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Pseudogene CYP4Z2P 3′UTR promotes angiogenesis in breast cancer

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ABSTRACT

Pseudogenes have long been marked as "false" genes, which are similar with real genes but have no apparent function. The 3′UTR is well-known to regulate gene expression post-transcriptionally. Our recent evidence, however, indicates novel functional roles of pseudogene CYP4Z2P 3′UTR (Z2P-UTR). We found that ectopic expression of Z2P-UTR in breast cancer cells significantly increased the expression of VEGF-A without affecting cell proliferation *in vitro*. Meanwhile, conditioned medium (CM) from Z2P-UTR overexpression cells enhanced proliferation, migration and tube formation of HUVEC, and promoted angiogenesis in *ex vivo* models. Also, CM increased the expression of VEGFR2 in HUVEC. Our data suggest that Z2P-UTR can promote breast cancer angiogenesis partly via paracrine pathway of VEGF-A/VEGFR2.

1. Introduction

Breast cancer is the leading cause of cancer-related deaths in women worldwide[1]. Angiogenesis plays a critical role in the growth and metastasis of breast cancer[2]. It is now widely accepted that the growth of malignant tumors require angiogenesis[3]. Given the poor diagnosis for breast cancer, it is imperative to identify new targets for therapy.

Angiogenesis generally occurs in various pathological processes[4], such as tumor progression and metastasis. In the meantime, angiogenesis-related molecules, such as VEGF, MMPs and TIMPs, exhibit meaningful changes along with progression of breast cancer, they also have been utilized as prognostic factors and therapeutic targets in breast cancer [5,6].

Pseudogenes are generally considered as non-functional copies because of either the lack of regulatory elements or the presence of frame-shift mutations[7]. Currently, the human genome is estimated to contain approximately 20,000 pseudogenes[29], a figure comparable to the number of protein-coding genes[8]. Therefore, the existence of abundant pseudogenes suggests they are preserved to have special roles. Topical papers indicate that pseudogenes exhibit functional roles in many diseases such as tumors[9]. In addition, pseudogenes can regulate gene expression by acting as small interfering RNA (siRNAs) [10] or competing endogenous mRNAs (ceRNAs) [11].

Functionalizing pseudogenes will undoubtedly lead to important insight about disease progression. We were interested in human pseudogene CYP4Z2P, which was first identified by Rieger [12]. It is highly expressed in breast cancer, whereas only marginal expression was found in all other tested tissues. These data indicate that CYP4Z2P may be involved in the pathogenesis of tumor progression. However, the precise role of CYP4Z2P is difficult to say. Others have reported that pseudogene could function as ceRNAs to regulate gene functions. For example, PTENP1, the pseudogene of tumor suppressor PTEN, has been shown to modulate PTEN functions [11]. In addition, long non-coding and protein-coding transcripts can co-regulate each other in ceRNA network via binding microRNAs [13]. Based on this reversed regulatory mechanism, RNA transcripts can reduce translational repression by sponging microRNA thereby enhancing the expression of microRNA targets, which has been confirmed by others [14]. Besides, most of noncoding RNAs are relatively small in size, making it convenient to express them in cells or animals. Therefore, using noncoding transcripts to study gene functions is very advantageous. Moreover, coding sequences of pseudogene CYP4Z2P shares high homology with CYP4Z1, making it impossible to clone by traditional molecular cloning technology. Herein, in the present study, we used Z2P-UTR as a model to study the role of CYP4Z2P indirectly and aimed to demonstrate that increased Z2P-UTR expression has potent pro-angiogenic activity *in vitro* and *ex vivo* models. Our results showed that Z2P-UTR could promote breast cancer-induced HUVECs proliferation, migration and tube formation via targeting VEGF/VEGFR2 pathway. Moreover, the expression of MMP-2/MMP-9 and TIMP-1/TIMP-2 was examined.

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2. Material and methods

2.1. Reagents, cell culture and patient samples

Human breast cancer cell lines (MCF-7, MDA-MB-231,SK-BR-3), MCF-10A, HEK293T cells and HUVECs were obtained from ATCC and maintained in DMEM medium (Life Technology, USA) supplemented with 10% FBS (Fetal Bovine Serum) at 37 °C. VEGF-A antibody (AB60788b) was purchased from Sangon Biotech (Shanghai, China), VEGFR2 antibody (catalog#2296-1) from Epitomics Inc. (Epitomics, California, USA). Eleven pairs of breast tumors with adjacent mammary gland epithelial tissues were obtained from 11 patients who underwent surgery at the Tumor Hospital of Jiangsu province from February 2014 to June 2014 and all the 11 cases had not metastasis. Approval from the Institute Research Ethics Committee was obtained for the use of these clinical materials for research purposes.

2.2. Plasmid constructions and transfection

Human CYP4Z2P cDNA was obtained by reverse transcription PCR of RNA prepared from SK-BR-3 cells, then amplified the Z2P-UTR based on cDNA template with the following primers: Z2P-UTR-F-BamHI (5′-CGCGGATCCGATGAAATCAGGGAACT CCTAGGG G-3′) and Z2P-UTR-R-HindIII (5′-CCCAAGCTTATTT CCCTTCGCAGT GGAAGTCAGC-3′), then subcloned into pSilencer 4.1-CMV neoVector (Psi) (Applied Biosystems, USA) to obtain the construct P2P. Two primers, Z2P-UTR-F-SpeI (5′-GGAC TAGTGATGAAATCAGGG AACTCCTAGGGG-3′) and Z2P-UTR-R-HindIII (5′-CCCAAGCT-TATTTCCCTTCGCAGTGGAAGTCA GC-3′), were used to generate the fragment of Z2P-UTR and inserted into PMiR-Report Fluc vectors (Ambion) to obtain a luciferase construct, lu-Z2P-UTR. Z2P-UTR overexpression and knockdown: The plasmids including P2P and Psi, siRNA and Normal Control (NC) were synthesized in Biomics Biotechnology Inc. (Biomics, Jiang Su, China), above of which were transfected into MCF-7 and MDA-MB-231 cells with TransIT®-BrCa Transfection Reagent (Mirus, USA) according to the manufacturer's protocol. After 48 h, total cellular RNA and protein were respectively extracted for the next detection.

2.3. In silico analyses

The sequences of human CYP4Z2P was subjected to secondary structure prediction using the minimum free energy (MFE) method implemented on the RNAfold WebServer (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi).

2.4. Luciferase siRNA target report assay

HEK293T cells were co-transfected with lu-Z2P-UTR with siRNA or NC, and β-gal control plasmid using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). The cells were lysed and luciferase activity was measured using a luminometer (Promega) 48 h later. An expression cassette for Rluc was co-transfected and employed to normalize the Fluc values expressed from the pMiR-Report constructs.

2.5. Tumor cell proliferation assays

MCF-7 and MDA-MB-231 cells transfected with P2P, Psi and control cells at 4×10^3/well were plated into 96-well plates and incubated for 24, 48 and 72 h. MTT assays were done according to manufacturer's instructions (Promega, USA).

2.6. Conditioned medium

CM were harvested as previously described [15]. Briefly, cells transfected with P2P, Psi, siRNA and NC were incubated for 48 h, the culture supernatants were harvested, and then subjected to centrifugation and filtration through an 0.22 μm filter (Navigator, USA) to remove any cell debris. Concentration of the CM was measured by the bicinchoninic acid (BCA) assay.

2.7. Assays for in vitro proliferation and tube formation of HUVECs

4×10^3 HUVECs per well in 96-well culture plates were treated with CM from different transfected cells, the CM from untransfected cells were used as control, and then the cells were incubated for 48 h. Relative cell proliferation was determined by MTT assay.

The extracellular matrix (ECM) gel-induced capillary tube formation assay was used as an *in vitro* measurement of angiogenesis. Briefly, a 96-well culture plate was coated with 12.5 μM/well ECM gel (Sigma) and allowed to stand for 30 min at 37 °C. After gel formation, 1×10^5 HUVECs were seeded on matrigel surfaces and grown in the absence or presence of CM. After 18 h, images were photographed at ×40 magnification, and tube formation was scored by a blinded observer as follows: A three-branch point event was scored as one tube. The experiment was repeated thrice with analogous results.

2.8. Transwell migration assay of HUVECs

Transwell migration assays were carried out using 24-well MILLIcell Hanging Cell Culture inserts 8 mm PET (MILLIPORE). Briefly, The lower chambers of the Transwell were seeded with MCF-7 and MDA-MB-231 cells transfected with P2P and Psi plasmid, siRNA and NC and 1×10^5 HUVECs were added to the upper chamber. The medium containing transfected cells in the lower chamber served as chemo-attractant. After co-culturing transfected breast cancer cells and HUVECs for 18 h, migrated cells were stained using methanol and viola crystalline solution. Five random fields from each of the triplicate invasion assays were counted at 40× objectives.

2.9. Chick embryo chorioallantoic membrane angiogenesis assay

Briefly, fertilized chick eggs which have been incubated 6 days were used for the experiment. A window was opened on the top of each egg covered with sterile tape. After 24 h, the transparent tape was cut open and a drug carrier plate with a 15 mm diameter was placed in the chamber. CM was added onto the plate. The eggs were incubated for 48 h and photographed. Blood vessels density was quantified by counting the number of branching blood vessels. Each experiment was conducted three times and represented as a bar diagram.

2.10. Rat aortic ring assay

Dorsal aortas from freshly sacrificed Sprague–Dawley rats were taken out in a sterile manner. They were then cut into 1 mm long rings and each ring was positioned in a collagen pre-coated 96-well plate. Different CM were added to the wells. On day 7th, the rings were analyzed by phase-contrast microscopy and microvessel outgrowths were quantified [16] and photographed. All experimental protocols were approved by the Ethics Committee for Animal Experimentation of China Pharmaceutical University.

2.11. qRT-PCR (quantitative real-time polymerase chain reaction)

Total cellular RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Total RNA was subjected to cDNA synthesis using M-MLV Reverse transcriptase. To determine mRNA expression levels, qRT-PCR was performed on ABI Prism 7500 Sequence Detector (Applied Biosystems, Life Technologies). qRT-PCR primers for VEGF-A, MMP-2, MMP-9, TIMP-1 and TIMP-2 were mentioned in the previous study [17]; The primers: Z2P-F, 5′-ACTACTGCTATCTCC TGGATCTTTT-3′ and Z2P-R, 5′-GAATT ACTTAATCCAGCTGA GAATG-3′ were used for CYP4Z2P; Z2P-UTR-F: 5′-CTTTCCAGA TGGACGCTCCTTACCT-3′ and Z2P-UTR-R: 5′-CCAGCAAGGA AATTAGAATTACTTAATCC-3′ were used for Z2P-UTR. cDNA templates (2 μl) were amplified in a final volume of 20 μl containing the SYBR Green PCR Master mix (Biomics, Jiang Su, China) and primers. The annealing temperature was 68 °C for CYP4Z2P and 58 °C for others. 55 cycles (CYP4Z2P) and 35 cycles (others) were performed. Relative quantification was performed using the $2^{-\Delta\Delta Ct}$ method.

2.12. Western blotting

Transfected and untransfected cells were lysed in lysis buffer RIPA (Beyotime, China). 50 μg of protein was analyzed on 10% SDS–PAGE and electrotransferred to PVDF membranes (Millipore, Bedford, Massachusetts). The membranes were blocked in 5% non-fat dried milk for 60 min at room temperature. Appropriate primary antibodies were applied to the membranes and incubated overnight at 4 °C, and then incubated with IgG-HRP secondary antibody (Bio-Rad, Hercules, California) for 1 h at room temperature. Detection of specific signals was performed using ECL chemiluminescence detection kit (Beyotime, China), followed by exposure to Kodak X-ray films subjected to SDS–PAGE. The protein expression level was normalized to β-actin.

3. Statistical analysis

The experimental results were shown as the mean ± SD. For three independent experiments. The differences between the groups were analyzed using a two-sided Student's *t*-test, and $P < 0.05$ was considered significant.

4. Results

4.1. Secondary RNA structure prediction of the Z2P-UTR and the potential mode of action

To study the roles of CYP4Z2P in breast cancer development, it would be helpful to up-regulate the expression of coding region. However, it was extremely difficult to clone the coding region due to its high homology with CYP4Z1. Previous attempts to clone the coding region resulted in either truncation or other products like CYP4Z1. Recent studies demonstrated that expression of the 3′UTR could bind microRNAs and free mRNA for translation [18]. Thus we hypothesized that expression of the Z2P-UTR in breast cancer cells would promote the expression of CYP4Z2P. Firstly, the mRNA level of Z2P-UTR was detected in breast cancer tissues and non-tumorigenic mammary gland epithelial tissues. As shown in Fig. 1A and B, qRT-PCR results exhibited higher Z2P-UTR expression level in breast cancer tissues than in non-tumorigenic mammary gland epithelial tissues, and the similar results were presented in cells, which were in line with the previous study [12]. And then the secondary RNA structure of Z2P-UTR was predicted in silico. As shown in Fig. 1C, Z2P-UTR was found to form a major loop stem structure in CYP4Z2P. The construct overexpression systems of Z2P-UTR was further attempted in breast cancer cells. As shown in Fig. 1D and E, cells transfected with P2P displayed approximately 50-fold greater Z2P-UTR expression levels in overexpression cells relative to control cells and increased the

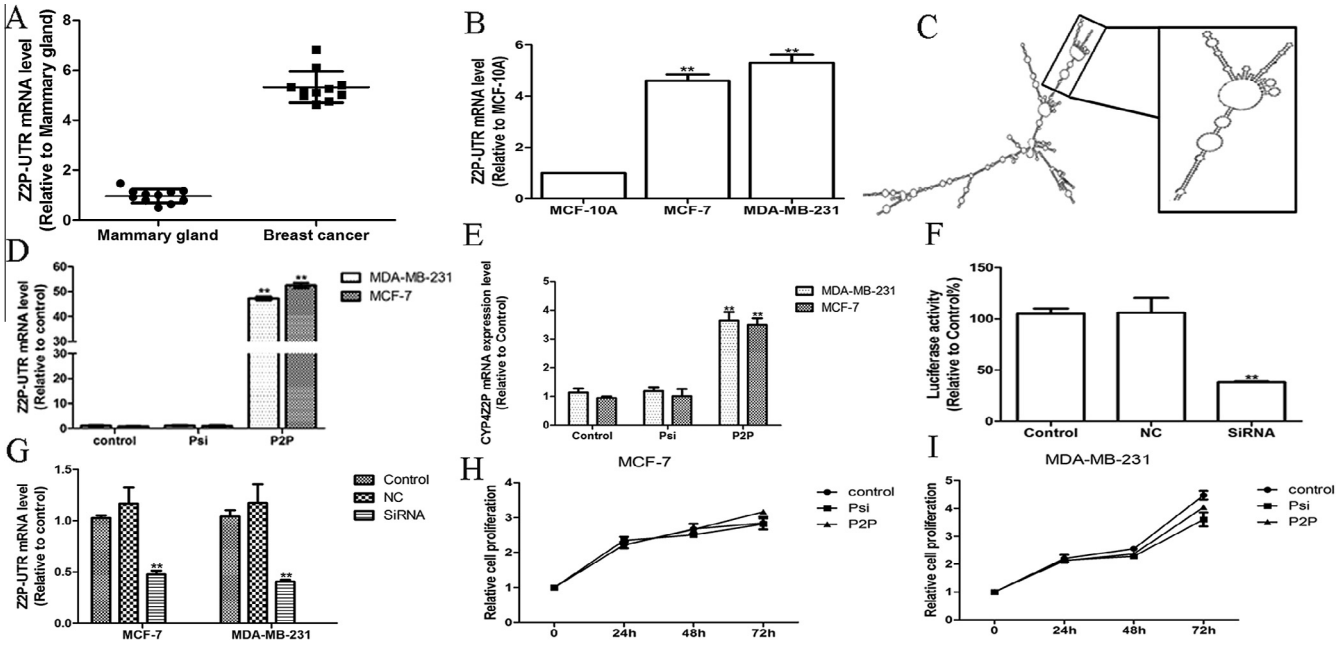

Fig. 1. Overexpression of Z2P-UTR in breast cancer cells does not affect cell growth *in vitro* (A) Z2P-UTR mRNA level is increased in human breast tumor samples. Nine pairs of breast tumors with adjacent mammary gland epithelial tissues were analyzed by qRT-PCR. (B) mRNA level of Z2P-UTR was determined in normal and breast cancer cells by qRT-PCR. (C) The secondary structure of Z2P-UTR was predicted by the minimum free energy (MFE) method implemented on the RNAfold WebServer. (D) Z2P-UTR mRNA expression in MCF-7 and MDA-MB-231 cells transfected or untransfected with P2P was determined by qRT-PCR. (E) CYP4Z2P mRNA expression in MCF-7 and MDA-MB-231 cells transfected or untransfected with P2P was determined by qRT-PCR. (F and G) The knockdown efficiency of Z2P-UTR-siRNA was examined by Luciferase siRNA Target Report Assay and qRT-PCR. (H and I) Different treated cells were plated into 96-well plates and incubated for 24, 48 and 72 h. Cell proliferation was determined by MTT assays. All experiments were detected and analyzed in triplicate, $^{**}P < 0.01$.

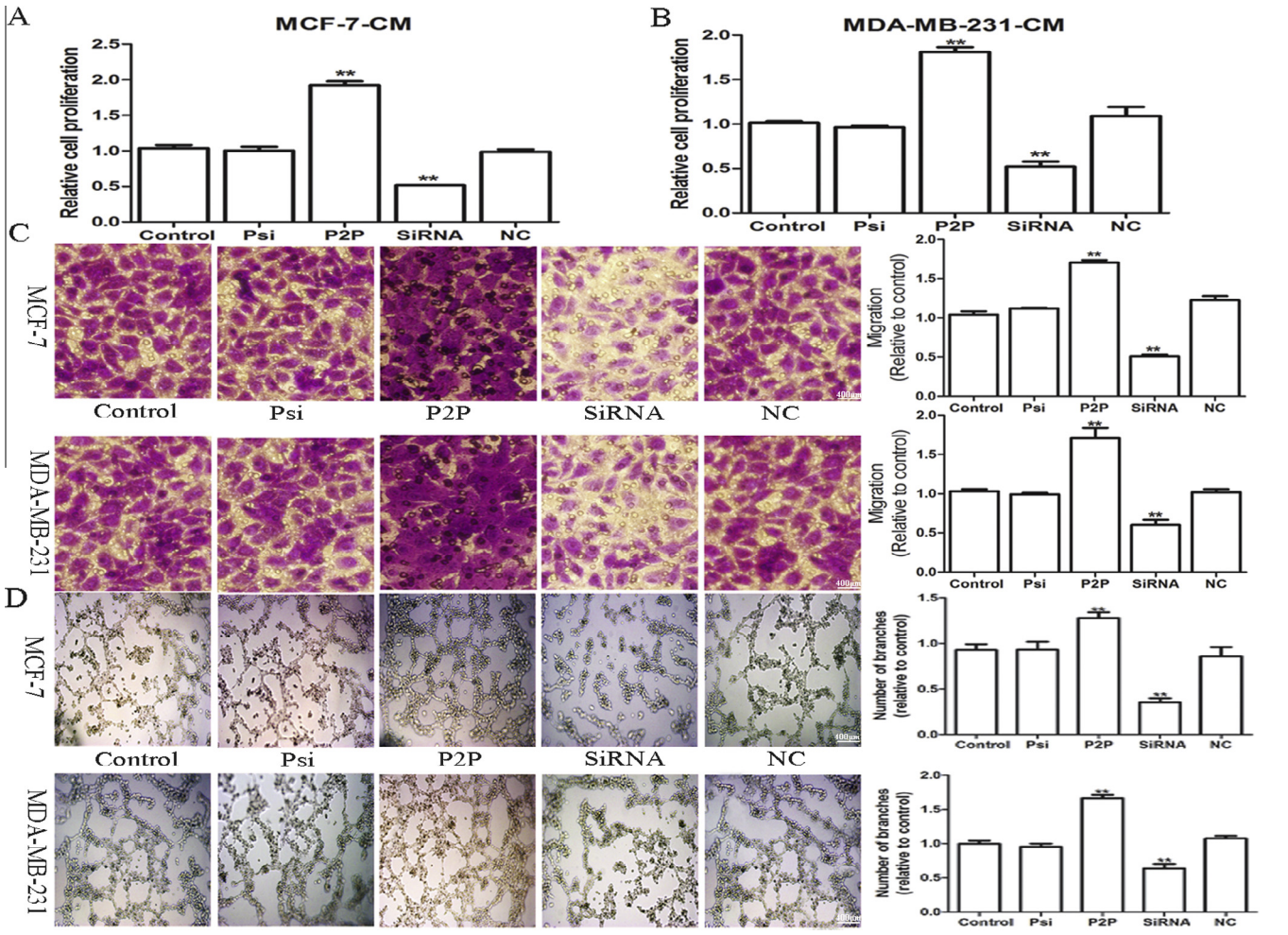

Fig. 2. Z2P-UTR could promote tumor-induced HUVEC cells proliferation, migration and tube formation. (A and B) HUVECs were plated at 96-well plates, and then treated with CM as in the presently described method. Cell proliferation was measured by MTT assay. (C) HUVEC migration assays were performed through co-culturing transfected cells with HUVECs in 24-well plates with transwell chambers, and cell migration was measured in 18 h Transwell assay in a blinded manner. (D) Tube formation assays. Cells were seeded on matrigel-coated wells in the presence of different CM, as indicated, and incubated for 18 h to form a capillary network. The total number of branched tubes was then counted. Values are mean ± SD. $^{**}P < 0.01$ ($n = 3$, Student's t-test).

level of CYP4Z2P, which built verification with our hypothesis. The knockdown efficiency of Z2P-UTR-siRNA was also examined. As shown in Fig. 1F and G, relative luciferase activity of lu-Z2P-UTR cotransfected with siRNA was obviously suppressed relative to control, and the mRNA level of Z2P-UTR transfected with siRNA was decreased more than 50% relative to control. Finally, Z2P-UTR was investigated whether it can promote the proliferation of breast cancer cells by MTT assay and we found there were no significant differences in cell viability between P2P-transfected cells and control cells (Fig. 1H and I), demonstrating the introduction of Z2P-UTR into MCF-7 and MDA-MB-231 cells does not affect cell proliferation.

4.2. Z2P-UTR overexpression promotes tumor-induced proliferation, migration and tube formation of HUVECs

Herein, Z2P-UTR was further investigated whether it has the pro-angiogenic activities. First, MTT assay was performed to explore the effect of Z2P-UTR on proliferation of endothelial cells. The extent of proliferation over 48 h was significantly increased in the groups treated with CM from cells transfected with P2P compared with vector control and this proliferation was reversed substantially by CM from siRNA groups (Fig. 2A and B). Second, transwell migration assays were conducted to examine the migration abilities of endothelial cells. After co-culturing transfected cells and HUVECs with the chamber for 18 h, the numbers of migrating HUVECs co-cultured with P2P-transfected groups were markedly increased and decreased with siRNA-transfected groups (Fig. 2C). Finally, a tube formation assay was carried out with HUVECs. As shown in Fig. 2D, CM from P2P-transfected cells could stimulate capillary tube formation of HUVECs compared with the CM from vector groups. On the contrary, treatment with the CM from siRNA-transfected cells significantly inhibited tumor-induced tube formation. Taken together, these results suggest that Z2P-UTR overexpression promote tumor-induced HUVECs proliferation, migration and tube formation *in vitro*.

4.3. Z2P-UTR promoted angiogenesis in chick embryo chorioallantoic membrane and rat aortic ring assay

The pro-angiogenic potential of Z2P-UTR was further explored via CAM and rat aortic ring assay. Firstly, the CM-impregnated filter disks were placed on blood vessels in avascular sections of CAM for 48 h. The disks and underlying CAM tissues were subsequently harvested. Angiogenesis was scored by counting vessel branches below the filter. Consistent with the data above, CM from P2P-treated cells led to a clearly increase in the development of new embryonic blood vessels as compared with vector control

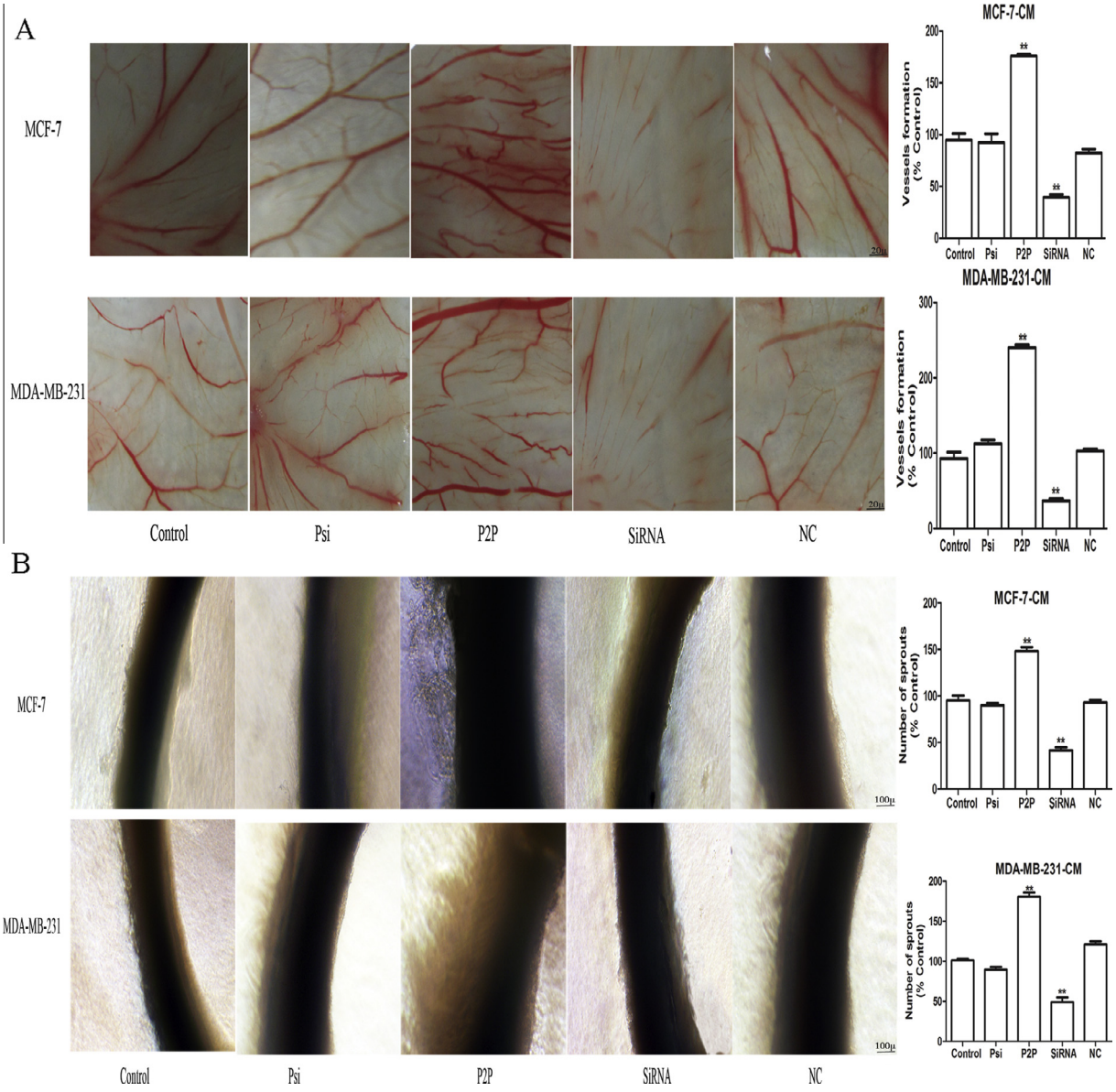

Fig. 3. Z2P-UTR overexpression promotes tumor-induced angiogenesis in CAM of the chick embryo and rat aortic ring assays. (A) Different CM was gently placed on the CAM. The eggs were incubated for 48 h and photographed. Blood vessel density was quantified by counting the number of branching blood vessels. (B) Each rat aortic ring was placed in a collagen pre-coated 96-well plate, different CM was added to the wells. On day 6, the rings were analyzed by phase-contrast microscopy and microvessel outgrowths were quantified and photographed. Values are mean ± SD. $^{**}P < 0.01$ ($n = 4$, Student's t-test).

(Fig. 3A). Conversely, the pro-angiogenic activity of Z2P-UTR was significantly reduced by the CM from siRNA groups. Secondly, as shown in Fig. 3B, the similar results were obtained in rat aortic ring assays. Collectively, these results confirmed the pro-angiogenic potential of Z2P-UTR using *ex vivo* and *ex vitro* models.

4.4. The pro-angiogenic effects of Z2P-UTR are associated with the activation of VEGF-A/VEGFR2 pathway and regulation of MMP-2/MMP-9 and TIMP1/TIMP-2 production

In order to further investigate the mechanism underlying the stimulative activity of Z2P-UTR. The effect of Z2P-UTR on the mRNA and protein level of VEGF-A and VEGFR2 was analyzed. Our results demonstrated the promotive effect of Z2P-UTR on mRNA level of VEGF-A and VEGFR2 (Fig. 4A and B) and hence the protein level (Fig. 4C and D). On the contrary, We found that treatment of MCF-7 or MDA-MB-231 with siRNA against Z2P-UTR markedly abrogated Z2P-UTR-mediated VEGF-A and VEGFR2 expression. Meanwhile, the effect of Z2P-UTR on the mRNA level of MMP-2/MMP-9 and TIMP-1/TIMP-2 were detected by qRT-PCR assay. As shown in Fig. 4E and F, Z2P-UTR could induced the gene expressions of MMP-2/MMP-9 and inhibited TIMP-1/TIMP-2 at the mRNA level. Taken together, our results demonstrate that Z2P-UTR could induce angiogenesis via activation of paracrine VEGF/VEGFR2 pathway and regulation of MMP-2/MMP-9 and TIMP-1/TIMP-2 production.

5. Discussion

Nowadays, it is widely admitted that disrupting the development of blood vessels can upset the growth of tumors [19]. Angiogenesis is a dynamic process regulated by a number of angiogenic molecules [20]. As such, targeting the angiogenesis-related markers can be employed as a strategy for cancer therapy.

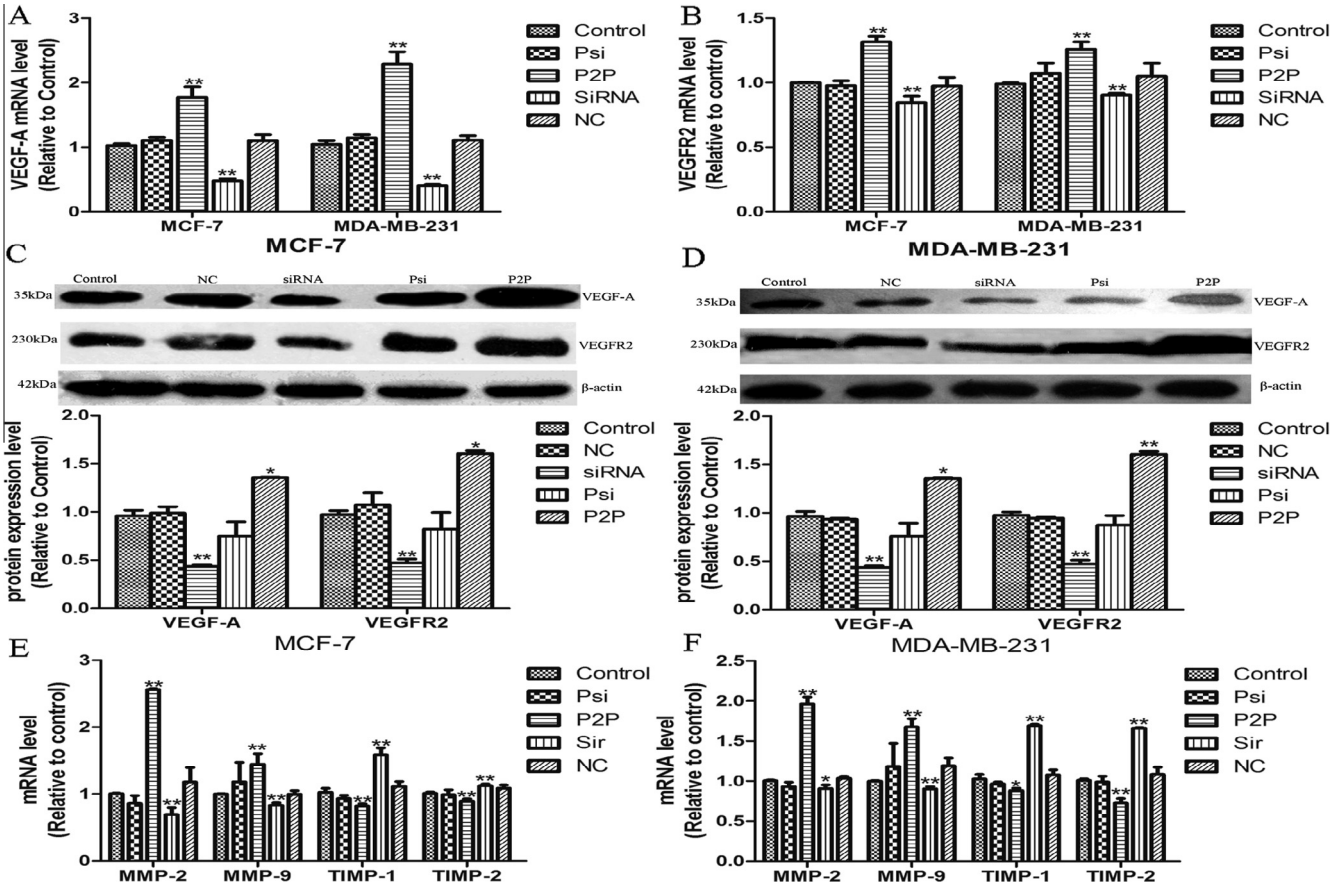

Fig. 4. Z2P-UTR overexpression promotes the mRNA and protein levels of VEGF-A and regulates MMP-2/MMP-9, TIMP-1/TIMP-2 mRNA levels in breast cancer cells in MCF-7 and MDA-MB-231 cells, hence the CM on VEGFR2 in HUVECs. (A) mRNA expression of VEGF-A from MCF-7 and MDA-MB-231 cells transfected or untransfected with P2P was examined using qRT-PCR assays. (B) mRNA level of VEGFR2 from HUVECs with different CM was examined using qRT-PCR assays. (C and D) The protein level of VEGF-A and VEGFR2 was determined by Western blot assays. (E) mRNA level of MMP-2/MMP-9 and TIMP-1/TIMP-2 from cells transfected or untransfected with P2P was examined using qRT-PCR. All experiments were detected and analyzed in triplicate. Values are mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ ($n = 3$, one-way ANOVA).

Over the past decades, the functional characterization of genes has primarily focused on the role of coding sequences without regarding to their 3′UTRs. Indeed, the recent explosion in knowledge demonstrating the importance of 3′UTRs in diseases, including cancer, has brought these heretofore neglected molecules to the forefront [21]. 3′UTRs contain multiple regulatory elements and have a widespread influence on mRNA translation, stability and subcellular localization [22]. Thus, aberrant oncogenes and tumor suppressor genes expression caused by 3′UTRs may increasingly prove to play a key role in cancer development [23]. Moreover, with the presentation of the ceRNA hypothesis, the classical dogma that a gene must be dependent on the coding sequences to exert its function has been challenged because microRNAs binding sites in 3′UTRs can sponge microRNAs and thereby lead to the derepression of microRNA target genes [24]. In addition, the potential employment of 3′UTRs as novel biomarkers in the clinical oncology setting is supported by the widespread observations of 3′UTRs reprogramming in cancers when compared with normal controls [25]. Therefore, it is becoming clear that insight into the widespread influences exerted by 3′UTRs may help us identify novel biomarkers for cancer diagnosis and present new opportunities for 3′UTR-based targeted therapy.

Here, we focus on discussing CYP4Z2P that maybe possess pro-cancerous effect since its expression level is often elevated in breast cancer tissues [12]. Due to its high coding sequences homology with CYP4Z1, it is presently not possible to clone by the traditional molecular technology. We have previously cloned its coding sequences but found it was difficult to obtain. This limitation can now be partially overcome by expressing its 3′UTR. Our studies showed that ectopic expression of the Z2P-UTR can lead to increased expression of endogenous CYP4Z2P, providing an ideal approach to study its functions. There are two novel observations made in our study. First, overexpression of Z2P-UTR in breast cancer cells could promote tumor angiogenesis evidenced by *in vitro* HUVECs models, *ex vivo* CAM and *ex vitro* rat aortic ring assay. To our knowledge, this is the first study that directly demonstrated the pro-angiogenic property of the 3′UTR of pseudogene CYP4Z2P. Second, we have demonstrated for the first time that Z2P-UTR overexpression led to breast cancer progression partly via targeting VEGF-A/VEGFR2 pathway and regulating the mRNA level of MMP-2/MMP-9 and TIMP-1/TIMP-2.

Our data showed that CM from Z2P-UTR-overexpression cells significantly enhanced proliferation, migration and tube formation of HUVECs. Meanwhile, Z2P-UTR overexpression could also increase new embryonic blood vessels in CAM mode, as well as sprouting of aortic ring in rat aortic ring assay. Conversely, we found the pro-angiogenic activities of Z2P-UTR were significantly inhibited by knockdown of Z2P-UTR. To confirm this conclusion, we detected angiogenic marker including MMP-2 and MMP-9 [26], as well as the anti-angiogenic marker like TIMP-1 and TIMP-2 [27] in breast cancer cells, which showed that Z2P-UTR was inversely correlated with TIMP-1 and TIMP-2, but positively correlated with MMP-2 and MMP-9 in mRNA level.

To elucidate the molecular mechanism underlying Z2P-UTR in angiogenesis, we focus on the paracrine VEGF-A/VEGFR2 signaling pathway, which has been the targeting pathway for the most

promise drug of anti-angiogenesis [28]. We found that Z2P-UTR overexpression could promote the expression of VEGF-A in breast cancer cells and vice versa. Also, CM from Z2P-UTR-overexpressed breast cancer cells could activate the expression of VEGFR2 in HUVEC cells, which can be rescued by CM from siRNA groups. Taken together, these results suggest that the activation of paracrine VEGF-A/VEGFR2 pathway is associated with Z2P-UTR-induced angiogenesis.

To the best of our knowledge, this is the first study to demonstrate the potential role of 3′UTR of pseudogene CYP4Z2P in the angiogenesis of breast cancer. These results provide strong evidence in support of the pro-angiogenic activities of Z2P-UTR in breast cancer, which is a characteristic that can be exploited and potentially applied to gene therapy. However, further investigation is needed to explore what factors could influence the function of Z2P-UTR as an activator on angiogenesis and whether targeting Z2P-UTR might be an effective novel method in breast cancer treatment.

Conflict of interest

The authors declare that they have no conflict of interests.

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