Ecotropic viral integration site 1 regulates EGFR transcription in glioblastoma cells

Asako Mizuguchi1 · Shinji Yamashita1 · Kiyotaka Yokogami1 · Kazuhiro Morishita2 · Hideo Takeshima1

Received: 11 August 2019 / Accepted: 3 October 2019 / Published online: 15 October 2019
© The Author(s) 2019

Abstract
Purpose Ecotropic viral integration site-1 (EVI1) is a transcription factor that contributes to the unfavorable prognosis of leukemia, some epithelial cancers, and glial tumors. However, the biological function of EVI1 in glioblastoma multiforme (GBM) remains unclear. Based on microarray experiments, EVI1 has been reported to regulate epidermal growth factor receptor (EGFR) transcription. Signal transduction via EGFR plays an essential role in glioblastoma. Therefore, we performed this study to clarify the importance of EVI1 in GBM by focusing on the regulatory mechanism between EVI1 and EGFR transcription.

Methods We performed immunohistochemical staining and analyzed the EVI1-expression in glioma tissue. To determine the relationship between EVI1 and EGFR, we induced siRNA-mediated knockdown of EVI1 in GBM cell lines. To investigate the region that was essential for the EVI1 regulation of EGFR transcription, we conducted promoter reporter assays. We performed WST-8 assay to investigate whether EVI1 affected on the proliferation of GBM cells or not.

Results It was observed that 22% of GBM tissues had over 33% of tumor cells expressing EVI1, whereas no lower-grade glioma tissue had over 33% by immunohistochemistry. In A172 and YKG1 cells, the expression levels of EGFR and EVI1 correlated. Analysis of the EGFR promoter region revealed that the EGFR promoter (from −377 to −266 bp) was essential for the EVI1 regulation of EGFR expression. We showed that EVI1 influenced the proliferation of A172 and YKG1 cells.

Conclusion This is the first study reporting the regulation of EGFR transcription by EVI1 in GBM cells.

Keywords Ecotropic viral integration site-1 · Epidermal growth factor receptor · Glioblastoma · Transcription factor · Tyrosine kinase receptor

Introduction
In 1988, ecotropic viral integration site-1 (EVI1) was originally identified as a common site of retroviral integration in murine myeloid tumors [1, 2]. The human EVI1 is located on chromosome 3q26 and produces a transcription factor that contains two DNA-binding zinc finger domains: one binds to a GATA-like consensus motif and the other binds to a v-ets erythroblastosis virus E26 oncogene homolog (ETS)-like motif [3, 4]. While regulating target gene expression, EVI1 interacts with transcription coregulators such as the C-terminal binding domain (CtBP), cAMP-responsive element-binding protein-binding protein (CBP), and p300/CBP-associated factor (P/CAF) [5]. EVI1 represses transforming growth factor-beta (TGF-β) signaling and activates PI3K/Akt/mTOR signaling [6, 7]. Elevated EVI1 expression is an unfavorable prognostic factor in human acute myeloid leukemia and some solid cancers [8–12].

EVI1 is closely related to embryonic neural development. In one study, the EVI1 homozygous mutant mouse embryos that died at 10.5 day post-coitus had a defect in the cranial ganglia and developed failure of the peripheral nervous system [13]. EVI1 is related to the Notch signaling pathway, which is important for cell-fate specification in a developing...
mammalian nervous system [5, 6]. This evidence showed that EVI1 is a key molecule in neurogenesis. Further, EVI1 has been reported to contribute to worsening of glial tumors [7, 8], suggesting that it might have an oncogenic role in glioma genesis. However, studies aiming to investigate EVI1 function in glioma have been few.

Recently, through cDNA microarray Gene Chips experiments, Chapeau et al. revealed that EVI1 regulated 621 cancer-associated genes in Hela and SKOV3 cells [9]. Among these EVI1 target genes, we were interested in epidermal growth factor receptor (EGFR). Signal transduction via the tyrosine kinase receptor EGFR contributes to cell proliferation, apoptosis inhibition, and angiogenesis in cancer cells. EGFR is the most important downstream target gene of the Notch signaling pathway [10, 11]. Since amplification and/or mutations in EGFR represent a genetic abnormality in primary glioblastoma multiforme (GBM), EGFR has been regarded as a pivotal target for GBM studies. Therefore, we presumed that EVI1 might have a significant role in GBM by regulating EGFR gene expression.

Materials and methods

Microarray data

We downloaded the four GEO series (GSE2223, GSE4271, GSE23806, and GSE43378) from the GEO database in NCBI (https://www.ncbi.nlm.nih.gov/geo/). Here we chose 29 GBM samples, except gliosarcoma, from GSE2223. In the same way, we chose 76 GBM samples from GSE4271, chose 32 conventional GBM cell line samples from GSE23806 and chose 32 GBM samples from GSE43378. We used pairs of probe-sets of MECOM and EGFR to evaluate the corresponding expression levels, demonstrated by scatter plot. (Supplementary Fig. 1).

Sample collection

This study was approved by the Ethics Committee of the University of Miyazaki Hospital, Miyazaki, Japan. All patients and their families gave informed consent for the use of the resected tissues. In this study, we included patients who had infiltrative glioma with histological grades II and III (lower-grade glioma, n = 27; 15 men and 12 women; median age, 41 years) or grade IV (GBM, n = 37; 27 men and 10 women; median age, 67 years). We used eight normal brain tissues as normal controls. Two or more pathologists examined all tumor tissue sections according to the WHO criteria. The tissue samples were obtained from tumors resected at the Department of Neurosurgery, University of Miyazaki Hospital between May 2014 and January 2017.

Immunohistochemical staining and evaluation of the paraffin-embedded tissues

To evaluate the expression of EVI1 using immunohistochemistry, we used the diluted EVI1 antibody (1:500, Cell Signaling Technology, #2593). To evaluate the expression of EGFR using immunohistochemistry, we used the diluted EGFR antibody (1:200, Leica microsystems, NCL-EGFR). The primary antibody was detected using the Dako EnVision™ + system—HRP Labeled Polymer (anti-rabbit) (Dako, K4002) and the Dako EnVision™ + system—HRP Labeled Polymer (anti-mouse) (Dako, K4000). The slides were counterstained using Meyer’s hematoxylin. Without knowledge on the clinical information, we photographed three regions of high cellularity in each tissue slide using a BZ-9000 microscope (Keyence, Osaka, Japan). The numbers of both EVI1-positive cells and cells in total were calculated using calculating using BZ-II Analyzer version 1.42 and BZ-II Dynamic Cell Count version 1.01 (Keyence). The mean values were defined as the EVI1-positive cell rate. The scores of the EVI1-positive tumor cells were defined as (−) if less than 5% (+) if 5–33% (++) if 33–50%, and (+++) if more than 50%. The threshold values were defined based on those described in the previous study by Hou et al. [8]

PCR and real-time quantitative PCR

Total RNA was isolated using the RNasy Mini Kit, according to the manufacturer’s instructions (Qiagen, No.74104). Total RNA in each sample was reverse transcribed using the Super Script VILO cDNA synthesis kit (Invitrogen, 11754-050). Quantitative PCR was performed on the StepOnePlus (Applied biosystems) using THUNDERBIRD SYBR qPCR Mix (TOYOBO, QPS-201). The primers used in this study are as described in Supplementary Fig. 2.

EVI1 RNA interference

The final RNA concentration was 16.6 nM. The siRNA sequences targeting human EVI1 were as described in Supplementary Fig. 2 [12]. The universal negative control siRNA was purchased from NIPPON GENE (NIPPON GENE CO. Ltd. Toyama, Japan).

Western blotting

We used the primary antibody (EGFR, Cell Signaling Technology #4267_1:1000 and b-Actin, SIGMA-ALDRICH_A5441_1:5000). The secondary antibody was
applied and the signal was visualized on ImageQuant LAS 4000 (GE Healthcare) using the Lumi-Light plus Western Blotting Substrate (Roche, 12015196001).

Constructs

All constructs that we used in this study are described in details in Electrical Supplementary Material 1.

 Luciferase assay

We performed the dual luciferase assays according to the manufacturer’s instructions (Promega, E1910).

 Cell proliferation assay

Cell proliferation assays were performed with a Cell Counting Kit-8 (Dojindo, Japan) according to the manufacturer’s protocol.

 Statistical analysis

The correlations between the expression levels of EVI1 and EGFR (GSE2223, GSE4271, GSE23806, and GSE43378) were calculated using Spearman’s rank correlation coefficient (Supplementary Fig. 1). The difference in the survival curves was analyzed by the log-rank test (Fig. 1c). We compared the EVI1-mRNA levels of the target genes between the GBM cell lines and the normal brain tissues using Mann–Whitney U-test (Fig. 2a). All statistical analyses were performed using the Student’s t test (Figs. 2, 3, 4). We used EasyR software for statistical analysis. P values <0.05 were considered significant.

 Results

Positive correlation between the expression levels of EVI1 and EGFR in GBM

To investigate whether EVI1 was associated with EGFR expression in GBM, we referred to GEO data and calculated the correlation coefficients of the expression levels of EVI1 and EGFR. A positive correlation was found between EVI1 and EGFR expressions in each of the GBM series, including GSE2223 (r = 0.429–0.51); GSE4271 (r = 0.379–0.462); GSE23806 (r = 0.445–0.606); and GSE43378 (r = 0.461–0.551) (Supplementary Fig. 1).

The EVI1 expression level was relatively high in GBM tissue and might contribute to worse prognosis

To clarify the role of EVI1 in glioma genesis, we performed immunohistochemical staining and analyzed the EVI1-positive cell rates in 37 GBM and 27 lower-grade glioma (LGG) tissue samples. EVI1 was detected in the nuclei of the glioma cells, and EVI1-positive tumor cells were observed diffusely in glioma tissue. We observed the heterogeneity of nuclei at the point of staining intensity (Fig. 1a). In addition, the cases those were stained well by EVI1 were also stained well by EGFR (Fig. 1b).

On examination of the 37 GBM tissue samples, the positive rate was 0–5% in 15 cases, 5–33% in 14 cases, 33–50% in six cases, and over 50% in two cases. On the other hand, among the 27 LGG tissue samples, the positive rate was 0–5% in 13 cases and 5–33% in 14 cases; none of the cases had over 33% positive rate (Table 1). Because no LGG tissues had over 33% of tumor cells expressing EVI1, we chose the 33% as the cut-off level in the survival curve. Of the 37 GBM cases, eight cases that had over 33% EVI1-positive rate showed significantly worse prognosis (P = 0.0109). The one-year survival rate was 12.5% for the cases with over 33% positive rate and 62.2% for the cases with 0–33% positive rate (Fig. 1c). On the other hand, we examined the relation between the level of EGFR-mRNA expression and post-operative days in these patients with GBM. However, the result showed no significant difference (Supplementary Fig. 3).

Among the 37 GBM tissue samples, the positive rate was over 33% in eight cases. However, among the 27 LGG tissue samples, none showed a positive rate of over 33%. The scores of the EVI1-positive tumor cells were defined as (−) if less than 5% (+) if 5% to 33% (++) if 33% to 50%, and (+++) if more than 50%.

The EVI1 expression levels were more varied in the GBM cell lines than in the normal brain tissue samples

To investigate the expression levels of EVI1 in various GBM cell lines, we performed quantitative PCR and western blotting using seven GBM cell lines (i.e., A-172, LN-18, LN-229, T98G, U-87MG, U-251MG, and YKG-1) and compared them with those in normal brain tissues (i.e., NB-1, NB-2, NB-3, NB-4, NB-5, NB-6, NB-7, and NB-8). The levels of EVI1-mRNA in the GBM cell lines varied and showed no significant difference; (P = 0.052) (Fig. 2a, left panel), however, the protein levels of EVI1 in GBM cell lines were higher compared with those in normal brain tissues (P <0.05) (Fig. 2a, right panel. The representative data was shown in Supplementary Fig. 4).

siRNA-mediated EVI1 knockdown decreased the expression levels of EGFR

To determine the regulatory mechanism between EVI1 and EGFR, we induced knockdown of EVI1 in GBM cells because EVI1 is a transcription factor. First, we induced
siRNA-mediated knock down in A172, LN18, LN229, T98G, U87MG, U251MG, and YKG1. As a result, the $EVI1$-mRNA levels in these GBM cell lines were reduced. In addition, we observed reduction in $EGFR$-mRNA and $EGFR$ protein expression in response to the decrease in $EVI1$ mRNA (Supplementary Figs. 5, 6). Among these GBM cell lines, the differences in $EGFR$-mRNA between knock down group and control group were bigger in A172 and YKG1 than other GBM cell lines. Thus, we chose these two cell lines for siRNA-mediated $EVI1$ knock down experiments.

Transfection with the siRNAs achieved >75% decrease in $EVI1$ mRNA expression in the A172 and YKG1 cells. In the A172 cells, $EGFR$-mRNA expression decreased by 35% and 45% after transfection with si$EVI1_B$ and si$EVI1_C$, respectively, whereas in the YKG1 cells, the expression decreased by 49% and 45% after transfection with si$EVI1_B$ and si$EVI1_C$, respectively (Fig. 2b).

---

**Fig. 1** Immunohistochemical staining for $EVI1$ in glioma tissue. 
(a) Representative HE staining (× 400) (i, iii) and $EVI1$ immunohistochemistry images (ii, iv) of GBM tissues; (i) and (ii) are GBM tissues expressing high levels of $EVI1$, whereas (iii) and (iv) are GBM tissues expressing low levels of $EVI1$. The scale bars represent 100 μm. 
(b) Representative immunohistochemistry images (× 200) of GBM tissues; (i) and (ii) are GBM tissues expressing high levels of $EVI1$, whereas (iii) and (iv) are GBM tissues expressing low levels of $EVI1$. (i, iii) were $EVI1$ immunohistochemistry, whereas (ii, iv) were $EGFR$ immunohistochemistry. The scale bars represent 100 μm. 
(c) Log-rank test for the survival curves stratified by $EVI1$ expression in 37 GBM tissues shows a worse overall survival rate in patients with high $EVI1$ expression ($EVI1$-positive cell rate of ≥33%; $n=8$) than in those with low $EVI1$ expression ($EVI1$-positive cell rate of <33%; $n=29$) ($P=0.0109$).
In the A172 cells transfected with siEVI1_C, reduced EVI1 and EGFR-mRNA levels were observed at 24, 48, 72, and 96 h; the largest difference in the EGFR-mRNA expression level, relative to the negative control, was observed at 96 h after transfection (66% reduction) (Fig. 2c). Therefore, we conducted western blot assay 96 h after transfection and found that the EGFR protein expression in GBM cells decreased after treatment with the siRNAs against EVI1, compared with the effects in the negative control. In the A172 cells, EGFR protein expression decreased by 65% and 79% after transfection with siEVI1_B and siEVI1_C, respectively, whereas in the YKG1 cells, the expression decreased by 27% and 47% after transfection with siEVI1_B and siEVI1_C, respectively (Fig. 2d).

The importance of the EVI1 regulatory region in the EVI1 regulation of EGFR transcription

To clarify the molecular mechanisms underlying the transcriptional regulation of EGFR by EVI1, we attempted to determine the essential promoter region of EGFR using luciferase assay. In the luciferase assays, we mainly used U87MG cells because these cells received few cell damages by the HilyMax compared to other GBM cell lines. The luciferase activity reduced significantly with pGL4-335 (59%) and pGL-265 (93%) than with pGL-377 (Fig. 3a). This result suggested that EGFR transcription was primarily driven by DNA derived from the 112 bp fragment, which was located −377 bp to −266 bp upstream of the EGFR promoter. When pGL4-377 was co-transfected with the EVI1 overexpression vector into
five GBM cell lines (A172, LN229, U-87MG, U-251MG, and YKG-1), exogenous EVI1 enhanced the luciferase activities by 2.9-fold in YKG1 and by 11.4-fold in U-251MG (Fig. 3b). Thus, EVI1 had a positive influence on EGFR transcription in GBM cells. To determine the more important region (−377 bp to −336 bp vs. −335 bp to −266 bp) for the EVI1 regulation of EGFR transcription, luciferase activities were measured after co-transfection of the pGL4-265, pGL4-335, and pGL4-377 plasmids with the EVI1 overexpression vector (pMXs-EVI1) into the U-87MG cells. The luciferase activities were enhanced by exogenous EVI1 in both the pGL4-335 and pGL4-377 plasmids. Furthermore, the luciferase activity was
Previous reports showed that the EVI1 N-terminal domains
including the N-terminal and C-terminal domains. Pre-
EVI1 contains two independent DNA-binding domains,
We focused on the EVI1-binding sequences in ERR.

![Fig. 3](image_url) The importance of ERR for EVI1 regulation of EGFR tran-
scription. All dual luciferase assays in the GBM cells were performed
in triplicate. The values and error bars depict the mean values ± SD. a
Various length fragments of the EVGFR promoter region were inserted
upstream of the luciferase gene in the reporter plasmid pGL4.10
[luc2]. The relative luciferase activity was normalized after transfection
in U-87MG. A pGL4.10 [luc2] plasmid was used as control
(pGL4-empty vector). **P<0.02. b The relative luciferase activities
after transfection of the pGL4-377 plasmid with the EVI1 overexpres-
sion vector (pCMV26-EVI1) or control vector (pCMV26-control).
The overexpression of EVI1 protein in GBM cells (U/87MG, YKG1)
was validated by western blot analysis (Supplementary Fig. 7). This
experiment was performed using the GBM cell lines LN229, YKG-1,
U-251MG, U-87MG, and A-172. **P<0.02. c The relative luciferase activities
in U-87MG after transfection of the luciferase reporter plas-
mids, with various lengths of EGFR promoter fragments integrated.
Each plasmid was transfected with the EVI1 overexpression vector
(pMXs-EVI1; black bars) or the control vector (pMXs-control; gray
bars). pGL265 with the control vector was used as control. All lucif-
erase reporter assays were performed in triplicate. The values
and error bars depict the mean ± SD. *P<0.05. d The panel shows the sequences of ERR (EVI1 regulatory region). The sequences are
numbered according to their positions, relative to the site of transla-
tion start (+ 1 at ATG). The two boxed sequences represent ERR1
(−377 bp to −336 bp) and ERR2 (−335 bp to −266 bp) sites.
The mutation regions that we used in the EGFR promoter mutant
carrier assay are shown in this schema. e The EGFR promoter
regions (−377 bp to −63 bp upstream of the translational starting
site), which contain mutations (X) in the putative EVI1 bind-
ing sites, are inserted upstream of the luciferase gene in the reporter
plasmid pGL4.10 [luc2]. The numbers placed on the X mark rep-
resent a region from the site of the translational start of the EGFR
promoter. Each plasmid is transfected with the EVI1 overexpression
vector (pMXs-EVI1, black bars) or the control vector (pMXs-control, gray
bars) into U-87MG. **P<0.02. f Enhancement of the promoter activity of EGFR by EVI1 via the C-terminal DNA-binding domain.
The structures of the wild-type EVI1 (EVI1 wt) and the mutant EVI1
containing a deletion of the C-terminal DNA-binding domains (EVI1
Δ8-10) are depicted. U-87MG, U-251MG, and YKG-1 cells were
co-transfected with the EGFR reporter vector (pGL4-377), pRL-TK,
and each EVI1 overexpression vector (pCMV26-EVI1/ pCMV26-
EVI1Δ8-10). pCMV26-control was used as control. All luciferase
reporter assays were performed in triplicate. The values and error
bars depict the mean ± SD. *P<0.05

significantly higher in pGL4-377 than in pGL4-335 (Fig. 3c).
The results suggested that both EGFR promoter regions were
important for the EVI1 regulation of EGFR transcription. We
designated the promoter region in −377 bp to −266 bp as the
EVI1 regulatory region (ERR). In ERR, −377 bp to −336
bp were designated as ERR1 and −335 bp to −266 bp were
designated as ERR2 (Fig. 3d).

The role of the polypyrimidine stretches in the EVI1
regulatory region in the EVI1 activation of the EGFR
promoter activity

We focused on the EVI1-binding sequences in ERR.
EVI1 contains two independent DNA-binding domains,
including the N-terminal and C-terminal domains. Pre-
vious reports showed that the EVI1 N-terminal domains
recognized a GATA-like motif and the C-terminal domains
recognized an ETS-like motif [3, 13]. Notably, ERR had
no GATA-like motif, but it was rich in polypyrimidine
stretches. We presumed that EVI1 needs at least two
regions of TC-rich direct-repeat sequences (general motif; TCCTCCTCC) in the EGFR promoter are essential for the
regulation of EGFR transcription [14, 15]. Therefore, we
generated eight EGFR mutant promoter constructs, viz.,
pGL4-377mt303, pGL4-377mt323, pGL4-377mt344,
pGL4-377mt349, pGL4-377mt363, pGL4-377mt303/343,
pGL4-377mt303/349, and pGL4-377mt303/363. We trans-
fected these eight plasmids with the EVI1 overexpression
vector into U-87 MG cells. As a result, the mutant
plasmid had reduced EGFR transcriptional activity. In
particular, transfection with pGL4-377mt303/343, pGL4-
377mt303/349, and pGL4-377mt303/363 completely abol-
ished EVI1 efficacy to enhance EGFR promoter activity
(Fig. 3e).

The promoter activity of EGFR is enhanced by EVI1 via
the C-terminal DNA-binding domain

The EVI1 mutant construct with deletion of the C-terminal
DNA-binding domain (pCMV26-EVI1Δ8-10) was trans-
fected with pGL4-377 into U-87MG, U-251MG, and YKG1
cells (Fig. 3f). EGFR promoter activity was enhanced in the
presence of transfected wild-type EVI1 but markedly
reduced after transfection of the EVI1 deletion mutant con-
struct (pCMV26-EVI1Δ8-10). This suggested that the loss of
the pCMV26-EVI1Δ8-10 transcriptional enhancer activity
could be attributed to losing the capacity to bind to the
EGFR promoter region.

EVI1 knockdown suppressed the proliferation of
GBM cells

We investigated whether EVI1 knockdown affected the pro-
lation of A172 and YKG-1 cells. On day 3, the transfec-
tion of siRNAs against EVI1 significantly suppressed the
EVI1 expression (Fig. 3f). EGFR promoter activity was
suppressed after EVI1 knockdown suppression. We used
siRNA-resistant MB cells expressing EVI1 as the control.

Discussion

In this study, we introduced the high tissue expression of
EVI1 in patients with GBM might relate to poor prog-
nosis. Next, we demonstrated that EVI1 regulated EGFR
transcription and affected the proliferation of GBM cells.
Further, we found that both TC-rich stretches in the EGFR promoter were essential for the EVI1 regulation of EGFR transcription. Only few reports on glioma focused on EVI1. In this study, we first reported that EVI1 regulated EGFR transcription in GBM cells.

EGFR amplification occurs in about 50% of primary GBM [16]. To date, several transcription factors have been reported to contribute to the regulation of EGFR transcription [14, 15, 17, 18]. Among these reports, the one by Johnson et al. is intriguing [14, 19] because they identified the two pairs of direct-repeat sequences, which conformed to the general motif TCCTCCTCC, on the EGFR promoter. We reported in this study that EVI1 also needed the TC-rich stretches in ERR, and the ability of EVI1 to regulate EGFR transcription depends on both ERR1 and ERR2 sites.

According to a previous study, the relations between EGFR expression and the prognosis of patients with GBM are controversial [20]. In this study, the result showed no significant relation between the EGFR-mRNA expression levels and the post-operative days (Supplementary Fig. 3). This result suggested that high-EVI1 expression could be correlated with worse prognosis of GBM depend on another mechanism unrelated to EGFR regulation.

According to the cBioPortal for cancer genomics data (https://www.cbioportal.org/index.do), the frequency of EVI1 genetic alteration was lower in glioma (2.1% of 283 cases) than in other solid cancers (lung squamous cell carcinoma, 44.1%; ovarian serous cyst adenoma, 33.8%; esophageal carcinoma, 25.5%; and neuroendocrine prostate cancer, 22.4%). Therefore, most researchers on glial tumors had less interest in this transcription factor. However, we showed that in patients with glioma, EVI1 might be related to poor prognosis.

There were some limitations to this report. First, our study population was relatively small and the design was retrospective. Second, our findings were only based on in vitro assays. To validate the role of EVI1 in GBM, in vivo assays are critical. Nevertheless, we showed the exciting potential of EVI1 to interrupt glioma cell proliferation. Therefore, further studies should clarify the role of EVI1 in patients with glioma.

### Conclusion

We reported in this study that EVI1 regulated EGFR transcription and effect on the cell proliferation in GBM cells. The TC-rich stretches in the EGFR promoter (from −377 to −266 bp, relative to the EGFR translational start site) was essential for the EVI1 regulation of EGFR expression.

### Acknowledgements

We would like to thank Dr. Kazuko Kaneda, Dr. Shingo Nakahata, Dr. Sohei Mizuguchi, and Ms. Ayumi Nagatomo for their assistance. We would like to thank Enago (www.enago.jp) for the English language review. This study was supported by a Grant-in-Aid for Clinical Research from Miyazaki University Hospital, JSPS KAKENHI Grant Number 25462275, 19K09486, and MSD [Affiliated company of Merck & Co., Inc., Whitehouse Station, NJ, USA].
Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This study was approved by the Ethics Committee of the University of Miyazaki Hospital, Miyazaki, Japan.

Informed consent All patients and their families gave informed consent for the use of the resected tissues.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Mucenski ML, McLain K, Kier AB, Swerdlow SH, Schreiner CM, Miller TA, Pietryga DW, Scott WJ Jr, Potter SS (1991) A functional c-myb gene is required for normal murine fetal hepatic hematopoiesis. Cell 65:677–689
2. Morishita K, Parker DS, Mucenski ML, Jenkins NA, Copeland NG, Ilehe JN (1988) Retroviral activation of a novel gene encoding a zinc finger protein in IL-3-dependent myeloid leukemia cell lines. Cell 54:831–840
3. Funabiki T, Kreider BL, Ilehe JN (1994) The carboxyl domain of zinc fingers of the Evi-1 myeloid transforming gene binds a consensus sequence of GAAGATGAC. Oncogene 9:1575–1581
4. Delwel R, Funabiki T, Kreider BL, Morishita K, Ilehe JN (1993) Four of the seven zinc fingers of the Evi-1 myeloid transforming gene are required for sequence-specific binding to GA(C/T)AAAGATTA. Mol Cell Biol 13:4291–4300
5. Li Y, Cheng CN, Verdun VA, Wingert RA (2014) Zebrafish nephrogenesis is regulated by interactions between retinoic acid, mecom, and Notch signaling. Dev Biol 386:111–122. https://doi.org/10.1016/j.ydbio.2013.11.021
6. Konantz M, Alghisi E, Muller JS, Lenard A, Esain V, Carroll KJ, Kanz L, North TE, Lengerke C (2016) Evi1 regulates Notch activation to induce zebrafish hematopoietic stem cell emergence. EMBO J. https://doi.org/10.15252/embj.201593454
7. Koos B, Bender S, Witt H, Mertsch S, Felsberg J, Beschhorn R, Korshunov A, Riesmeier B, Pilster S, Paulus W, Hasselblatt M (2011) The transcription factor evi-1 is overexpressed, promotes proliferation, and is prognostically unfavorable in infratentorial ependymomas. Clin Cancer Res 17:3631–3637. https://doi.org/10.1158/1078-0432.CCR-11-0175
8. Hou A, Zhao L, Zhao F, Wang W, Niu J, Li B, Zhou Z, Zhu D (2016) Expression of MECOM is associated with unfavorable prognosis in glioblastoma multiforme. Onco Targets Ther 9:315–320. https://doi.org/10.2147/OTT.S95831
9. Bard-Chapeau EA, Jeyakani J, Kok CH, Muller J, Chua BQ, Gunaratne J, Batagov A, Jenjaroenpun P, Kuznetsov VA, Wei CL, D’Andrea RJ, Bourque G, Jenkins NA, Copeland NG (2012) Ecotropic viral integration site 1 (EVII) regulates multiple cellular processes important for cancer and is a synergistic partner for FOS protein in invasive tumors. Proc Natl Acad Sci USA 109:2168–2173. https://doi.org/10.1073/pnas.1119229109
10. Purow BW, Sundararesan TK, Burdick MJ, Kefa BA, Comeau LD, Hawkinson MP, Su Q, Kotliarov Y, Lee J, Zhang W, Fine HA (2008) Notch-1 regulates transcription of the epidermal growth factor receptor through p53. Carcinogenesis 29:918–925. https://doi.org/10.1093/carcin/bgn079
11. Cenciarelli C, Marei HE, Zonfrillo M, Casalbore P, Felsani A, Giannetti S, Trevisi G, Althani A, Mangiola A (2017) The interference of Notch1 target Has1 affects cell growth, differentiation and invasiveness of glioblastoma stem cells through modulation of multiple oncogenic targets. Oncotarget 8:17873–17886. https://doi.org/10.18632/oncotarget.15013
12. Jazaeri AA, Ferriss JS, Bryant JL, Dalton MS, Dutta A (2010) Evaluation of EVII and EVIIx (Delta324) as potential therapeutic targets in ovarian cancer. Gynecol Oncol 118:189–195. https://doi.org/10.1016/j.ygyno.2010.04.007
13. Yuasa H, Oike Y, Iwama A, Nishikata I, Sugiyama D, Perkins A, Mucenski ML, Suda T, Morishita K (2005) Oncogenic transcription factor Evi1 regulates hematopoietic stem cell proliferation through GATA-2 expression. EMBO J 24:1976–1987. https://doi.org/10.1038/sj.emboj.7600679
14. Johnson AC, Jinno Y, Merlino GT (1988) Modulation of epidermal growth factor receptor proto-oncogene transcription by a promoter site sensitive to S1 nuclease. Mol Cell Biol 8:4174–4184
15. Englert C, Hou X, Maheswaran S, Bennett P, Ngwu C, Re GG, Garvin AJ, Rosner MR, Haber DA (1995) WT1 suppresses synthesis of the epidermal growth factor receptor and induces apoptosis. EMBO J 14:4662–4675
16. Furnari FB, Cloughesy TF, Cavenee WK, Mischel PS (2015) Heterogeneity of epidermal growth factor receptor signalling networks in glioblastoma. Nat Rev Cancer 15:302–310. https://doi.org/10.1038/nrc3918
17. Ludes-Meyers JH, Subler MA, Shivakumar CV, Munoz RM, Jiang P, Bigger JE, Brown DR, Deb SP, Deb S (1996) Transcriptional activation of the human epidermal growth factor receptor promoter by human p53. Mol Cell Biol 16:6009–6019
18. Vaughan CA, Pearsall I, Singh S, Windle B, Deb SP, Grossman SR, Yeadall WA, Deb S (2016) Addiction of lung cancer cells to GOF p53 is promoted by up-regulation of epidermal growth factor receptor through multiple contacts with p53 transactivation domain and promoter. Oncotarget 7:12426–12446. https://doi.org/10.18632/oncotarget.6998
19. Johnson AC (1996) Activation of epidermal growth factor receptor gene transcription by phorbol 12-myristate 13-acetate is mediated by activator protein 2. J Biol Chem 271:3033–3038
20. Ohgaki H, Dessen P, Bourdeau H, Horstmann S, Nishikawa T, Di Patre PL, Burkhard C, Schuler D, Probst-Hensch NM, Maiorka PC, Baesa N, Pisani P, Yonekawa Y, Yasargil MG, Lutolf UM, Kleihues P (2004) Genetic pathways to glioblastoma: a population-based study. Cancer Res 64:6892–6899. https://doi.org/10.1158/0008-5472.CAN-04-1337

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.