Biochemical and Biophysical Research Communications 416 (2011) 239–245

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Angiopoietin1 contributes to the maintenance of cell quiescence in EVI1^{high} leukemia cells

Emi Ichihara, Kazuko Kaneda, Yusuke Saito, Norio Yamakawa, Kazuhiro Morishita *

Division of Tumor and Cellular Biochemistry, Department of Medical Sciences, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

ARTICLE INFO

Article history:
Received 8 October 2011
Available online 18 October 2011

Keywords:
EVI1
AML
Ang1
p18
Cell cycle

ABSTRACT

Ecotropic viral integration site-1 (EVI1) is an oncogenic transcription factor in human acute myeloid leukemia (AML) associated with poor prognosis. Because the drug-resistance of leukemia cells is partly dependent on cell quiescence in the bone marrow niche, EVI1 may be involved in cell cycle regulation in leukemia cells. As a candidate regulator of the cell cycle in leukemia cells with high EVI1 expression (EVI1^{high}), we analyzed angiopoietin1 (Ang1), which is a down-regulated gene in EVI1-deficient mice and is involved in the quiescence of hematopoietic stem cells. The results of real-time PCR analyses showed that Ang1 is highly expressed in leukemia cell lines and primary AML cells with EVI1^{high} expression. Introduction of shRNA against EVI1 into EVI1^{high} leukemia cells down-regulated Ang1 expression. Moreover, knockdown of Ang1 in EVI1^{high} leukemia cells promoted cell cycle progression and down-regulated the CDK inhibitor p18 (INK4c). Treatment with a decoy Tie2/Fc protein also down-regulated the expression of p18. These results suggest that Ang1/Tie2 signaling may suppress cell cycle progression via maintenance of G0/G1 phase through up-regulation of p18 expression. This mechanism may help to maintain EVI1^{high} leukemia cells in the bone marrow niche and promote resistance to anti-cancer drugs.

1. Introduction

The murine ecotropic viral integration site-1 (EVI1) gene was isolated from a common site of retroviral insertion in AKXD murine myelogenous leukemias [1,2]. The homologous human gene EVI1 is located on chromosome 3q26, and chromosomal abnormalities at 3q26 lead to aberrant expression of EVI1 in myeloid malignancies, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and blastic crisis of chronic myeloid leukemia (CML) [3,4]. AML with high expression of EVI1 (EVI1^{high}) accounts for approximately 8–10% of all cases of AML, and it exhibits a poor prognosis because of resistance to chemotherapy [5–7]. The gene expression profiles of EVI1^{high} AML patients are quite similar to those of control CD34^{+} cells [6], and furthermore, analysis of EVI1-deficient mice has shown that EVI1 is required for the maintenance of hematopoietic stem cells (HSCs), suggesting that EVI1^{high} leukemia cells may have stem cell-like phenotypes.

We and other groups have shown that EVI1 is predominantly expressed both in embryonic HSCs and HSCs in adult bone marrow [8–10]. EVI1 maintains the self-renewal capacity of embryonic HSCs by activating Gata2 transcription [11], and ablation of EVI1 in adult bone marrow also leads to a significant decrease in HSCs [12]. Taken together, these results suggest that EVI1 is indispensable for HSC maintenance and that EVI1 may also play an important role in the maintenance of cell quiescence as stem cell-like phenotypes in leukemia cells, thereby contributing to their chemoresistance.

In this manuscript, we focused on angiopoietin1 (Ang1) as a candidate gene whose high expression may be involved in the maintenance of cell quiescence in EVI1^{high} leukemia cells. Ang1 was identified as a ligand of the tyrosine kinase receptor Tie2 [13,14], and it belongs to the angiopoietin family, which also includes Ang2, Ang3, and Ang4. Ang1 and Ang4 activate the Tie2 receptor as agonists, whereas Ang2 and Ang3 act as antagonists [15,16]. Interactions between Ang1 and the receptor tyrosine kinase Tie2 promote HSC quiescence and are important for the maintenance of long-term repopulation in vivo [17,18]. In our previous study, Ang1 expression was found to be down-regulated in embryonic HSCs from EVI1-deficient mice, as was Gata2 [11]. Therefore, we hypothesized that the expression of Ang1 in EVI1^{high} leukemia cells might be regulated by EVI1 and that Ang1 would promote cell quiescence in EVI1^{high} leukemia cells.

In this study, we initially examined the expression of Ang1 in leukemia cells and found that Ang1 was strongly expressed in leukemia cell lines and primary AML cells with EVI1^{high} expression.

* Corresponding author. Address: Division of Tumor and Cellular Biochemistry, Department of Medical Sciences, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki, Japan. Fax: +81 985 85 2401.
E-mail address: kmorishi@medmiyazaki-u.ac.jp (K. Morishita).

0006-291X/$ - see front matter © 2011 Published by Elsevier Inc.
doi:10.1016/j.bbrc.2011.10.061

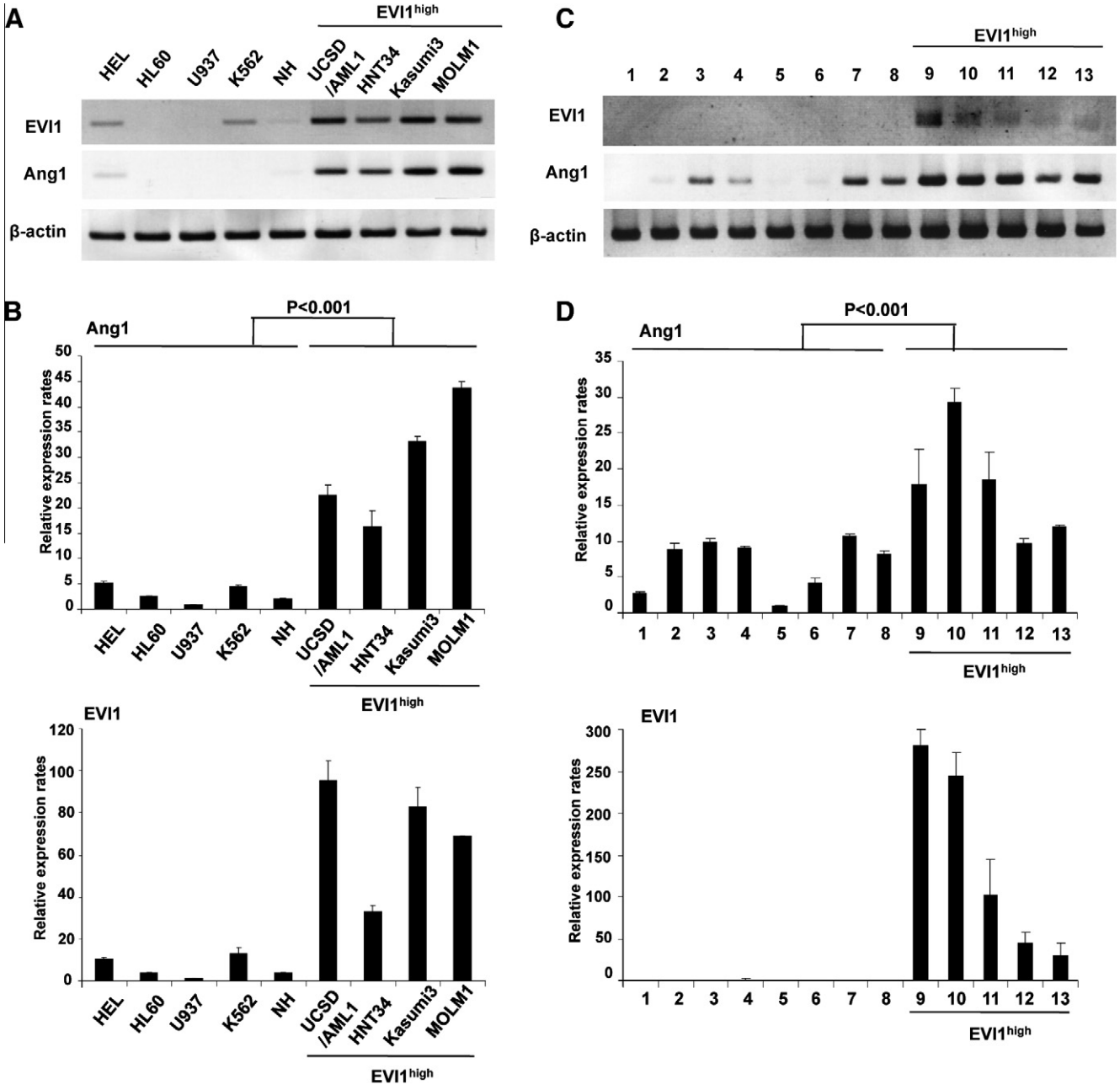

Fig. 1. Ang1 is highly expressed in EVI1^{high} leukemia cells. (A) Semi-quantitative reverse-transcription PCR (RT-PCR) analysis of EVI1 and Ang1 in various types of human leukemia cell lines. (B) Quantitative real-time RT-PCR analysis of EVI1 and Ang1 in leukemia cell lines. The data are presented as relative fold changes compared with the expression level in U937 cells. The data are expressed as the mean ± standard deviation (s.d.). Student's *t*-test was used for statistical analysis. (C) Semi-quantitative RT-PCR analysis of EVI1 and Ang1 in AML patient samples. (D) Quantitative real-time RT-PCR analysis of EVI1 and Ang1 in AML patient samples. The data are presented as relative fold changes compared with the expression in sample No. 5. The data are expressed as the mean ± s.d. Student's *t*-test was used for statistical analysis.

Introduction of shRNA targeting EVI1 into EVI1^{high} leukemia cells resulted in down-regulation of Ang1 expression. Ang1-knockdown experiments revealed that Ang1 suppresses cell cycle progression via maintenance of G0/G1 phase in EVI1^{high} leukemia cells. Moreover, knockdown of Ang1 expression and treatment with a Tie2 inhibitor (chimeric Tie2/Fc protein) down-regulated CDK inhibitor p18 (INK4c) expression. Therefore, Ang1/Tie2 signaling may contribute to cell quiescence through up-regulation of p18 expression in EVI1^{high} leukemia cells. This may enhance the maintenance of EVI1^{high} leukemia cells with resistance to chemotherapy in the bone marrow niche.

2. Materials and methods

2.1. Cell lines

UCSD/AML1 [19,20], HNT34 [21], and Kasumi-3 [22] cells were cultured in RPMI 1640 (Wako, Osaka, Japan) supplemented with 10% fetal calf serum (FCS) and 1 ng/mL human granulocyte–macrophage colony stimulating factor (hGM-CSF). HEL [23], HL60 [24], U937 [25], K562 [26], NH and MOLM1 [27] cells were cultured in RPMI 1640 supplemented with 10% FCS. 293T cells were cultured in DMEM (Wako) supplemented with 10% FCS. Detailed information