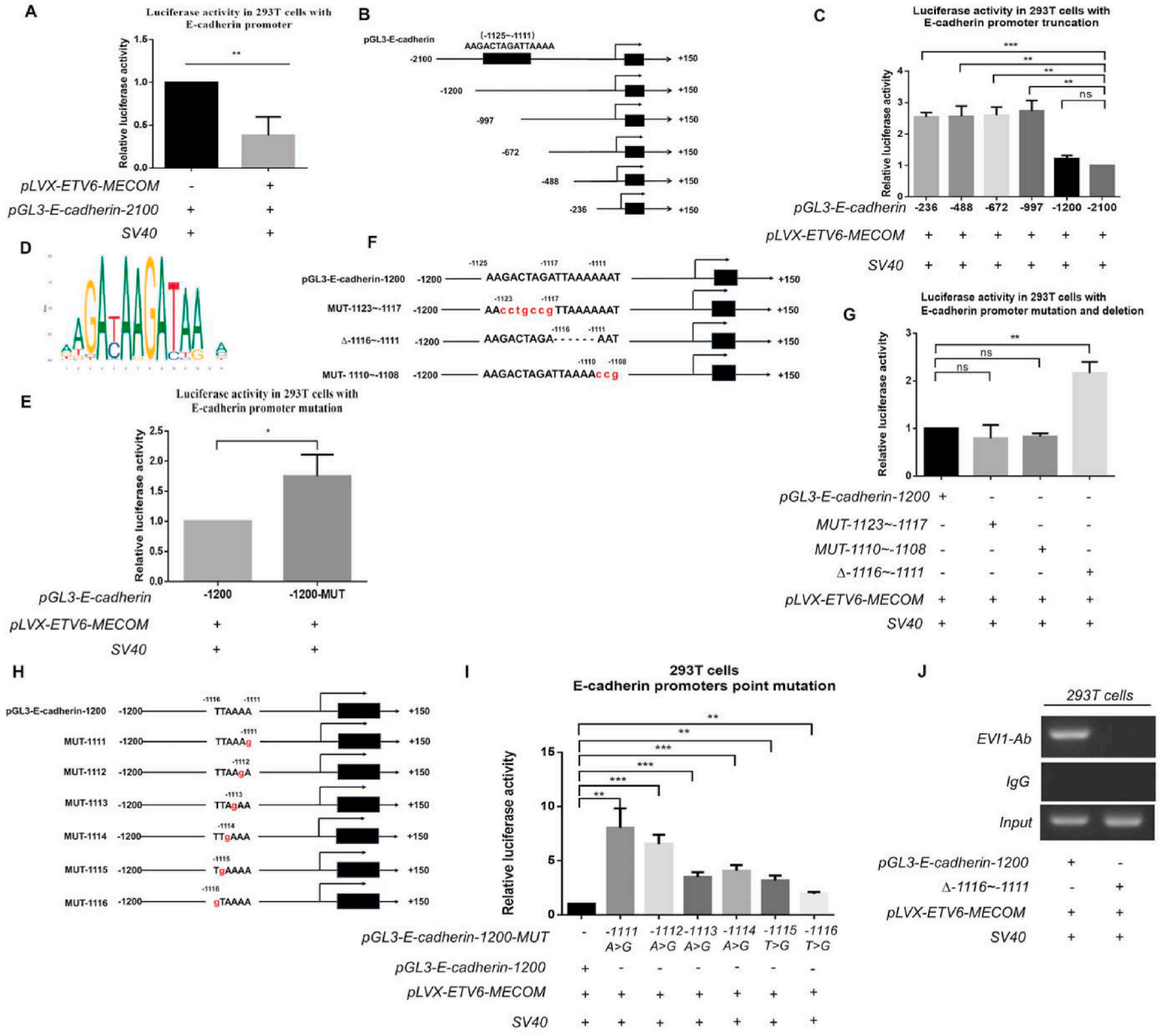

Fig. 4. ETV6-MECOM binds to $^{-1116}$TTAAAA^{-1111} in the E-cadherin promoter. (A) ETV6-MECOM inhibited the transactivation of E-cadherin promoter. (B) A diagram showing pGL3-E-cadherin truncations (−2100, −1200, −997, −672, −488 and −236). (C) Luciferase activity assay results showing that ETV6-MECOM regulated E-cadherin at −1200 to −997. (D) Bioinformatic analysis predicting putative binding sequences in the E-cadherin promoter. (E) Luciferase activity assay results showing the putative sequence 5′-AAGACTAGATTAAAA-3′ as the regulatory region of the ETV6-MECOM oncoprotein. (F) A diagram showing pGL3-E-cadherin-1200 mutations (MUT-1123 to −1117 and MUT-1110 to −1108) and deletion (Δ-1116 to −1111). (G) Luciferase assay results showing that only the Δ-1116 to −1111 plasmid inactivated the E-cadherin promoter. (H) A diagram showing pGL3-E-cadherin-1200 point-mutations (MUT-1116[T > G], MUT-1115 [T > G], MUT-1114 [A > G], MUT-1113 [A > G], MUT-1112 [A > G], and MUT-1111 [A > G]). (I) Luciferase assay results showing that each nucleotide responded to the transcriptional activation. (J) ChIP results showing interaction of ETV6-MECOM with different E-cadherin promoters. Data were presented as mean ± SEM. n = 3. *P < 0.05, **P < 0.01, ***P < 0.001 and ns: not significant.

The plasmids pGL3-E-cadherin-1200 or pGL3-E-cadherin-Δ-1116~-1111, pLVX-ETV6-MECOM, and SV40 were co-transfected into 293T cells. In addition, ChIP was performed with the anti-EVI1 antibody. The PCR band in agarose gel electrophoresis was positive in 293T cells with pGL3-E-cadherin-1200, but negative in pGL3-E-cadherin-Δ-1116~-1111. This indicated that ETV6-MECOM bound to the DNA motif $^{-1116}$TTAAAA^{-1111} of E-cadherin (Fig. 4J) to repress its transactivation, resulting in the downregulation of E-cadherin expression at the transcriptional level.

4. Discussion

The ETV6-MECOM fusion gene is associated with disease progression and poor prognosis, with a median survival time of 6.3 months [4]. However, the function of the ETV6-MECOM oncoprotein in leukemia remains unknown. In the present study, the ETV6-MECOM fusion protein remarkably induced EMT properties, including leukemic stemness-like properties. It further increased migration and invasion abilities through transcriptional repression of E-cadherin by binding to the core motif $^{-1116}$TTAAAA^{-1111} of this promoter. The unique transcriptional regulatory pathways identified in this study may contribute

to the development of novel targeted therapies for ETV6-MECOM rearrangements.

Aberrant EMT reactivation is emerging as an important phenomenon in leukemia biology. However, the contribution of key regulators and oncoproteins to hematopoietic malignancies remains unclear. EMT confers motility, stem cell-like properties, and therapeutic resistance to various cancerous tissues during cancer progression and metastasis [16]. Our data show that the ETV6-MECOM fusion protein is functionally relevant for leukemia, resulting in obvious morphological changes and promoting the migration and invasion of K562 cells. The abnormal phenotypes caused by ETV6-MECOM indicated that this oncoprotein induces EMT traits. Our findings suggest that this protein reprogrammed K562 cells into an undifferentiated morphological state. Cancer stem cells (CSCs), defined by self-renewal, dedifferentiation, and tumorigenicity, often exhibit slowed proliferation and can re-enter rapid proliferation in a new tumor microenvironment during EMT [17,18]. Consistent with these findings, spheroid formation assays demonstrated that K562-ETV6-MECOM cells possess self-renewal ability and cell lineage multipotency in the present study.

SNAIL1/2/3, ZEB1/2, and TWIST1/2 primarily modulate the transcriptional pathway that drives EMT by regulating motility and stemness [19]. In current work, the expression of ETV6-MECOM oncoprotein in K562 cells downregulated E-cadherin, upregulated N-cadherin, SNAIL, SLUG, ZEB2 and TWIST2, did not affect Vimentin. Accumulating evidence has implicated these EMT-TFs in regulating the malignancy of leukemia cells [20]. For instance, increased SNAIL expression contributes to impaired differentiation, enhanced self-renewal, and proliferation of immature myeloid cells [14]. The absence of SLUG impairs the self-renewal ability of leukemia stem cells and delays leukemia progression [15]. ZEB2 affects the differentiation and proliferation of leukemia cells [21]. Similarly, TWIST2 regulates the differentiation of bone marrow cells and inhibits the proliferation of granulocyte-macrophage progenitor cells [22]. These reports suggest that master EMT-TFs play crucial roles in the occurrence and progression of leukemia. However, the downregulation of SNAIL, SLUG, ZEB2, or TWIST2 did not affect the regulatory role of ETV6-MECOM in the EMT-related properties of leukemia cells in the present study. This indicated that EMT-TFs are not key regulators of EMT traits in leukemia when ETV6-MECOM is present, which is markedly different from their classical regulatory role in the development of EMT in cancers.

E-cadherin, a hallmark of EMT, is generally regulated by EMT-TFs such as SNAIL, SLUG, ZEB2, and TWIST2. These EMT-TFs can independently bind to E-box response elements in the E-cadherin promoter to repress its expression [23–26]. Our results showed that ETV6-MECOM inhibits E-cadherin expression. We found no obvious changes in E-cadherin expression when SNAIL, SLUG, ZEB2, or TWIST2 expression was downregulated in the presence of ETV6-MECOM. Lu et al. [27] found that EVI1, SNAIL, and HDAC1 formed a co-repressor complex to repress E-cadherin expression and ultimately contributed to EMT and CSC properties in nasopharyngeal carcinoma cells. In colon cancer, EVI1 inhibited EMT by directly inhibiting to the transcriptional activity of SLUG and the downregulation of MECOM expression decreased the expression of E-cadherin [10]. In AML, EVI1 regulated the proliferation and invasion of AML via MS4A3-mediated TGFβ/EMT signaling [9]. These findings indicated that ETV6-MECOM and EVI1 have different regulatory mechanisms, which may lead to different therapeutic strategies. The downregulation of E-cadherin expression served as a fundamental event of EMT, which occurs frequently during tumor metastasis [28]. In this study, E-cadherin overexpression confirmed that EMT traits were inhibited in K562-ETV6-MECOM cells. These findings suggest that the ETV6-MECOM fusion protein directly regulates E-cadherin expression and the EMT traits in K562 leukemia cells.

The dual-luciferase assay showed that ETV6-MECOM directly inhibits the transactivation of the E-cadherin promoter. We further identified the putative sequence $^{-1125}$AAGACTAGATTAAAA^{-1111} as the binding site of ETV6-MECOM in E-cadherin. EVI1 regulates gene transcription by recognizing and binding to GATA-like motifs [29]. The ETV6-MECOM fusion protein contains the entire functional domains of EVI1. In addition, the sequence $^{-1125}$AAGACTAGATTAAAA^{-1111} in E-cadherin contains the motif $^{-1123}$GACTAGA^{-1117}, which is highly homologous to the GATA-like motif. However, ETV6-MECOM fusion did not recognize the GATA-like motif similar to EVI1. Therefore, the sequence $^{-1116}$TTAAAA^{-1111} in the E-cadherin promoter was identified as the core binding site of ETV6-MECOM, and every nucleotide was identified as crucial for E-cadherin promoter transactivation. Furthermore, ChIP confirmed that the ETV6-MECOM oncoprotein binds to the sequence $^{-1116}$TTAAAA^{-1111} in E-cadherin to downregulate its expression. However, further research is needed to fully elucidate why transcriptional regulation patterns differ between ETV6-MECOM and EVI1 even though they have the same terminal sequences.

To our knowledge, our study is the first to explore the function and mechanism of the ETV6-MECOM fusion protein in the development of EMT-related properties of myeloid leukemia cells. Moreover, isolating the primary cells endogenously ETV6-MECOM rearrangement from leukemia patients and comparing the real situation with that of the enforced ETV6-MECOM expression in cells, and even making them into immortalized cells will be in a great help in the research field. E-cadherin serves as a downstream target and may be beneficial in determining available therapeutic options for patients with leukemia characterized by ETV6-MECOM rearrangement and improving the patient outcomes.

Funding

This study was supported by Liaoning province governmental guidance fund for local development of science and technology (Grant number. 2023JH6/100100019).

CRediT authorship contribution statement

Qian Li: Writing – original draft, Methodology, Data curation. **Furong Wang:** Resources, Methodology. **Xuehong Zhang:** Formal analysis, Data curation. **Shuqing Liu:** Writing – original draft, Validation, Methodology. **Ming-Zhong Sun:** Supervision, Methodology, Investigation. **Jinsong Yan:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

Acknowledgement statement

We thank Yinggang Zhang for helping to establish the K562-ETV6-MECOM cell lines. Thanks laboratory colleagues for helping in research skills.

Abbreviations:

AML,	acute myeloid leukemia
MDS1	myelodysplasia syndrome 1
EVI1	ecotropic viral integration site 1
MECOM	MDS1 and EVI1 complex locus
MDS	myelodysplastic syndromes
CML,	chronic myelogenous leukemia
ZFD	zinc finger domains
EMT	epithelial-to-mesenchymal transition
FBS	fetal bovine serum
DMEM	Dulbecco's modified Eagle's medium

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101667.

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