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PEA3 activates VEGF transcription in T47D and SKBR3 breast cancer cells

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Vascular endothelial growth factor (VEGF) is a potent stimulator of angiogenesis and a prognostic factor for many tumors, including those of endocrine-responsive tissues such as the breast and uterus. In this study, we found that overexpression of *PEA3* could increase *VEGF* mRNA levels and *VEGF* promoter activity in human T47D and SKBR3 breast cancer cells. Chromatin immunoprecipitation assay demonstrated that *PEA3* could bind to the *VEGF* promoter in the cells transfected with PEA3 expression vector. PEA3 small interfering RNA attenuated *VEGF* promoter activity and the binding of PEA3 to the *VEGF* promoter in T47D and SKBR3 cells. These results indicated that PEA3 could activate *VEGF* promoter transcription.

Keywords PEA3; VEGF promoter; transcription regulation

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Introduction

Angiogenesis is the formation of new blood vessels from a pre-existing endothelium, which involves proliferation of capillary endothelial cells and their migration toward the angiogenic stimulus [1–3]. Vascular endothelial growth factor (VEGF) plays a pivotal role in the induction of increased microvascular permeability and in angiogenesis [4,5]. Deregulated VEGF expression contributes to the development of solid tumors by promoting tumor angiogenesis and to the etiology of several additional diseases that are characterized by abnormal angiogenesis. Consequently, inhibition of VEGF signaling abrogates the development of a wide variety of tumors [6,7].

Some transcription factors can influence VEGF transcription. It is reported that the effects of estradiol on VEGF expression in human cancer cells involve estrogen receptor alpha (ER alpha) interactions not with estrogen response elements but with Sp1 and Sp3 on a proximal, GC-rich segment (−66 to −47) of the promoter [8,9]. VEGF expression is also strongly induced in cells by hypoxia, and this occurs via a hypoxia response element on the VEGF promoter, which binds the transcription factor hypoxia-inducible factor 1 [10]. ER alpha induces VEGF transcription activation, and BRCA1 significantly inhibits VEGF gene transcription activation and VEGF protein secretion via direct interaction between BRCA1 and the estrogen receptor [11]. PEA3 is also a transcription factor, and so far there are no reports as to whether PEA3 can activate VEGF transcription.

The purpose of this study is to explore the activity of PEA3 on VEGF transcription and to reveal the role of PEA3 involved in VEGF-mediated angiogenesis. Our results showed that PEA3 played an important role in inducing VEGF promoter activity by directly binding to the VEGF promoter, which is helpful to understand the angiogenic mechanism.

Materials and Methods

Cell lines, culture, plasmids, and transfection

Human breast cancer cell lines, T47D and SKBR3, were maintained in RPMI 1640 containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Cells were checked routinely and found to be free of contamination by *Mycoplasma* or fungi.

VEGF promoter/luciferase construct pGL3-VEGF (−2352 to +955) was kindly provided by Dr Lee

M. Ellis (Department of Surgical Oncology, UTMD Anderson Cancer Center, Houston, USA). PEA3 expression vector was kindly provided by Dr Hassell (Department of Biology, McMaster University of Canada). Transfections were conducted by the lipofectamine method. Briefly, for transient transfection, cells were seeded in six-well plates at a density of 4×10^5 cells/well. The following day, cells were transfected with 4 μg of PEA3 expression vector or pcDNA3 using Lipofectamine 2000 (Gibco BRL, Carlsbad, USA). Following transfection, cells were maintained in RPMI 1640 medium containing 10% FBS and cultured for 48 h.

Reverse transcription–polymerase chain reaction

Total RNA was extracted from cells with TRIzol reagent (Invitrogen Life Technologies, Inc., Carlsbad, CA, USA) and quantified by UV absorbance spectroscopy. The reverse transcription reaction was performed using the Superscript First-Strand Synthesis System (Invitrogen Life Technologies, Inc.) in a final volume of 20 μl containing 5 μg of total RNA, 200 ng of random hexamers, 1× reverse transcription buffer, 2.5 mM $MgCl_2$, 1 mM deoxynucleotide triphosphate mixture, 10 mM DTT, RNaseOUT Recombinant Ribonuclease Inhibitor (Invitrogen), 50 U of superscript reverse transcriptase, and diethylpyrocarbonate-treated water. After incubation at 42°C for 50 min, the reverse transcription reaction was terminated by heating at 85°C for 5 min. The newly synthesized cDNA was amplified by PCR. The reaction mixture contained 2 μl of cDNA template, 1.5 mM $MgCl_2$, 2.5 U of Taq polymerase, 0.5 μM of VEGF primer (5′-GGATGTCTATCAGCGCAGCTAC-3′; 5′-TCA CCGCCTCGGCTTGTCACATC-3′), and PEA3 primer (5′-CAGCTCAGCTTCTTCCTAGGTC-3′; 5′-CCTCTCT GCTTATACCCAGCAC-3′). The *GAPDH* primer (5′-G CCAAAAGGGTCATCATCTC-3′; 5′-GTAGAGGCAGG GATGATGTTC-3′) was used as an internal control. Amplification cycles were: 94°C for 3 min, then 33 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 1.5 min, followed by 72°C for 10 min. Aliquots of PCR product were electrophoresed on 1.5% agarose gels, and PCR fragments were visualized by ethidium bromide staining.

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were carried out according to the manufacturer's protocol (Active motif, Carlsbad, CA, USA). Briefly, cells in 150 mm tissue culture dishes were fixed with 1% formaldehyde and incubated for 10 min at 37°C. The cells were then washed twice with ice-cold phosphate-buffered saline (PBS), harvested, and re-suspended in ice-cold TNT lysis buffer [20 mM Tris–HCl (pH 7.4), 200 mM NaCl, 1% Triton X-100, 1 mM PMSF, and 1% aprotinin]. The lysates were sonicated to shear the DNA to fragments of 200 − 600 bp and subjected to immunoprecipitation with the following antibodies, respectively, PEA3 or IgG (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). For each immunoprecipitation, 3 μg of antibodies were used. The antibody/protein complexes were collected by Protein G beads and washed three times with ChIP washing buffer (5% SDS, 1 mM EDTA, 0.5% bovine serum albumin, and 40 mM $NaHPO_4$, pH 7.2). The immune complexes were eluted with 1% SDS and 1 M $NaHCO_3$, and the cross-links were reversed by incubation at 65°C for 4 h in the presence of 200 mM NaCl and RNase A. The samples were then treated with proteinase K for 2 h, and then DNA was purified by mini-column, ethanol precipitation, and re-suspended in 100 ml of H_2O. The primer corresponding to the VEGF promoter region (−339 to −159) (sense: 5′-AGAGGGAACGGCTCTCAGGC-3′; antisense: 5′-CTCTGCGGACGCTCAGTGAA-3′) was used for PCR to detect the presence of the VEGF promoter DNA.

Small interfering RNA preparation and transfection

Cells in the exponential phase of growth were seeded in six-well plates at a concentration of 5×10^5 cells/well. After incubation for 24 h, the cells were transfected with small interfering RNA (siRNA) specific for PEA3 [12] (catalog number: 115237) (Ambion, Austin, TX, USA) and non-targeting siRNA at a final concentration of 100 nM using oligofectamine and OPTI-MEMI-reduced serum medium (Invitrogen Life Technologies, Inc.), according to the manufacturer's protocol. Silencing was examined 48 h after transfection by reverse transcription–polymerase chain reaction (RT–PCR) and western blotting.

Western blot analysis

Cells were washed twice with PBS containing 1 mM phenylmethylsulphonyl fluoride, lysed in mammalian protein extraction buffer (Pierce, Rockford, IL, USA). The lysates were transferred to Eppendorf tubes and clarified by centrifugation at 12,000 *g* for 40 min at 4°C. Equal amounts (50 μg of protein) of cell lysates were resolved by SDS–PAGE. The proteins were transferred to nitrocellulose membranes. Membranes were incubated in blocking solution consisting of 5% powered milk in PBST (PBS plus 0.1% Tween 20) at room temperature for 1 h, and then immunoblotted with anti-PEA3

antibody (Santa Cruz Biotechnology, Inc.) or anti-tubulin antibody (Sigma-Aldrich, St Louis, MO, USA), respectively. Detection by enzyme-linked chemiluminescence was performed according to the manufacturer's protocol (ECL; Amersham Pharmacia Biotech, Piscataway, NJ).

Luciferase reporter gene assay

T47D or SKBR3 cells were seeded in six-well plates at a density of $1-2\times10^5$ cells/well and cultured for 24 h. Cells were then transfected with the VEGF promoter/luciferase construct pGL3-VEGF (0.5 μg/well) or co-transfected with 0.5 μg of pcDNA3.0, or PEA3 expression vector, together with 20 ng of control Renilla luciferase reporter construct, pRL-TK (Promega, Madison, WI, USA). The total amount of DNA per well was adjusted to 1.5 μg by the addition of sonicated salmon sperm DNA. Luciferase assays were performed as recommended by the manufacturer (Promega) and normalized relative to protein concentration determined by bicinchoninic acid protein assay (Pierce).

Results

Overexpression of PEA3 induced *VEGF* mRNA level in T47D and SKBR3 cells

To identify the role of PEA3 in regulating *VEGF* transcription, PEA3 expression vector or pcDNA3 was transfected into T47D and SKBR3 cells and *VEGF* mRNA was detected. **Fig. 1(A)** showed that as compared with T47D cells transfected with pcDNA3, the level of *VEGF* mRNA in the cells transfected with PEA3 expression vector increased as determined by reverse transcription–polymerase chain reaction (RT–PCR). **Fig. 1(B)** showed that as compared with SKBR3 cells transfected with pcDNA3, the level of *VEGF* mRNA in the cells transfected with PEA3 expression vector increased as determined by RT–PCR. In this experiment, exogenous PEA3 could induce *VEGF* mRNA, indicating PEA3 played a role in regulating VEGF transcription.

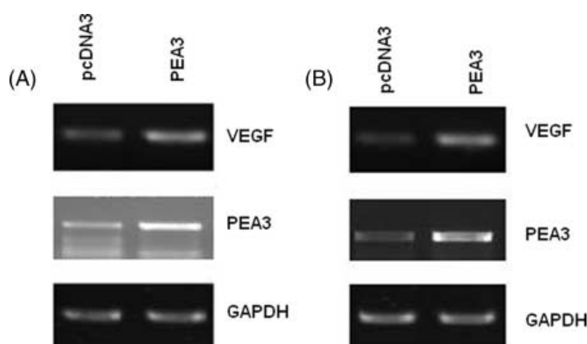

Fig. 1 Overexpression of PEA3 induced VEGF mRNA level in T47D and SKBR3 cells T47D (A) and SKBR3 (B) cells were plated in six-well tissue culture plates, then transfected with 4 μg of PEA3 expression vector, cultured for 48 h, and mRNA expression levels of *PEA3* and *VEGF* were detected.

PEA3 activated *VEGF* promoter activity in T47D or SKBR3 cells

To identify the role of PEA3 in regulating *VEGF* promoter transcription, we co-transfected the VEGF promoter/luciferase construct with PEA3 expression vector or pcDNA3 in T47D and SKBR3 cells and detected VEGF promoter activity. **Fig. 2(A)** and **(B)** showed that the luciferase activity was enhanced by PEA3 both in T47D cells and in SKBR3 cells, further indicating that PEA3 could activate VEGF promoter activity. In this experiment, exogenous PEA3 could activate VEGF promoter activity, suggesting that PEA3 played a role in regulating *VEGF* transcription.

PEA3 bound to the *VEGF* promoter in PEA3-overexpressed T47D or SKBR3 cells

To investigate if PEA3 bound to the *VEGF* promoter in the cells transfected with PEA3 expression vector, we performed ChIP experiments. The results showed that PEA3 could bind to the *VEGF* promoter both in T47D and SKBR3 cells transfected with PEA3 expression vector **[Fig. 3(A)** and **(B)]**. In this experiment, PEA3 could bind to the *VEGF* promoter in PEA3-overexpressed T47D or SKBR3 cells, indicating PEA3 activated *VEGF* transcription by binding directly to the *VEGF* promoter.

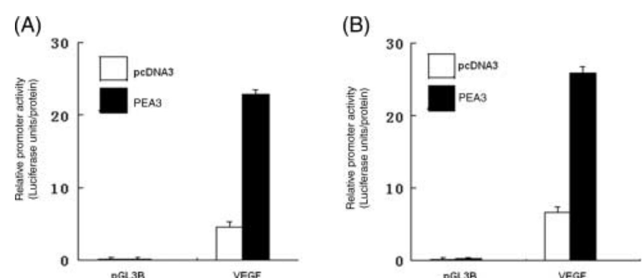

Fig. 2 PEA3 activated VEGF promoter activity in T47D and SKBR3 cells T47D (A) and SKBR3 (B) cells were plated in six-well tissue culture plates, then co-transfected with 0.5 μg of pGL3-VEGF with 0.5 μg of PEA3 expression vector or pcDNA3.0 control vector for 48 h, and luciferase activity was detected.

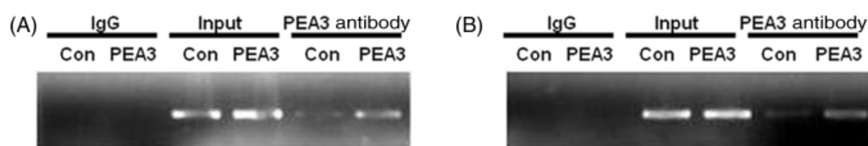

Fig. 3 PEA3 bound to the VEGF promoter after overexpression of PEA3 in T47D and SKBR3 cells T47D (A) and SKBR3 (B) cells were plated in six-well tissue culture plates, then transfected with 4 μg of PEA3 expression vector, and cultured for 48 h. Nucleic extracts were prepared from T47D and SKBR3 cells with or without PEA3 transfection. Chromatin immunoprecipitation (ChIP) assays were performed using antibody against PEA3 and IgG. The primers corresponding to the VEGF promoter region (−339 to −159) upstream of the transcriptional start site were used for PCR to detect the presence of the VEGF promoter DNA. Con, control.

PEA3 small interfering RNA inhibited PEA3 expression in T47D or SKBR3 cells

To further identify the role of PEA3 in regulating VEGF transcription, we knocked down the expression of PEA3 with a gene-specific siRNA and measured PEA3 mRNA and protein. As shown in **Fig. 4(A)** and **(B)**, PEA3 siRNA inhibited *PEA3* mRNA significantly in T47D and SKBR3 cells after transfection with PEA3 siRNA for 48 h. As indicated in **Fig. 4(C)** and **(D)**, PEA3 siRNA inhibited PEA3 protein significantly in T47D and SKBR3 cells after transfection with PEA3 siRNA for 48 h. This experiment indicated that PEA3 siRNA could knock down PEA3 expression efficiently.

PEA3 small interfering RNA repressed VEGF promoter activity in T47D and SKBR3 cells

To determine if the decrease in PEA3 would reduce *VEGF* gene transcription, we knocked down the expression of PEA3 and measured VEGF promoter activity. As determined in **Fig. 5(A)** and **(B)**, PEA3 siRNA attenuated VEGF promoter activity in normal T47D and SKBR3 cells after transfection with PEA3 siRNA for 48 h. This experiment indicated that when endogenous PEA3 was knocked down by siRNA, the promoter activity of endogenous VEGF also decreased.

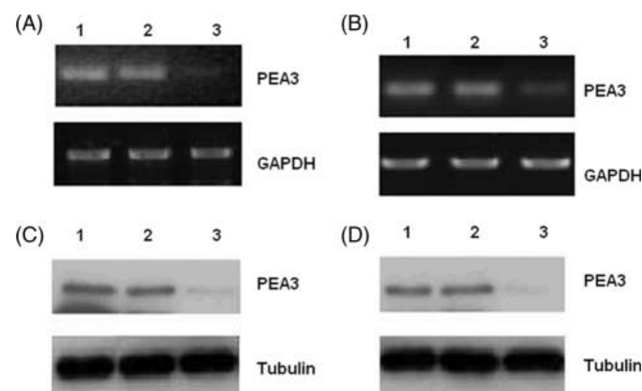

Fig. 4 PEA3 small interfering RNA (siRNA) inhibited PEA3 expression in T47D or SKBR3 cells (A and B) PEA3 siRNA inhibited PEA3 mRNA in T47D and SKBR3 cells. (C and D) PEA3 siRNA inhibited PEA3 protein in T47D and SKBR3 cells. T47D (A and C) and SKBR3 (B and D) cells were treated with 100 nM of PEA3 siRNA or non-targeting siRNA for 48 h, and reverse transcription–polymerase chain reaction and western blotting were performed. GAPDH or tubulin was used as an internal control. 1, control cells; 2, cells transfected with non-targeting siRNA; 3, cells transfected with PEA3 siRNA.

PEA3 small interfering RNA attenuated the binding of PEA3 to the VEGF promoter

To determine if the decrease in PEA3 would influence the binding of PEA3 on the *VEGF* promoter, we knocked down the expression of PEA3 and measured the binding status of PEA3 on the *VEGF* promoter. As determined in **Fig. 6(A)** and **(B)**, PEA3 siRNA attenuated the binding of PEA3 to the VEGF promoter in normal T47D and SKBR3 cells after transfection with PEA3 siRNA for 48 h. This experiment showed that when endogenous PEA3 was knocked down by siRNA, the binding of PEA3 to the VEGF promoter decreased, also indicating that PEA3 regulated VEGF transcription by binding directly to the VEGF promoter.

Discussion

The PEA3 subfamily includes PEA3, ER81, and ERM [13–15]. PEA3 may play a role in human breast cancer. The human *PEA3* gene is transcriptionally upregulated in breast tumor cell lines [16] and in 93% of HER2/Neu-positive human breast tumors [17]. PEA3 expression is also found in the majority of both clinical specimens and cell lines of lung and oral carcinoma [18–20]. PEA3 can regulate the transcription of several such proteinases including the matrix metalloproteinases (MMP) collagenase-IV/gelatinase B (MMP-9), matrilysin (MMP-7), and stromelysin-3 (MMP-11), and the serine protease urokinase-type plasminogen activator [21,22]. However, transfection of oral carcinoma cells with an antisense sequence of *PEA3* can result in the inhibition

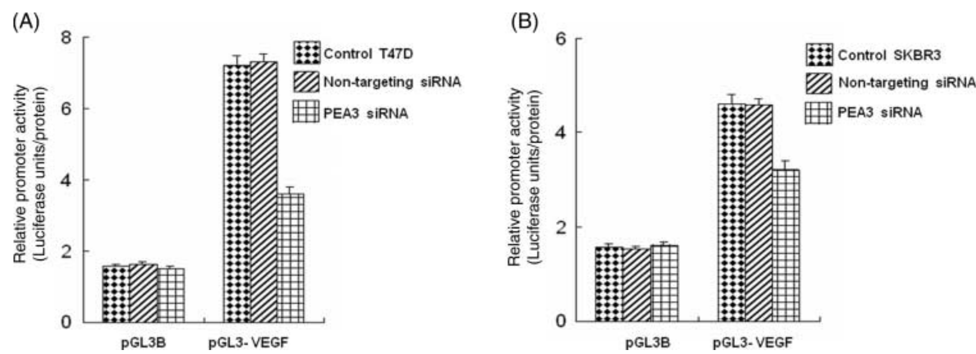

Fig. 5 PEA3 siRNA repressed VEGF promoter activity in T47D or SKBR3 cells T47D (A) and SKBR3 (B) cells were plated in six-well tissue culture plates, then pGL3B or VEGF promoter construct (pGL3-VEGF) was co-transfected with PEA3 siRNA or non-targeting RNA into cells, and cultured for 48 h. Luciferase activity was detected.

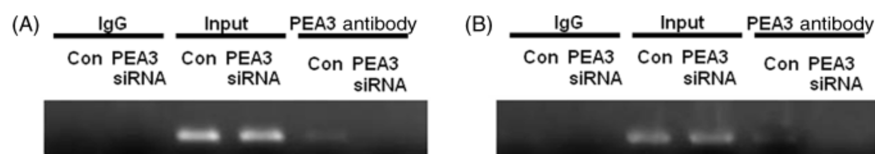

Fig. 6 PEA3 siRNA attenuated the binding of PEA3 to the VEGF promoter T47D (A) and SKBR3 (B) cells were treated with 100 nM of PEA3 siRNA or non-targeting RNA for 48 h and ChIP assay was performed.

of invasion and MMP expression [23]. Transfection with *PEA3* results in enhanced motility and invasion in lung cancer cells and human SKBR3 breast cancer cells [18,23]. Expression of PEA3 dominant-negative form reduces tumor growth in this model [24]. All these reports indicated that PEA3 is related to cancer motility and invasion, but there are no reports about the relationship between PEA3 and VEGF transcription in breast cancer.

In our studies, overexpression of PEA3 could increase the *VEGF* mRNA level in T47D and SKBR3 cells. In order to analyse the putative effects of PEA3 on VEGF transcription, we performed luciferase assay, and our results demonstrated that PEA3 activated VEGF promoter activity. ChIP assay demonstrated that PEA3 could bind to the VEGF promoter in the PEA3-overexpressed cells. Bioinformatic analysis of the 5′-flanking region of the human VEGF gene showed that there existed a PEA3 binding site (TTTCCT) in the VEGF promoter region from −298 to −293. It has been reported that PEA3 could elevate *PEG-3* promoter activity by binding to the PEA3 binding site (TTTCCT) in the *PEG-3* promoter [25,26].

A functional interaction between *PEA3* and *VEGF* promoter was strengthened by *PEA3* siRNA. We further found that *PEA3* siRNA attenuated VEGF promoter activity in T47D and SKBR3 cells. At the same time, *PEA3* siRNA attenuated the binding of *PEA3* to the *VEGF* promoter. These results further indicated that PEA3 could influence *VEGF* promoter activity by binding to the *VEGF* promoter.

We conclude that PEA3 plays an important role in inducing *VEGF* promoter activity by directly binding to the *VEGF* promoter. These investigations are important and offer potential for defining the angiogenic mechanism regulated by VEGF and PEA3. With this information, it will be possible to demarcate potential targets and define appropriate reagents, such as antisense or small molecule antagonists, for inhibiting or preventing cancer development and progression.

Funding

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References

1 Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995, 1: 27–31.

2 Battegay EJ. Angiogenesis, mechanistic insights, neovascular diseases, and therapeutic prospects. J Mol Med, 1995, 73: 333–346.

3 Gao X and Xu Z. Mechanisms of action of angiogenin. Acta Biochim Biophys Sin 2008, 40: 619–624.
4 Ferrara N. Molecular and biological properties of vascular endothelial growth factor. J Mol Med 1999, 77: 527–543.
5 Neufeld G, Cohen T, Gengrinovitch S and Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 1999, 13: 9–22.
6 Kim KJ, Li B, Houck K, Winer J and Ferrara N. The vascular endothelial growth factor proteins: identification of biologically relevant regions by neutralizing monoclonal antibodies. Growth Factors 1992, 7: 53–64.
7 Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS and Ferrara N. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 1993, 362: 841–844.
8 Stoner M, Wang F, Wormke M, Nguyen T, Samudio I, Vyhlidal C and Marme D, *et al*. Inhibition of vascular endothelial growth factor expression in HEC1A endometrial cancer cells through interactions of estrogen receptor alpha and Sp3 proteins. J Biol Chem 2000, 275: 22769–22779.
9 Stoner M, Wormke M, Saville B, Samudio I, Qin C, Abdelrahim M and Safe S. Estrogen regulation of vascular endothelial growth factor gene expression in ZR-75 breast cancer cells through interaction of estrogen receptor alpha and SP proteins. Oncogene 2004, 23: 1052–1063.
10 Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD and Semenza GL. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 1996, 16: 4604–4613.
11 Kawai H, Li H, Chun P, Avraham S and Avraham HK. Direct interaction between BRCA1 and the estrogen receptor regulates vascular endothelial growth factor (VEGF) transcription and secretion in breast cancer cells. Oncogene 2002, 21: 7730–7739.
12 Cowden Dahl KD, Zeineldin R and Hudson LG. PEA3 is necessary for optimal epidermal growth factor receptor-stimulated matrix metalloproteinase expression and invasion of ovarian tumor cells. Mol Cancer Res 2007, 5: 413–421.
13 Xin JH, Cowie A, Lachance P and Hassel JA. Molecular cloning and characterization of PEA3, a new member of the Ets oncogene family that is differentially expressed in mouse embryonic cells. Genes Dev 1992, 6: 481–496.
14 Monte D, Coutte L, Baert JL, Angeli I, Stehelin D and de Launoit Y. Molecular characterization of the Ets-related human transcription factor ER81. Oncogene 1995, 11: 771–779.
15 Monte D, Baert JL, Defossez PA, de Launoit Y and Stehelin D. Molecular cloning and characterization of human ERM, a new member of the Ets family closely related to mouse PEA3 and ER81 transcription factors. Oncogene 1994, 9: 1397–1406.
16 Scott GK, Daniel JC, Xiong X, Maki RA, Kabat D and Benz CC. Binding of an ETS-related protein within the DNase I hypersensitive site of the HER2/neu promoter in human breast cancer cells. J Biol Chem 1994, 269: 19848–19858.
17 Benz CC, O'Hagan RC, Richter B, Scott GK, Chang C-H, Xiong X and Chew K, *et al*. HER2/Neu and the Ets transcription activator PEA3 are coordinately upregulated in human breast cancer. Oncogene 1997, 15:1513–1525.
18 Hiroumi H, Dosaka-Akita H, Yoshida K, Shindoh M, Ohbuchi T, Fujinaga K and Nishimura M. Expression of E1AF/PEA3, an Ets-related transcription factor in human non-small-cell lung cancers: its relevance in cell motility and invasion. Int J Cancer 2001, 93: 786–791.
19 Hida K, Shindoh M, Yasuda M, Hanzawa M, Funaoka K, Kohgo T and Amemiya A, *et al*. Antisense E1AF transfection restrains oral cancer invasion by reducing matrix metalloproteinase activities. Am J Pathol 1997, 150: 2125–2132.
20 Hida K, Shindoh M, Yoshida K, Kudoh A, Furaoka K, Kohgo T and Fujinaga K, *et al*. Expression of E1AF, an ets-family transcription factor, is correlated with the invasive phenotype of oral squamous cell carcinoma. Oral Oncol 1997, 33: 426–430.
21 Matrisian LM. Matrix metalloproteinase gene expression. Ann N Y Acad Sci 1994, 732: 42–50.
22 Higashino F, Yoshida K, Noumi T, Seiki M and Fujinaga K. Ets-related protein E1A-F can activate three different matrix metalloproteinase gene promoters. Oncogene 1995, 10: 1461–1463.
23 Kaya M, Yoshida K, Higashino F, Mitaka T, Ishii S and Fujinaga K. A single ets-related transcription factor, E1AF, confers invasive phenotype on human cancer cells. Oncogene 1996, 12: 221–227.
24 Shepherd TG, Kockeritz L, Szrajber MR, Muller WJ and Hassell JA. The pea3 subfamily ets genes are required for HER2/Neu-mediated mammary oncogenesis. Curr Biol 2001, 11: 1739–1748.
25 Su Z, Shi Y, Friedman R, Qiao L, McKinstry R, Hinman D and Dent P, *et al*. PEA3 sites within the progression elevated gene-3 (PEG-3) promoter and mitogen-activated protein kinase contribute to differential PEG-3 expression in Ha-*ras* and v-*raf* oncogene transformed rat embryo cells. Nucleic Acids Res 2001, 29: 1661–1671.
26 Su ZZ, Shi YJ and Fisher PB. Cooperation between AP1 and PEA3 sites within the progression elevated gene-3 (PEG-3) promoter regulate basal and differential expression of PEG-3 during progression of the oncogenic phenotype in transformed rat embryo cells. Oncogene 2000, 19: 3411–3421.