PLAU is associated with cell migration and invasion and is regulated by transcription factor YY1 in cervical cancer

YANJUN GAO[1*], XINMEI MA[2*], HUANXI LU[3], PAN XU[2] and CHENGLING XU[1]

[1]Clinical Laboratory and [2]Central Laboratory, Liaocheng People's Hospital; [3]Department of Gynecology, Dongchangfu People's Hospital, Liaocheng, Shandong 252000, P.R. China

Received April 17, 2022; Accepted September 30, 2022

DOI: 10.3892/or.2022.8462

Abstract. Cervical cancer, one of the most common malignancies, has a poor survival rate. The identification of more biomarkers for cervical cancer diagnosis and therapy is urgently needed. Plasminogen activator urokinase (PLAU) exerts multiple biological effects in various physiological and pathological processes; however the role of PLAU in cervical cancer progression is not fully understood. In the present study, the involvement and transcriptional regulation of PLAU in cervical cancer were explored. The expression of PLAU in cervical cancer was first analyzed, and PLAU was found to be overexpressed. *In vitro* experiments demonstrated that the migration and invasion of HeLa and HT3 cells were significantly suppressed by PLAU knockdown. Additionally, the core promoter of PLAU was confirmed, and the transcription factor YinYang 1 (YY1) was found to regulate PLAU mRNA expression. Overall, the present study elucidated the direct association between PLAU and cervical cancer, suggesting the YY1/PLAU axis as a potential novel therapeutic target for cervical cancer.

Introduction

Cervical cancer is the fourth most common malignancy in women (1,2). Although therapeutic methods and treatment techniques for tumors have improved recently, the outcomes for patients with cervical cancer remain poor (3). The main reasons for poor outcomes are recurrence and metastasis, which remain huge challenges in the treatment of cervical cancer. Therefore, it is necessary to identify biomarkers for cervical cancer diagnosis and treatment.

Plasminogen activator urokinase (PLAU), also known as name urokinase-type plasminogen activator (uPA), exerts multiple biological effects in various physiological and pathological processes, such as keratinocyte proliferation (4), air-way inflammation (5) and rheumatoid arthritis (6). Additionally, PLAU is an important modulator of tumorigenesis and progression. PLAU is overexpressed in several tumors, including head and neck squamous cell carcinoma (HNSCC) (7), gastric adenocarcinoma (8) and breast cancers (9). PLAU participates in tumor progression by promoting tumor cell proliferation, cell migration, invasion, and epithelial-mesenchymal transition (10-12). Given the oncogenic role of PLAU, it is considered a potential prognostic marker for tumors (7,13,14). To date, PLAU has been reported to be involved in cervical cancer progression. PLAU is upregulated and plays a vital role in the invasion and metastasis of advanced cervical cancer (15-17). Therefore, it is necessary to elucidate the mechanisms by which PLAU expression is regulated.

PLAU is regulated by several factors. Certain miRNAs have been reported to regulate PLAU expression: miR-23b-3p upregulates PLAU, thereby affecting HNSCC progression (7); meanwhile, the lncRNA TRPM2-AS promotes the progression of gastric adenocarcinoma by regulating PLAU (8). In addition to miRNAs, transcription factors are important for gene regulation. Transcription factors are the main elements of transcription, which is the process of RNA synthesis according to the genomic DNA sequence (18). Transcription factors can bind to specific sequences in the promoter of target genes, thereby inducing the upregulation or downregulation of target genes (18-20). Several transcription factors regulate PLAU at the transcriptional level. Specificity protein 1 (Sp1) and Sp3 transcription factors can aid in the transcription of PLAU (21,22). Transcription factors Ets1 and Ets2 can bind to the enhancer region of PLAU, thereby increasing transcription (23). However, the mechanisms underlying PLAU transcription remain unclear.

YinYang1 (YY1), a member of the GLI-Krüppel family of zinc-finger DNA-binding proteins, is a transcription factor widely expressed in various tissues. YY1 can activate the transcription of several target genes, such as FOXE1, TNK2-AS1 and LINC00466 (24-26). By regulating these target genes,

Correspondence to: Dr Chengling Xu, Clinical Laboratory, Liaocheng People's Hospital, 67 Dongchangxi Road, Liaocheng, Shandong 252000, P.R. China
E-mail: xucllcrmyy@sina.com

Dr Pan Xu, Central Laboratory, Liaocheng People's Hospital, 67 Dongchangxi Road, Liaocheng, Shandong 252000, P.R. China
E-mail: panxulc@126.com

*Contributed equally

Key words: cervical cancer, plasminogen activator urokinase, promoter, transcription, transcription factor YinYang 1

YY1 participates in various biological functions, including cell proliferation (27,28), apoptosis (29), invasion and migration (30), radioresistance (31) and drug resistance (32). However, it is unknown whether YY1 is associated with PLAU transcription.

In the current study, the expression of PLAU in cervical cancer was analyzed and the role of PLAU in cervical cancer cell proliferation, migration and invasion, as well as the location of the PLAU core promoter, were determined. An important transcription factor, YY1, which is involved in PLAU transcription, was identified.

Materials and methods

Clinical specimens. Cervical cancer tissues (16 pairs) and the matched adjacent normal tissues were collected at Liaocheng People's Hospital (Liaocheng, China) from February 2021 to November 2021. All the cases have not received any treatment before hospitalized and were diagnosed as cervical cancer by two pathologists. The present study was approved (approval no. LC2021015) by the Ethics Committee of Liaocheng People's Hospital (Liaocheng, China). Written informed consent was provided by all patients.

Cell lines and cell culture. Both HeLa and HT3 cell lines were purchased from the American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS (both from Thermo Fisher Scientific, Inc.) in an incubator with 5% CO_2 at 37˚C.

Analyzing the expression of PLAU in cervical cancer based on UALCAN database. The UALCAN database (http://ualcan.path.uab.edu/) is a comprehensive web resource for analyzing cancer OMICS data, which can be used for performing pan-cancer gene expression analysis (33).

RNA extraction. For cervical cancer tissues and matched adjacent normal tissues, tissues were placed into microtubes containing 1 ml precooled TRIzol® (Thermo Fisher Scientific, Inc.) and smashed in a tissue grinding machine. For cells cultured in six-well plates, after the media were discarded, 1 ml TRIzol reagent was added to each well. After the tissues or cells were lysed using TRIzol reagent, the lysates were transferred to new microtubes. The RNA was extracted by adding 500 μl chloroform to each tube. The RNA was then separated by adding 200 μl isopropanol. RNA concentration was measured using an ultramicro spectrophotometer (Implen GmbH).

Reverse transcription-quantitative PCR (RT-qPCR). RNA (2 μg) was reverse-transcribed into cDNA using a Titan One Tube RT-PCR kit (Roche Diagnostics) following the manufacturer's instruction. YY1 and PLAU mRNA expression in cervical cancer tissues and adjacent normal tissues were measured with TaqMan Real-Time PCR Assays using a BeyoFast™ Probe qPCR Mix kit (Beyotime Institute of Biotechnology) following the manufacturer's protocol. The primers and probes used were listed in Table SI. The thermocycling conditions of TaqMan Real-Time PCR assays were as follows: 95˚C, 10 min; 95˚C, 15 sec; and 60˚C, 60 sec for 40 cycles. The expression of YY1, PLAU, MMP-9, E-cadherin, and Cyclin D1 in HeLa cells and HT3 cells were measured with SYBR Green Real-Time PCR Assays using a FastStart Universal SYBR Green Master (ROX) kit (Roche Diagnostics) following the manufacturer's protocol with the following conditions: 95˚C, 10 min; 95˚C, 15 sec; 60˚C, 60 sec for 40 cycles, and the primers used were listed in Table SII. The expression of target genes was quantified using the $2^{-\Delta\Delta Cq}$ method, in accordance with a previous study (34).

PLAU and YY1 knockdown by lentiviral infection. The lentiviral vectors (Lv)-small hairpin RNA (shRNA) based on a 3rd generation lentiviral system targeting PLAU (Lv-shSPP1), Lv-shYY1, and negative control (Lv-shCon) were purchased from Shanghai GeneChem Co., Ltd. Briefly, 1.5 μg shRNA vector and 1.5 μg package mix plasmids were transfected into 293T cells (cat. no. C6008; Beyotime Institute of Biotechnology) with Lipofectamine 3000® reagent (cat. no. L3000001; Thermo Fisher Scientific, Inc.) for 24 h at 37˚C, and the medium was then harvested and the virus was extracted by 80,000 x g-ultracentrifugation at 4˚C for 2 h in a centrifuge (Beckman Coulter, Inc.). For lentiviral transduction, the cells were seeded in six-well plates at a density of 50,000 cells/well. The lentiviral vector was added at a multiplicity of infection of 15, with 5 μl polybrene (Sigma-Aldrich; Merck KGaA) per well. After 72 h, cells infected with the lentiviral vectors were selected using 1 μg/ml puromycin (Beyotime Institute of Biotechnology). Knockdown efficiency was evaluated using RT-qPCR.

Transwell assay. Transwell migration assays were performed using Transwell plates (Corning, Inc.) with 8 μm-wells in the membrane. For the migration assay, cells in 200 μl DMEM without FBS were seeded into the upper chamber of the plate at 100,000 cells/well. In the lower chamber, 500 μl DMEM media with 15% FBS was added. After 12 h, the cells remaining in the upper chamber were wiped away and the cells transferred to the lower chamber were stained with crystal violet (Beyotime) at 25˚C for 3 min. Then the stained cells were observed using a T2R inverted (T2R, Nikon Corporation) fluorescence microscope at x200 magnification under brigbtfield counted in three randomly selected visual fields. The invasion assay was similar to the transwell migration assay, except that the upper chambers were pre-coated with 20 μg of ECM gel (MilliporeSigma).

Cell Counting Kit-8 (CCK-8) proliferation assay. HeLa and HT3 Cells were seeded in 96-well plate at 5,000 cells per-well and cultured in an incubator with 5% CO_2 at 37˚C. After being cultured for 0, 24 and 48 h, 10 μl of CCK-8 reagents (cat. no. GB707; Dojindo, Laboratories, Inc.) were added into each well, and incubated for 1 h at 37˚C. Then the optical density was measured at 450 nm in a spectrophotometer (BioTek Instruments, Inc.,).

Construction of PLAU promoter reporter. The sequence of the PLAU promoter was obtained from NCBI (https://www.ncbi.nlm.nih.gov/nuccore/NG_011904.1?from=5001&to=11398&report=genbank). The region of -1500/+18 region (transcription starting site was considered as +1) was cloned from the

genomic DNA of HeLa cells and inserted into the pGL3-basic vector. The promoter-reporter was confirmed by Sanger sequencing in an Illumina NextSeq 500 instrument (Illumina, Inc.) at Sangon Biotech Co., Ltd. and named pGL3-PLAU.

Deletion mutation and site-directed mutation. In accordance with a previous study (35), the deletion mutation was performed by self-linking the fragment of pGL3-PLAU with the exception of the region needing to be deleted. The fragment was obtained using a PCR procedure (95˚C, 1 min; 95˚C, 15 sec; 55˚C, 30 sec; 72˚C, 5 min for 30 cycles; 72˚C, 10 min) with pGL3-PLAU as the template. The forward primer was designed based on the sequence at the downstream of the deletion region of pGL3-PLAU, and the reverse primer was designed based on the sequence at the upstream of the deletion region of pGL3-PLAU. The primers were listed in Table SIII. Subsequently, the PCR products were extracted using an Agarose Gel Extraction kit (cat. no. D0056; Beyotime Institute of Biotechnology). Following incubation with T4 polynucleotide kinase (New England BioLabs, Inc.) at 37˚C for 1 h, the product was self-linked using a T4 DNA ligase (New England BioLabs, Inc.) at 37˚C for 1 h. The deletion mutants were confirmed by sequencing at Sangon Biotech Co., Ltd. The primer used for DNA sequencing was: 5'-CTAGCAAAATAG GCTGTCCC-3'.

According to a previous study (35), site-directed mutagenesis was performed using PCR procedure (95˚C, 1 min; 95˚C, 15 sec; 55˚C, 30 sec; 72˚C, 5 min for 30 cycles; 72˚C, 10 min) with pGL3-PLAU (-900/-1100) as the template; and the primers used are listed in Table SIV. pGL3-PLAU-MTA and pGL3-PLAU-MTB were constructed based on the pGL3-PLAU as template. pGL3-PLAU-MTA were constructed based on the pGL3-PLAU-MTA as template. After digestion with Dpn I-restricted enzyme (New England BioLabs, Inc.), the product was transferred to the Top 10 bacterial *E. coli* cells (Beyotime Institute of Biotechnology) for amplification. The mutations were confirmed by Sanger sequencing.

Luciferase activity assay. HeLa cells were seeded in 12-well plates at 50,000 cells/well. After the cells reached 80% confluence, 1 μg of promoter-reporter and 1 μg pRL *Renilla* Luciferase Control Reporter Vectors (negative control) were transfected into the cells using Lipofectamine 3000 reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After 24 h, the cells were lysed in the lysis buffer of the Promega E1500 Luciferase Assay System (Promega Corporation) and the cell lysates were incubated with the luciferin of the Promega E1500 Luciferase Assay System. The activity of *Renilla* luciferase was measured using a *Renilla*-Glo® Luciferase Assay Kit (cat. no. E2710; Promega Corporation). Luciferase activity was measured using the Promega LumiPro instrument (Promega Corporation). The promoter activity was normalized as follows: Promoter-reporter activity/*Renilla* luciferase activity.

Statistical analysis. Transwell assay, CCK-8 assay, RT-qPCR and luciferase activity assays were biological repeated three times and the data are presented as the mean ± SD. Statistical analysis was performed using the SPSS software (version 16.0 IBM). Differences of gene expression between normal tissues and cervical cancer tissue of TCGA were analyzed with unpaired Student's t-test. The significance of the differences in the expression of YY1 and PLAU between the cervical cancer tissues and the paired normal tissues was determined using a paired t-test. Tukey's post hoc test following one-way ANOVA was used to evaluate the significance of the differences among multiple independent groups. The correlation between PLAU expression and YY1 was analyzed using Spearman's rank correlation coefficient analysis with Rho and P-values as indicated. P<0.05 was considered to indicate a statistically significant difference.

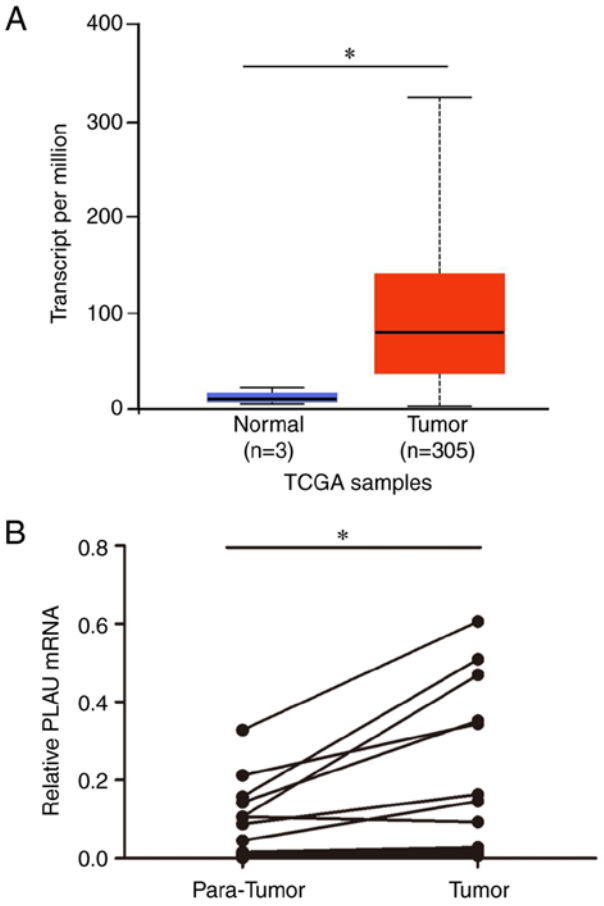

Figure 1. PLAU is overexpressed in cervical cancer. (A) PLAU expression in normal tissues and cervical cancer tissues based on the UALCAN database. (B) Expression of PLAU in 16 cervical cancer tissues and matched tumor-adjacent normal tissues was detected using reverse transcription-quantitative PCR. *P<0.05. PLAU, plasminogen activator urokinase; TCGA, The Cancer Genome Atlas.

Results

PLAU is overexpressed in cervical cancer. The expression of cervical cancer in UALCAN (http://ualcan.path.uab.edu/) was analyzed based on The Cancer Genome Atlas (TCGA) database. As shown in Fig. 1A, cervical cancer tissues showed higher PLAU expression than normal cervical tissues. To further verify the PLAU expression, PLAU expression was measured and compared in 16 cervical cancer tissues and matched para-cancer tumors using RT-qPCR. As revealed in Fig. 1B, PLAU was upregulated in cervical cancer tissues compared with that in matched para-cancer normal tissues.

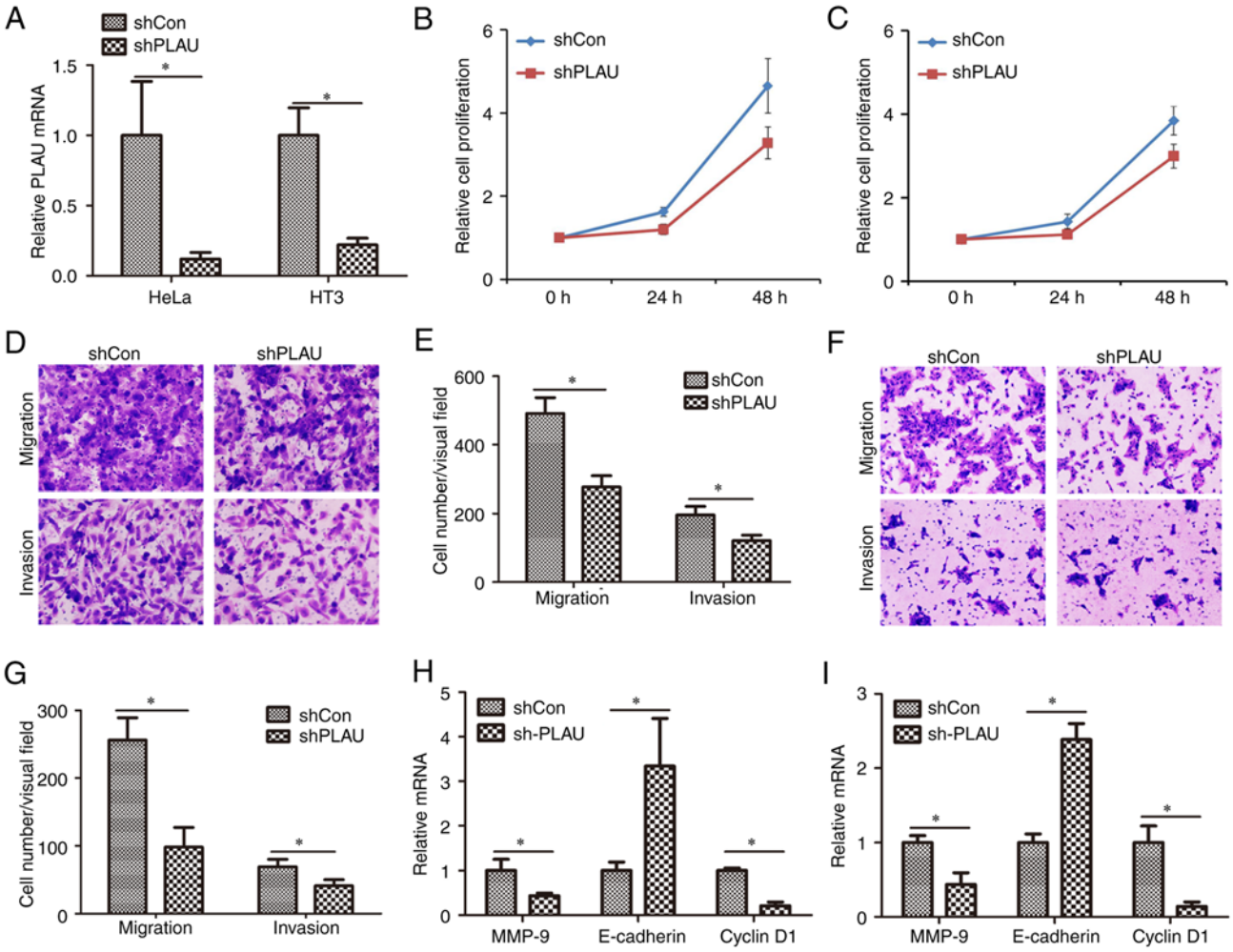

Figure 2. Knockdown of PLAU inhibits cell proliferation, migration and invasion of cervical cancer. (A) PLAU mRNA expression in HeLa and HT3 cells infected with Lv-shCon or Lv-shPLAU. (B and C) The proliferation of (B) HeLa and (C) HT3 cells with Lv-shCon or Lv-shPLAU infection. (D) The migration and invasion of HeLa cells with Lv-shCon or Lv-shPLAU infection. (E) Counting of migratory and invasive HeLa cells. (F) Cell migration and invasion of HT3 cells with Lv-shCon or Lv-shPLAU infection. (G) Counting of migratory and invasive HT3 cells. (H and I) Expression of cyclin D1, MMP-9, and E-cadherin in (H) HeLa and (I) HT3 cells with Lv-shCon or Lv-shPLAU infection. *P<0.05. PLAU, plasminogen activator urokinase; Lv, lentiviral; sh-, short hairpin.

Knockdown of PLAU inhibits cell proliferation, migration and invasion of cervical cancer. PLAU was knocked down in two cervical cancer cell lines, HeLa and HT3, by lentiviral infection. As revealed in Fig. 2A, PLAU expression was significantly decreased in HeLa cells infected with Lv-shPLAU compared with PLAU expression in those infected with Lv-shCon; similarly, PLAU expression was significantly decreased in HT3 cells infected with Lv-shPLAU compared with PLAU expression in those infected with Lv-shCon. Cell proliferation was measured using CCK-8 assay (Fig. 2B and C). It was demonstrated that proliferation of cells infected with Lv-shPLAU was significantly inhibited compared with those infected with Lv-shCon. HeLa cells infected with Lv-shCon or Lv-shPLAU were subjected to Transwell assays to evaluate cell migration and invasion capacities. As revealed in Fig. 2D and E, in HeLa cells, Lv-shPLAU infection induced weaker migration and invasion capacities than Lv-shCon infection. Similarly, Lv-shPLAU infection weakened the migration and invasion capacities of HT3 cells compared with Lv-shPLAU infection (Fig. 2F and G). Furthermore, the mechanism of PLAU regulating cell proliferation, migration and invasion was determined by measuring the mRNA expression levels of several proliferation, migration and invasion-associated genes. Knockdown of PLAU induced downregulation of MMP-9 and cyclin D1 mRNA levels, as well as upregulation of E-cadherin mRNA levels in HeLa cells (Fig. 2H) and HT3 cells (Fig. 2I). These results suggested that PLAU knockdown inhibits the migration and invasion of cervical cancer cells.

Identification of the PLAU core promoter. Considering the overexpression and important role of PLAU in cervical cancer, it is necessary to elucidate the regulation of PLAU. A PLAU promoter-reporter, pGL3-PLAU, was constructed; and based on that a series of deletion mutant types of the PLAU promoter-reporter were constructed. The activity of these reporters was compared and it was found that when the -1100/-901 region was deleted, the promoter activity significantly decreased (Fig. 3A). The -1100/-901 region was cloned into the pGL3-basic vector and the activities of pGL3-PLAU and pGL3-PLAU (-1100/-901) were compared. As revealed in Fig. 3B, until it was narrowed to -1100/-901, the promoter still possessed 78.13% activity of the full-length promoter,

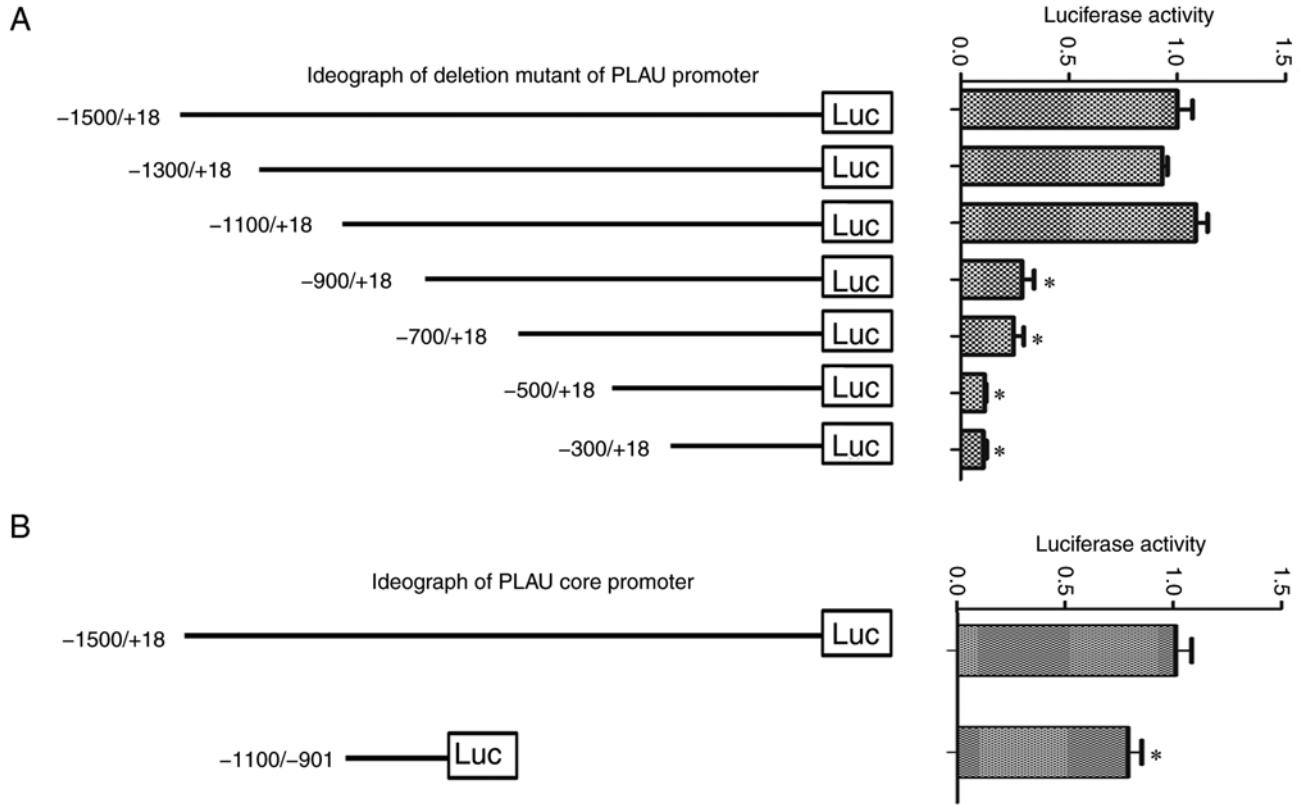

Figure 3. Identification of the PLAU core promoter. (A) Based on the pGL3-PLAU, a series of deletion mutants of the PLAU promoter (-1500/+18, -1300/+18, -1100/+18, -900/+18, -700/+18, -500/+18, -300/+18) were constructed and transfected into HeLa cells to measure the luciferase activity. Left panel, the ideograph of the deletion mutants of the PLAU promoter; right panel, relative luciferase activity of each mutant. (B) Left panel, ideograph of the PLAU core promoter; right panel, relative luciferase activity. *P<0.05 vs. the -1500/+18. PLAU, plasminogen activator urokinase; luc, luciferase.

suggesting that the core promoter of PLAU is located in the -901/-1100 regions.

YY1 regulates PLAU expression at the transcriptional level. The -1100/-901 sequence was submitted to the online PROMO software (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3). Several transcription factors were predicted, including TF II D, YY1, GATA1 and GR-β (Fig. 4A). The expression of the predicted transfection factors was analyzed (Fig. S1). The expression of C/EBPβ, GATA1, TBP (encoding TBP, the key subunit of TF II D), PR (encoding PRA and PRB), SULTIE1 (encoding c-Est-2) and GR (encoding GR and GR-α) showed no statistically significant difference between normal and cervical cancer tissues. However, the expression of YY1 was overexpressed in cervical cancer compared with normal tissues (Fig. 4B). YY1 expression was further evaluated in the collected cervical tissues. As revealed in Fig. 4C, YY1 in cervical cancer was higher than in adjacent normal tissues. In addition, it was found that the expression of PLAU is positively correlated to YY1 in cervical cancer tissues (Fig. 4D). Furthermore, the role of YY1 in cervical cancer cell migration and invasion was evaluated. Cervical cancer cells were infected with the lentivirus Lv-shYY1 and it was observed that knockdown of YY1 inhibited cell migration and invasion of HeLa (Fig. 4E and F) and HT3 cells (Fig. 4H and I). Besides, knockdown of YY1 induced downregulation of MMP-9 and cyclin D1 mRNA levels, as well as upregulation of E-cadherin mRNA levels in HeLa (Fig. 2G) and HT3 cells (Fig. 2J). PLAU mRNA was also measured; as demonstrated in Fig. 4K, Lv-shYY1 infection decreased PLAU mRNA, indicating that YY1 is a regulator of PLAU. To evaluate whether YY1 regulates PLAU through transcription, the PLAU core promoter-reporter was transfected into cells with or without YY1 knockdown and promoter activity was determined. The results showed that following Lv-shYY1 infection, core promoter activity was downregulated in HeLa cells (Fig. 4L), indicating that YY1 regulates the transcription of PLAU.

Identification of the YY1 binding site on the PLAU promoter. According to PROMO software, there are two putative binding sites in the core promoter region of PLAU: Site A is located at -1024/-1030, while Site B is located at -974/-980. To determine which site was responsible for YY1 regulating PLAU, Site A and Site B were mutated separately and Site A and Site B were double-mutated; they were named pGL3-PLAU-MTA, pGL3-PLAU-MTB and pGL3-PLAU-MTA&B, respectively (Fig. 5A). pGL3-PLAU-MTA, pGL3-PLAU-MTB and pGL3-PLAU-MTA&B were transfected into HeLa cells with or without YY1 knockdown and promoter activity was measured. As revealed in Fig. 5B, YY1 knockdown induced a decrease in the activity of pGL3-PLAU and pGL3-PLAU-MTA, instead of PGL3-PLAU-MTB and pGL3-PLAU-MTA&B, indicating that Site B is the main site responsible for YY1 regulating PLAU.

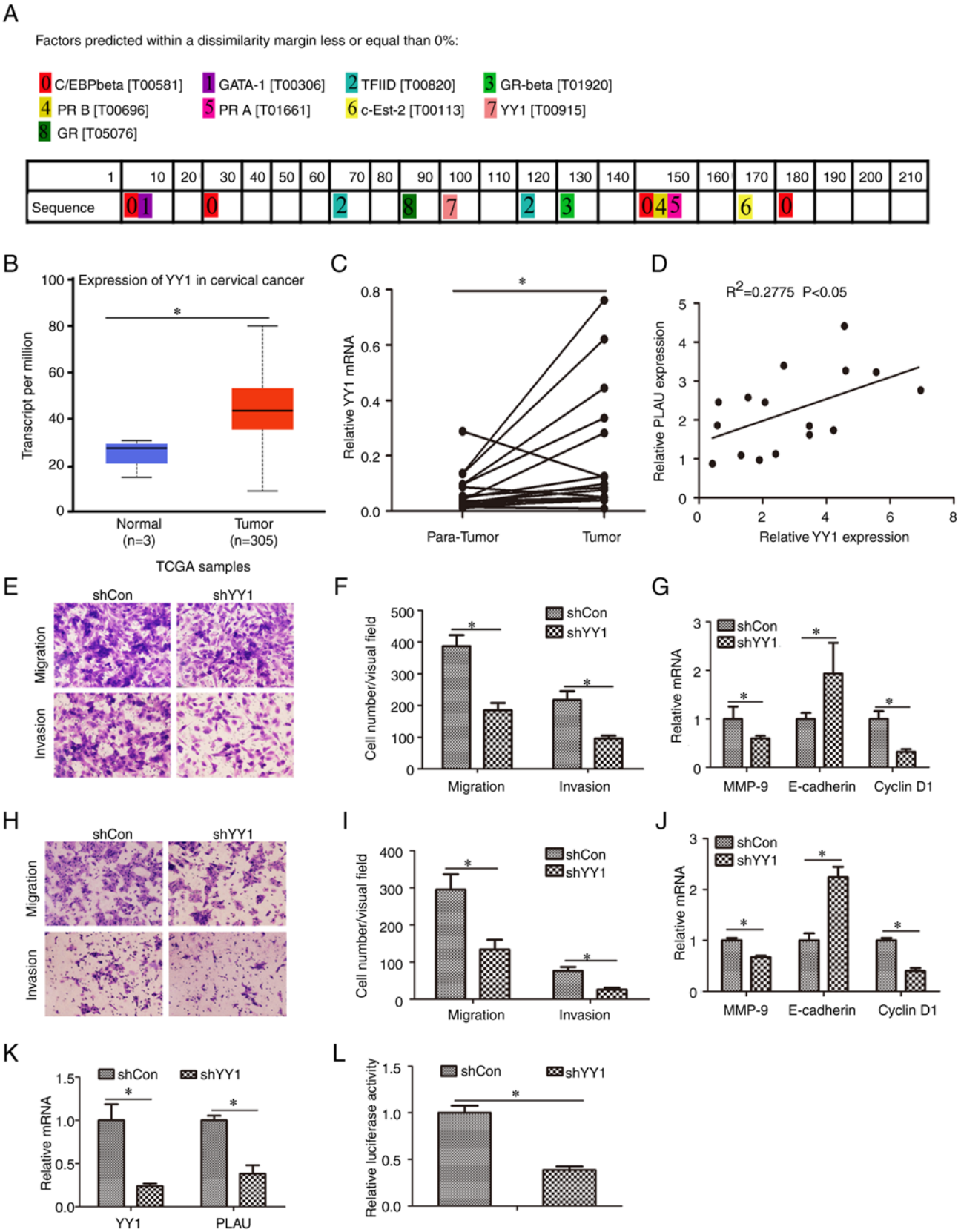

Figure 4. YY1 regulates PLAU expression at the transcriptional level. (A) A total of 8 transcriptional factors were predicted to bind to the PLAU core promoter according to PROMO software. (B) YY1 expression in normal and cervical cancer tissues based on the UALCAN database. (C) Expression of YY1 in 16 cervical cancer tissues and matched tumor-adjacent normal tissues was detected using reverse transcription-quantitative PCR. (D) Correlation of PLAU to YY1 expression in cervical cancers. (E) The migration and invasion of HeLa cells with Lv-shCon or Lv-shYY1 infection. (F) Counting of migratory and invasive HeLa cells. (G) mRNA expression levels of cyclin D1, MMP-9, and E-cadherin in HeLa cells with Lv-shCon or Lv-shYY1 infection. (H) The migration and invasion of HT3 cells with Lv-shCon or Lv-shYY1 infection. (I) Counting of migratory and invasive HT3 cells. (J) mRNA expression levels of cyclin D1, MMP-9 and E-cadherin in HT3 cells with Lv-shCon or Lv-shYY1 infection. (K) PLAU mRNA expression in HeLa cells with Lv-shCon or Lv-shYY1 infection. (L) PLAU core promoter activity in HeLa cells with Lv-shCon or Lv-shYY1 infection. *P<0.05. YY1, transcription factor YinYang 1; PLAU, plasminogen activator urokinase; Lv, lentiviral; sh-, short hairpin.

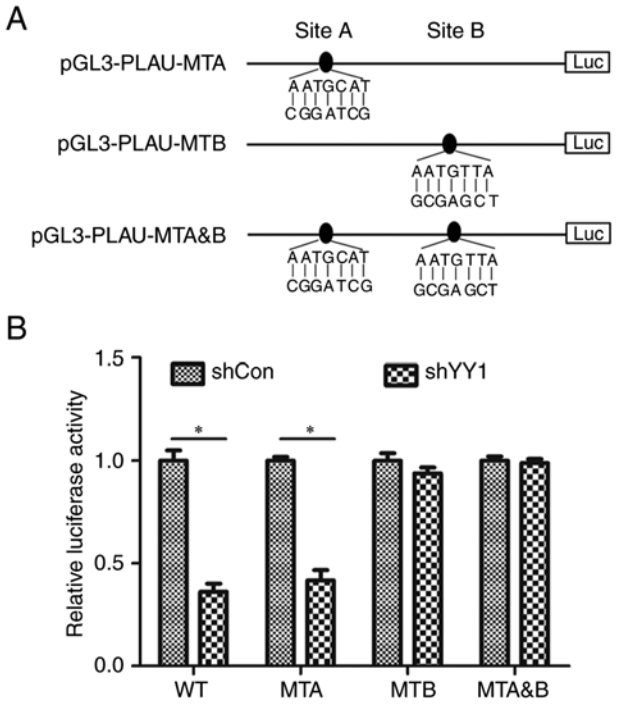

Figure 5. Identification of the binding site of YY1 on the PLAU promoter. (A) Site A and Site B were mutated separately or double-mutated. (B) Luciferase activity of the PLAU promoter with separate Site A and Site B mutation, and with double mutation of Site A and Site B were measured in HeLa cells with Lv-shCon or Lv-shYY1 infection. *P<0.05. YY1, transcription factor YinYang 1; PLAU, plasminogen activator urokinase; Lv, lentiviral; sh-, short hairpin.

Discussion

In the current study, it was found that PLAU was overexpressed in cervical cancer and knockdown of PLAU weakened the cell proliferation, migration and invasion capacities of cervical cancer cells. The location of the PLAU core promoter and an important transcription factor, YY1, which regulates PLAU promoter activity, were also identified.

Using the UALCAN database and RT-qPCR assay, it was found that PLAU was overexpressed in cervical cancer and promoted cell, proliferation, migration and invasion, indicating that PLAU promotes cervical cancer progression. Actually, upregulation of PLAU, as well as its oncogenic role, has already been observed in other tumor types, such as HNSCC and gastric cancer (7,8). In cervical cancer, PLAU has been reported to regulate tumor invasion and metastasis (15-17). Our study further revealed the important role of PLAU in tumor progression. The present results verified previous studies about higher expression of PLAU in cervical cancer and its role in cervical cancer cell migration and invasion (15-17); therefore, not only for HNSCC and gastric cancer, but also for cervical cancer PLAU can serve as a potential prognostic marker and therapeutic target, and this is just the reason why the regulation of PLAU transcriptional regulation was investigated in cervical cancer.

Transcription is the process by which RNA is synthesized according to the genomic sequence of DNA. The transcription process requires two key elements, the promoter and transcription factor binding to the promoter (18,36,37). Revealing the mechanisms by which an oncogene is regulated will help to further understand the process of tumorigenesis and progression, as well as to determine novel therapeutic targets (35). In the present study, a PLAU promoter-reporter was constructed and the core promoter located in the region -901/-1100 was identified. Based on the sequence of the core promoter, it was predicted that several transcription factors putatively bind to the core promoter; however, among these transcription factors, only the expression of YY1 showed significant difference between normal tissues and cervical cancers. YY1, a member of the GLI-Krüppel family of zinc-finger DNA-binding proteins, is a ubiquitously expressed transcription factor. YY1 has been reported to activate the transcription of several target genes, such as FOXE1, TNK2-AS1, and LINC00466 (24-26). In the current study, it was demonstrated that YY1 knockdown induced downregulation of PLAU promoter activity and mRNA expression, indicating that PLAU is a newly identified target gene of YY1. YY1 plays a critical role in tumorigenesis and progression by participating in various biological processes, including cell proliferation (27,28), apoptosis (29), invasion and migration (30), radioresistance (31), and drug resistance (32).

It was observed that PLAU is correlated with YY1 expression in cervical cancer in the cervical cancer tissues collected in Liaocheng People's Hospital. In addition, it was found that knockdown of YY1 induced downregulation of PLAU mRNA and promoter activity; therefore, the regulatory role of the transcription factor YY1 on PLAU expression was confirmed. To the best of our knowledge, this is the first study to demonstrate how PLAU is regulated at the transcriptional level. Our finding that YY1 regulates PLAU reveals a new mechanism of YY1-associated cervical cancer progression, suggesting a strategy for targeting PLAU, which is short of an applicable drug in cervical cancer patients, through inhibiting YY1. Although the YY1-regulating PLAU in cervical cancer was confirmed, whether this regulation was unique in cervical cancer or a universal effect in different tumor types is unknown.

As predicted by PROMO software, there are two potential binding sites for YY1 in the PLAU core promoter. Using site-directed mutation and luciferase activity assay, it was revealed that mutation of Site B (-974/-980) not Site A (-1024/-1030) abolished YY1's effects on PLAU promoter activity, suggesting that Site B is the main site responsible for YY1-mediated regulation of PLAU. Blocking Site A may abolish the regulatory effects of YY1 on PLAU and may be a promising target for cervical cancer therapy. There are unclear details about YY1 regulating PLAU. YY1 can regulate target genes with various co-factors, such as p300 and HDAC1 (38-40); however, whether YY1 regulates PLAU expression needing such co-factors remains unknown.

HPV infection is a risk factor of cervical cancer. In the present study, the HPV was not in our concern, thus it was not analyzed whether the samples were infected with HPV. It was determined that YY1/PLAU influences the migration and invasion in HeLa and HT-3 cells. HeLa cells are infected with HPV18, while HT-3 cells are not infected with HPV; therefore, the present results suggested that YY1/PLAU regulates cell migration and invasion independent of HPV infection.

There are several limitations to the present study. Firstly, 16 pairs of cervical cancers and the matched adjacent normal tissues were only collected, and extension the number of specimen pairs will be helpful in further confirming the conclusion of the present study. Secondly, the mRNA levels of YY1 and PLAU were evaluated; results would provide an improved understanding if protein levels are also detected. Nevertheless, it is considered that the mRNA level, to a certain extent, can confirm the expression of PLAU and YY1. Thirdly, our study detected the role of PLAU in regulating cervical cancer cell migration and invasion and explored the mechanism of how PLAU is regulated, but the mechanism of how PLAU regulates cervical cancer cell migration and invasion was not detected; our future studies will elucidate the mechanism by which PLAU regulates cell migration and invasion.

In conclusion, it was demonstrated that PLAU is overexpressed in cervical cancer and the promotion role of PLAU in cervical cancer was observed. The core promoter of PLAU was identified and it was determined that YY1 may be a crucial transcription factor of PLAU. The findings of the present study suggested that YY1/PLAU may be potential therapeutic targets for cervical cancer treatment.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Figshare repository (https://figshare.com/s/1966432ad77921b295cd).

Authors' contributions

CX designed the study. YG and PX performed the cell culture and western blotting experiments, and drafted the manuscript. HL and XM collected the tissue samples and performed the RT-qPCR experiments, and the construction of PLAU promoter-reporter. PX performed the statistical analysis, the luciferase assay and participated in data analysis. YG and CX confirm the authenticity of the raw data. All authors have read and approved the final version of the manuscript, and agree to be accountable for all aspects of the present research, ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved (approval no. LC2021015) by the Ethics Committee of Liaocheng People's Hospital (Liaocheng, China). Written informed consent was provided by all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Siegel RL, Miller KD, Fuchs HE and Jemal A: Cancer statistics, 2022. CA Cancer J Clin 72: 7-33, 2022.
3. Benard VB, Watson M, Saraiya M, Harewood R, Townsend JS, Stroup AM, Weir HK and Allemani C: Cervical cancer survival in the United States by race and stage (2001-2009): Findings from the CONCORD-2 study. Cancer 123 (Suppl 24): S5119-S5137, 2017.
4. Corley SM, Mendoza-Reinoso V, Giles N, Singer ES, Common JE, Wilkins MR and Beverdam A: Plau and Tgfbr3 are YAP-regulated genes that promote keratinocyte proliferation. Cell Death Dis 9: 1106, 2018.
5. Peng W, Wu Y, Zhang G, Zhu W, Chang M, Rouzi A, Jiang W, tong J, Wang Q, Liu J, *et al*: GLIPR1 protects against cigarette smoke-induced airway inflammation via PLAU/EGFR signaling. Int J Chron Obstruct Pulmon Dis 16: 2817-2832, 2021.
6. Buckley BJ, Ali U, Kelso MJ and Ranson M: The urokinase plasminogen activation system in rheumatoid arthritis: Pathophysiological roles and prospective therapeutic targets. Curr Drug Targets 20: 970-981, 2019.
7. Huo Z, Li X, Zhou J, Fan Y, Wang Z and Zhang Z: Hypomethylation and downregulation of miR-23b-3p are associated with upregulated PLAU: A diagnostic and prognostic biomarker in head and neck squamous cell carcinoma. Cancer Cell Int 21: 564, 2021.
8. Sun J, Zhou F, Xue J, Ji C, Qu Y and Pan Y: Long non-coding RNA TRPM2-AS regulates microRNA miR-138-5p and PLAU (plasminogen activator, urokinase) to promote the progression of gastric adenocarcinoma. Bioengineered 12: 9753-9765, 2021.
9. Moquet-Torcy G, Tolza C, Piechaczyk M and Jariel-Encontre I: Transcriptional complexity and roles of Fra-1/AP-1 at the uPA/Plau locus in aggressive breast cancer. Nucleic Acids Res 42: 11011-11024, 2014.
10. Chen G, Sun J, Xie M, Yu S, Tang Q and Chen L: PLAU promotes cell proliferation and epithelial-mesenchymal transition in head and neck squamous cell carcinoma. Front Genet 12: 651882, 2021.
11. Wang S, Jiang L, Han Y, Chew SH, Ohara Y, Akatsuka S, Weng L, Kawaguchi K, Fukui T, Sekido Y, *et al*: Urokinase-type plasminogen activator receptor promotes proliferation and invasion with reduced cisplatin sensitivity in malignant mesothelioma. Oncotarget 7: 69565-69578, 2016.
12. Zou B, Li J, Xu K, Liu JL, Yuan DY, Meng Z and Zhang B: Identification of key candidate genes and pathways in oral squamous cell carcinoma by integrated bioinformatics analysis. Exp Ther Med 17: 4089-4099, 2019.
13. Wu M, Wei B, Duan SL, Liu M, Ou-Yang DJ, Huang P and Chang S: Methylation-driven gene PLAU as a potential prognostic marker for differential thyroid carcinoma. Front Cell Dev Biol 10: 819484, 2022.
14. Wang H, Lu L, Liang X and Chen Y: Identification of prognostic genes in the pancreatic adenocarcinoma immune microenvironment by integrated bioinformatics analysis. Cancer Immunol Immunother 71: 1757-1769, 2022.
15. Daneri-Navarro A, Macias-Lopez G, Oceguera-Villanueva A, Del Toro-Arreola S, Bravo-Cuellar A, Perez-Montfort R and Orbach-Arbouys S: Urokinase-type plasminogen activator and plasminogen activator inhibitors (PAI-1 and PAI-2) in extracts of invasive cervical carcinoma and precursor lesions. Eur J Cancer 34: 566-569, 1998.
16. Kobayashi H, Fujishiro S and Terao T: Impact of urokinase-type plasminogen activator and its inhibitor type 1 on prognosis in cervical cancer of the uterus. Cancer Res 54: 6539-6548, 1994.
17. Koelbl H, Kirchheimer JC, Tatra G, Christ G and Binder BR: Increased plasma levels of urokinase-type plasminogen activator with endometrial and cervical cancer. Obstet Gynecol 72: 252-256, 1988.

18. Kou XX, Hao T, Meng Z, Zhou YH and Gan YH: Acetylated Sp1 inhibits PTEN expression through binding to PTEN core promoter and recruitment of HDAC1 and promotes cancer cell migration and invasion. Carcinogenesis 34: 58-67, 2013.
19. de Jonge WJ, Patel HP, Meeussen JVW and Lenstra TL: Following the tracks: How transcription factor binding dynamics control transcription. Biophys J 121: 1583-1592, 2022.
20. Hong JH and Zhang HG: Transcription factors involved in the development and prognosis of cardiac remodeling. Front Pharmacol 13: 828549, 2022.
21. Su SC, Lin CW, Yang WE, Fan WL and Yang SF: The urokinase-type plasminogen activator (uPA) system as a biomarker and therapeutic target in human malignancies. Expert Opin Ther Targets 20: 551-566, 2016.
22. Mahmood N, Mihalcioiu C and Rabbani SA: Multifaceted role of the urokinase-type plasminogen activator (uPA) and its receptor (uPAR): Diagnostic, prognostic, and therapeutic applications. Front Oncol 8: 24, 2018.
23. Nagamine Y, Medcalf RL and Muñoz-Cánoves P: Transcriptional and posttranscriptional regulation of the plasminogen activator system. Thromb Haemost 93: 661-675, 2005.
24. Li S, Zhang Z, Peng H and Xiao X: YY1-induced up-regulation of FOXE1 is negatively regulated by miR-129-5p and contributes to the progression of papillary thyroid microcarcinoma. Pathol Res Pract 221: 153337, 2021.
25. Yao W, Yan Q, Du X and Hou J: TNK2-AS1 upregulated by YY1 boosts the course of osteosarcoma through targeting miR-4319/WDR1. Cancer Sci 112: 893-905, 2021.
26. Li F, Shen ZZ, Xiao CM and Sha QK: YY1-mediated up-regulation of lncRNA LINC00466 facilitates glioma progression via miR-508/CHEK1. J Gene Med 23: e3287, 2021.
27. Zhang Q, Wan M, Shi J, Horita DA, Miller LD, Kute TE, Kridel SJ, Kulik G and Sui G: Yin Yang 1 promotes mTORC2-mediated AKT phosphorylation. J Mol Cell Biol 8: 232-243, 2016.
28. Wu S, Wang H, Li Y, Xie Y, Huang C, Zhao H, Miyagishi M and Kasim V: Transcription factor YY1 promotes cell proliferation by directly activating the pentose phosphate pathway. Cancer Res 78: 4549-4562, 2018.
29. Zhai Z, Ren Y, Shu C, Chen D, Liu X, Liang Y, Li A and Zhou J: JAC1 targets YY1 mediated JWA/p38 MAPK signaling to inhibit proliferation and induce apoptosis in TNBC. Cell Death Discov 8: 169, 2022.
30. Guan C, Liu L, Zhao Y, Zhang X, Liu G, Wang H, Gao X, Zhong X and Jiang X: YY1 and eIF4A3 are mediators of the cell proliferation, migration and invasion in cholangiocarcinoma promoted by circ-ZNF609 by targeting miR-432-5p to regulate LRRC1. Aging (Albany NY) 13: 25195-25212, 2021.
31. Zhao L, Li R, Qiu JZ, Yu JB, Cao Y and Yuan RT: YY1-mediated PTEN dephosphorylation antagonizes IR-induced DNA repair contributing to tongue squamous cell carcinoma radiosensitization. Mol Cell Probes 53: 101577, 2020.
32. Zhao L, Li R and Gan YH: Knockdown of Yin Yang 1 enhances anticancer effects of cisplatin through protein phosphatase 2A-mediated T308 dephosphorylation of AKT. Cell Death Dis 9: 747, 2018.
33. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK and Varambally S: UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 19: 649-658, 2017.
34. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
35. Bai G, Wei N, Li F, Zhao P, Meng Z, Zou B, Liu Y, Xu K, Li K, Yao C and Yang P: Function and transcriptional regulation of TCTN1 in oral squamous cell carcinoma. Oncol Rep 47: 26, 2022.
36. Lynch TR, Xue M, Czerniak CW, Lee C and Kimble J: Notch-dependent DNA cis-regulatory elements and their dose-dependent control of C. elegans stem cell self-renewal. Development 149: dev200332, 2022.
37. Yin S, NandyMazumdar M, Paranjapye A and Harris A: Cross-talk between enhancers, structural elements and activating transcription factors maintains the 3D architecture and expression of the CFTR gene. Genomics 114: 110350, 2022 (Epub ahead of print).
38. Nakayama T, Fukutomi T, Terao Y and Akagawa K: Syntaxin 1A gene is negatively regulated in a cell/tissue specific manner by YY1 transcription factor, which binds to the -183 to -137 promoter region together with gene silencing factors including histone deacetylase. Biomolecules 11: 146, 2021.
39. Hou X, Li Y, Huang Y, Zhao H and Gui L: Adenosine receptor A1-A2a heteromers regulate EAAT2 expression and glutamate uptake via YY1-induced repression of PPARγ transcription. PPAR Res 2020: 2410264, 2020.
40. Wei X, Liu F, Jiang X, Xu X, Zhou T and Kang C: YY1 promotes telomerase activity and laryngeal squamous cell carcinoma progression through impairment of GAS5-mediated p53 stability. Front Oncol 11: 692405, 2021.