# High expression of PDIA4 promotes malignant cell behavior and predicts reduced survival in cervical cancer

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**Abstract.** The protein disulfide isomerase (PDI) gene family plays important roles in the maintenance of several cellular functions. Previous studies have showed that protein disulfide isomerase family A member 4 (PDIA4) is aberrantly expressed in several types of cancer, and correlates with prognosis of patients. However, the role of PDIA4 in cervical cancer remains unclear. In the present study, the expression pattern of PDIA4 from both public database and immunohistochemical analysis in cervical samples was analyzed. Cell Counting Kit-8 and Transwell assays were performed to determine the effect of PDIA4 on cervical cancer cell proliferation and migration. Gene set enrichment analysis (GSEA) was used to provide the associated enriched pathways of PDIA4 in regulating cervical tumorigenesis. It was observed that mRNA expression and protein level of PDIA4 were upregulated in cervical cancer tissues. High expression of PDIA4 was significantly associated with poor overall survival (P=0.0095) and relapse-free survival (P=0.0019) in The Cancer Genome Atlas cohort. Knockdown of PDIA4 inhibited cervical cancer cell proliferation and migration. Moreover, PDIA4 affected the expression of proliferation-related molecules (cyclin D1 and PCNA) and migration-related molecules (E-cadherin and Vimentin). Additionally, GSEA revealed that PDIA4 was significantly associated with gene signatures involving glycan biosynthesis, glycosaminoglycan degradation and protein export. In conclusion, the present findings highlighted the importance of PDIA4 in cervical oncogenesis, and suggested that targeting PDIA4 may be a potential therapeutic application for cervical cancer.

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## Introduction

Cervical cancer is the fourth leading cancer in women with estimated 342,000 cancer-related deaths per year globally (1). In the past decades, with the development of cervical cancer prevention and screening measures, the survival rate of patients with cervical cancer has been improved. However, the prognosis of advanced cervical cancer is far from satisfactory. So far, the main treatment methods for advanced cervical cancer include radical surgery, radiotherapy, chemotherapy and immunotherapy. However, the curative effect is limited and the potential adverse reactions are also very serious. At present, targeted therapy is gradually emerging in cervical cancer (2). Therefore, identification of novel molecular targets may help to improve the survival of patients with cervical cancer.

Protein disulfide isomerase (PDI) is a redox-dependent protein with both chaperone and oxidoreductase activities, and is originally discovered in the endoplasmic reticulum (ER) and participates in protein folding (3). Protein disulfide isomerase family A member 4 (PDIA4) is one of the PDI family members, and has been revealed to exert functions in the pathogenesis of various diseases (4). Similar to other PDI members, PDIA4 can enhance thrombus formation and initiate coagulation through a series of cascade reactions (4). Aberrant expression of PDIA4 has been proved as a self-protection response (5). In human malignancies, PDIA4 was found to be overexpressed in esophageal squamous cell carcinoma (6). High expression of PDIA4 was associated with poor survival rate in glioma (7). Modulating the expression of PDIA4 in cancer cells reveled that PDIA4 facilitated cell growth via regulating activity of caspases 3 and 7 (8). PDIA4 inhibited prostate cancer cell apoptosis and drove docetaxel resistance via activating the Akt-signaling pathway (9), whereas PDIA4 inactivation could restore a classical mitochondrial apoptosis pathway (10). Additionally, PDIA4 also mediated miR-378a-3p activating PI3K/AKT signaling to promote the growth of ovarian cancer cells (11). However, the role of PDIA4 in cervical cancer remains unknown.

In the present study, the expression pattern of PDIA4 in cervical cancer was explored through bioinformatics and immunohistochemical analysis. Proliferation and migration assays were performed to reveal the effect of PDIA4 on cervical cancer cells. Furthermore, Gene set enrichment analysis (GSEA) and protein-protein interaction network showed that numerous cancer related pathways may be associated with PDIA4.

## Materials and methods

*Immunohistochemical (IHC) analysis*. The commercial cervical tissue microarray, including 10 normal cervix samples, 27 cervical intraepithelial neoplasia (CIN) I samples, 55 CIN II-III samples and 10 cervical squamous cell cancer samples, was purchased from Shanghai Landian Biotechnology (Shanghai, China). The clinicopathological data of these samples are presented in Table SI. The IHC staining of PDIA4 was performed according to standard protocols. In brief, the paraffin-embedded tissues (5-$\mu$m) were deparaffinized with xylene and rehydrated with descending ethanol series. The microarray was microwave-treated with citrate buffer (pH=6.0) for 30 min. The endogenous peroxidase activity was blocked at room temperature using 3% $H_2O_2$ in methanol for 15 min, and the slide was blocked with 5% skimmed milk at room temperature for 15 min. Then the microarray was incubated with PDIA4 polyclonal antibody (1:100; cat. no. 14712-1-AP; ProteinTech Group, Inc.) at 4˚C overnight. Subsequently, the microarrays were incubated with goat anti-rabbit secondary antibody (1:500; cat. no. ab6721; Abcam) for 1 h at room temperature. The IHC staining was interpreted by two professional pathologists by using a light microscope (Olympus Corporation). The immunoreactivity score was used to identify the IHC staining according to the following criteria: staining extent x staining intensity. The staining intensity was evaluated as 0=negative, 1=weak, 2=moderate and 3=strong. The staining extent was evaluated as 0=no positive cells, 1<10%, 2=10-33%, 3=34-66% and 4≥67%. The present study was approved (approval no. 2021KN81) by the Institutional Ethics Committee of Shanghai Tenth People's Hospital (Shanghai, China).

*Cell lines, small interfering (siRNA) and reverse transcription-quantitative (RT-q) PCR*. Cervical cancer cell lines SiHa (cat. no. TCHu113), Me180 (cat. no. TCHu177) and HeLa (cat. no. TCHu187) were purchased from the Type Culture Collection of the Chinese Academy of Science (Shanghai, China). All cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; both from Invitrogen; Thermo Fisher Scientific, Inc.) and 1% streptomycin/penicillin, in a humidified atmosphere incubator containing 5% $CO_2$ at 37˚C.

The following siRNA oligonucleotide sequences against PDIA4 were used in the present study: siRNA against PDIA4-1, 5'-CCTGAGAGAAGATTACAAATT-3'; PDIA4-2, 5'-GCAAGGTGTCAAACGATGCTA-3'; and control siRNA, 5'-AAGAACAACACAAAGGACAG-3'. Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used to transfect cervical cancer cells with siRNA in a 12-well plate according to the manufacturer's protocol. The cells were co-cultured with lipofectamine/siRNA at room temperature for 30 min, and then cultured in the incubator at 37˚C for 6 h. Final siRNA concentrations were 6 nM. Following a 48-h siRNA transfection, the cells were used for subsequent experiments.

RT-qPCR was used to identify the knockdown efficiency of siPDIA4. Total RNA was isolated from transfected SiHa, ME180 or HeLa cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The cDNA was synthesized by using the PrimeScript™ Reverse Transcriptase (cat. no. 2690S; Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. SYBR qPCR Mix (cat. no. Q712-02; Nanjing Vazyme Biotech Co., Ltd.) was used to perform the RT-qPCR. The primer sequences used in the present study were as follows: PDIA4 forward, 5'-CCACCGCAGAAACAGACCT-3' and reverse, 5'-GGGCCGTTGTAGTCATAAGGC-3'; CCND1 forward, 5'-GCTGCGAAGTGGAAACCATC-3' and reverse, 5'-CCTCCTTCTGCACACATTTGAA-3'; PCNA forward, 5'-ACACTAAGGGCCGAAGATAACG-3' and reverse, 5'-ACAGCATCTCCAATATGGCTGA-3'; CDH1 forward, 5'-CGAGAGCTACACGTTCACGG-3' and reverse, 5'-GGG TGTCGAGGGAAAAATAGG-3'; VIM forward, 5'-GAC GCCATCAACACCGAGTT-3' and reverse, 5'-CTTTGTCGT TGGTTAGCTGGT-3'; and GAPDH forward, 5'- CTGGGC TACACTGAGCACC-3' and reverse, 5'-AAGTGGTCGTTG AGGGCAATG-3'.

The RT-qPCR thermocycling conditions were as follows: Initial denaturation at 95˚C for 5 min, 40 cycles of 95˚C for 15 sec and 58˚C for 30 sec, followed by a melting curve ranging from 95˚C for 15 sec, 60˚C for 1 min, to 95˚C for 15 min. Relative quantitation was performed using the comparative $2^{-\Delta\Delta Cq}$ method (12).

*Western blotting*. Total protein was extracted by using RIPA lysis buffer (cat. no. P0013B; Beyotime Institute of Biotechnology). After using the bicinchoninic acid method to determine the protein concentration, 4x loading buffer was used to prepare the protein samples. Equal amounts of proteins (20 $\mu$g) were separated on 10% SDS-PAGE gel and transferred onto a nitrocellulose membrane at 300 mA for 90 min. The membranes were blocked with 5% bovine serum albumin (BSA; cat. no. E661003; Sangon Biotech, Co., Ltd.) for 1 h at room temperature, and then incubated with the following primary antibodies at 4˚C overnight: PDIA4 polyclonal antibody (1:500; cat. no. 14712-1-AP; ProteinTech Group, Inc.), cyclin D1 (1:1,000; cat. no. 55506S), PCNA (1:1,000; cat. no. 13110), E-cadherin (1:1,000; cat. no. 14472) and Vimentin (1:1,000; cat. no. 5741; all from Cell Signaling Technology, Inc.). Next day, the membranes were incubated with Tween-20 (TBST) for 45 min at room temperature and then incubated with secondary antibody (HRP-conjugated Affinipure goat anti-rabbit IgG (H+L); 1:5,000; cat. no. SA00001-2; ProteinTech Group, Inc.) for 1 h at room temperature. The proteins were detected by the electrochemiluminescence imaging system (Tanon Science and Technology Co.). Immunoreactive bands were quantified using ImageJ software (Version 1.8.0.172; National Institutes of Health).

*Cell Counting Kit-8 (CCK-8) assay*. CCK-8 (cat. no. C0037; Beyotime Institute of Biotechnology) was used to assess the proliferation of SiHa, ME180 or HeLa cells. The transfected cells were placed on a 96-well plate at a density of 1,000 cells per well. At 72 h after incubation at 37˚C, 10 $\mu$l of 5 mg/ml CCK-8 reagent was added to the plate well. The culture was terminated 1 h after adding CCK-8 reagent, and the optical density value was detected by a microplate reader at 450 nm. The experiments were repeated in triplicate independently.

*Transwell assay*. Transwell assay was used to determine the migration of cervical cancer cells. Cells ($1x10^5$) in serum-free medium were added to the top of Transwell chamber (cat. no. 3422; Corning, Inc.). The lower chamber was filled with DMEM medium containing 10% FBS as a chemoattractant. After 24 h of incubation at 37˚C, migratory cells were fixed with methanol for 20 min and stained with 5% crystal violet for 10 min at room temperature. The number of migratory cells was counted from five randomly selected fields by using a light microscope.

*Bioinformatics analysis*. UALCAN platform (http://ualcan.path.uab.edu/) was used to identify the expression of PDIA4 in The Cancer Genome Atlas (TCGA) cancer samples (13). Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) was used to reveal the expression of PDIA4 in different cervical tissues. The data of GSE7803 was obtained from the study of Zhai et al (14), which contained 10 normal squamous cervical epithelial samples and 21 invasive squamous cell carcinomas of the cervix. The data of GSE9750 was obtained from the study of Scotto *et al* (15), which contained 24 normal cervical epithelium and 33 cervical cancer samples. The data of GSE7410 was obtained from the study of Biewenga *et al* (16), which contained 5 non-cervical carcinoma samples and 35 cervical cancer samples.

The Kaplan-Meier plotter (http://kmplot.com/analysis/) was used to explore the prognostic value of PDIA4, PLOD3, GALNT10, GLB1, MOGS, POFUT1 and SEC63 in patients with cervical cancer (17). It divided all the samples into lower and upper expression groups based on the auto cut-off plot. The multivariate Cox regression analysis was performed to reveal the association between PDIA4 expression and overall survival (OS) in different clinical features by using TCGA data.

LinkedOmics website (http://linkedomics.org/login.php) was used to perform GSEA based on TCGA data to demonstrate the association between the expression of PDIA4 and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, Panther pathways, Gene Ontology biological process and Gene Ontology molecular function (18). False discovery rate (q-value) was shown, which was the probability estimation of the possible false positive results of the standardized enrichment score.

The TIMER website (https://cistrome.shinyapps.io/timer/) was used to show the association between the expression of PDIA4 and glycan biosynthesis-related genes, glycosaminoglycan-related genes or protein export-related genes (19).

The GeneMANIA website (http://genemania.org/) was used to validate the gene interaction network (20). The BioGRID database (https://thebiogrid.org/) was used to show the biomedical interaction network (21).

The GEPIA2021 website (http://gepia2021.cancer-pku.cn/) was used to visualize the expression of PDIA4 in each immune cell type available in TCGA/GTEx sub-datasets (22).

*Statistical analysis*. In the present study, GraphPad Prism (Version 8.0; GraphPad Software, Inc.) was used to perform statistical analyses. Paired student's t-test was used to compare the difference between two different groups. Welch's ANOVA test was used to analyze the difference among IHC scores of multiple groups. Kaplan-Meier analysis was used to show the survival rate of patients with cervical cancer. The R package survival was applied in multivariate Cox regression analysis. Data were presented as the mean ± SD. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*The expression of PDIA4 is upregulated in cervical cancer*. To determine the mRNA expression of PDIA4 in cervical cancer, the UALCAN platform was first used to study the expression of PDIA4 between normal and cancer samples. As revealed in Fig. 1A, PDIA4 was not only upregulated in cervical cancer, but also highly expressed in other types of cancer, such as bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSC), lung adenocarcinoma (LUAD) and uterine corpus endometrial carcinoma (UCEC). GEO database showed that the mRNA expression levels of PDIA4 were increased in cervical cancer tissues compared with normal cervix tissues (GSE7803, GSE9750 and GSE7410; Fig. 1B-D). Then, IHC analysis was used to demonstrate the protein level of PDIA4 in cervical samples. Similar to mRNA expression pattern, the protein level of PDIA4 was elevated in CIN and cervical cancer tissues (Fig. 1E and F). In addition, the mRNA expression of PDIA4 was also analyzed in different immune cells. The results revealed that the expression of PDIA4 in $CD4^+$ T cells and $CD8^+$ T cells was higher than that in B cells and natural killer cells (Fig. S1).

*High expression of PDIA4 predicts worse survival in patients with cervical cancer*. Next, the predictive value of PDIA4 in cervical cancer was analyzed. Kaplan-Meier survival curves revealed that high mRNA expression of PDIA4 was associated with worse OS [P=0.0095; hazard ratio (HR)=2.19; 95% confidence interval (CI)=1.19-4.02] and relapse-free survival (P=0.0019; HR=3.22; 95% CI=1.48-7.03) of patients with cervical cancer (Fig. 2A and B) based on the auto cut-off plot. Then the prognostic value of PDIA4 was examined by using subgroup analysis. The association of PDIA4 mRNA expression with OS in different clinical features (such as age of patient, human race, clinical stage and histological grade) was examined via univariate Cox analysis. High expression of PDIA4 indicated poor clinical outcome (Fig. 2C).

*PDIA4 promotes malignant behavior of cervical cancer cells*. Then, the effect of PDIA4 on the biological activity of cervical cancer cells was analyzed. CCK-8 assay demonstrated that silencing of PDIA4 inhibited proliferation of Siha, ME180 and Hela cell lines (Figs. 3A and S2A). Furthermore, knockdown

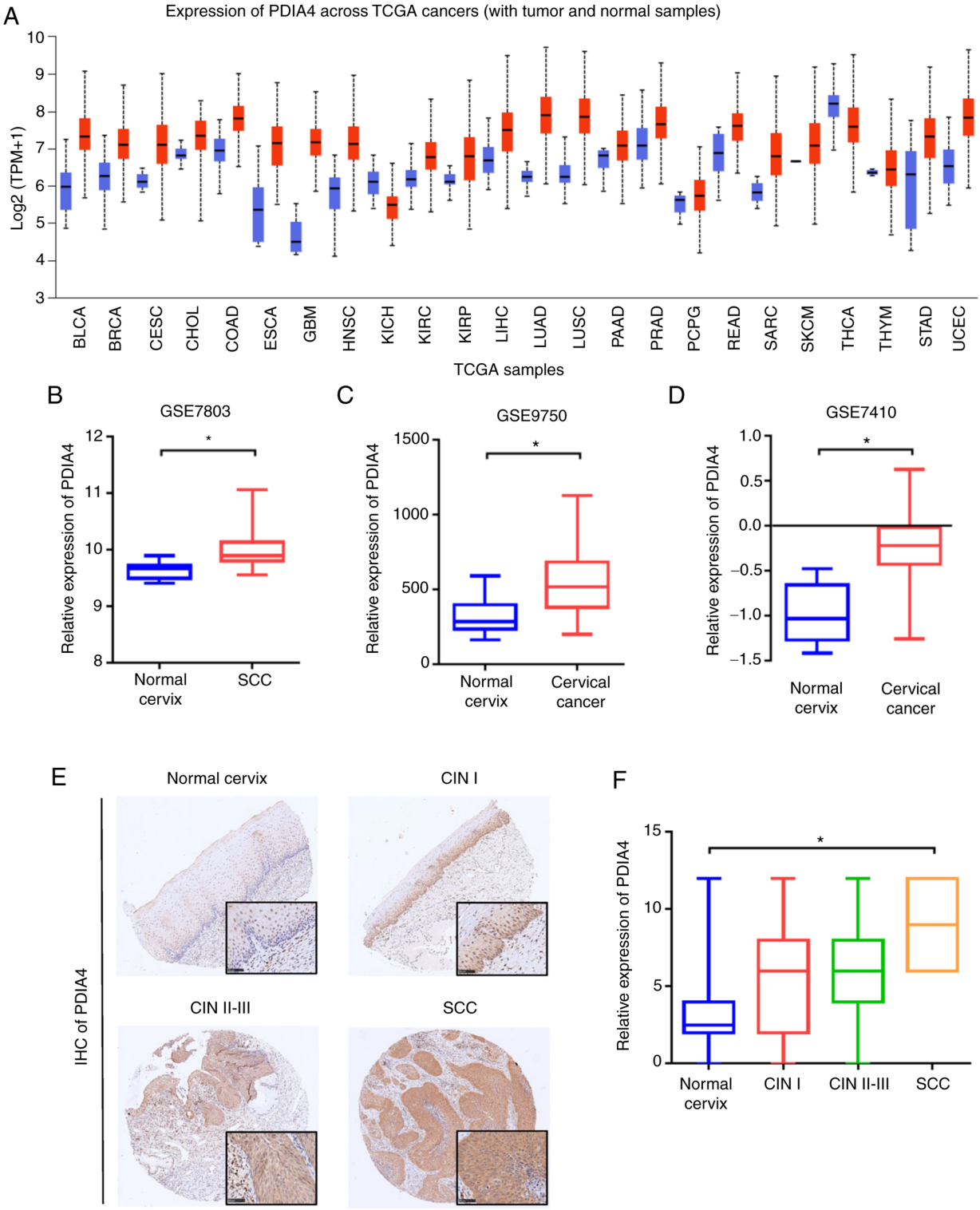


Figure 1. Expression of PDIA4 is upregulated in cervical cancer. (A) The expression of PDIA4 between normal and cancer samples from UALCAN platform (http://ualcan.path.uab.edu/index.html). (B) The expression of PDIA4 in normal cervix and cervical squamous cell carcinoma tissues from GSE7803. (C and D) The expression of PDIA4 in normal cervix and cervical cancer tissues from (C) GSE9750 and (D) GSE7410. (E) The protein level of PDIA4 in normal cervix, CIN and cervical cancer tissues detected by IHC analysis. (F) IHC scores of normal cervix, CIN I, CIN II-III and SCC tissues. Scale bar=50 $\mu$m. *P<0.05. PDIA4, protein disulfide isomerase family A member 4; CIN, cervical intraepithelial neoplasia; IHC, immunohistochemical; SCC, squamous cervical cancer.

of PDIA4 also reduced the migration of cervical cancer cells as revealed by Transwell assay (Fig. 3B). Additionally, the expression of proliferation-related molecules (cyclin D1 and PCNA) and migration-related molecules (E-cadherin and Vimentin) were detected. The mRNA and protein expression levels of cyclin D1, PCNA and Vimentin were decreased in both SiHa and ME180 cells after PDIA4 silencing, whereas the mRNA expression and protein level of E-cadherin were

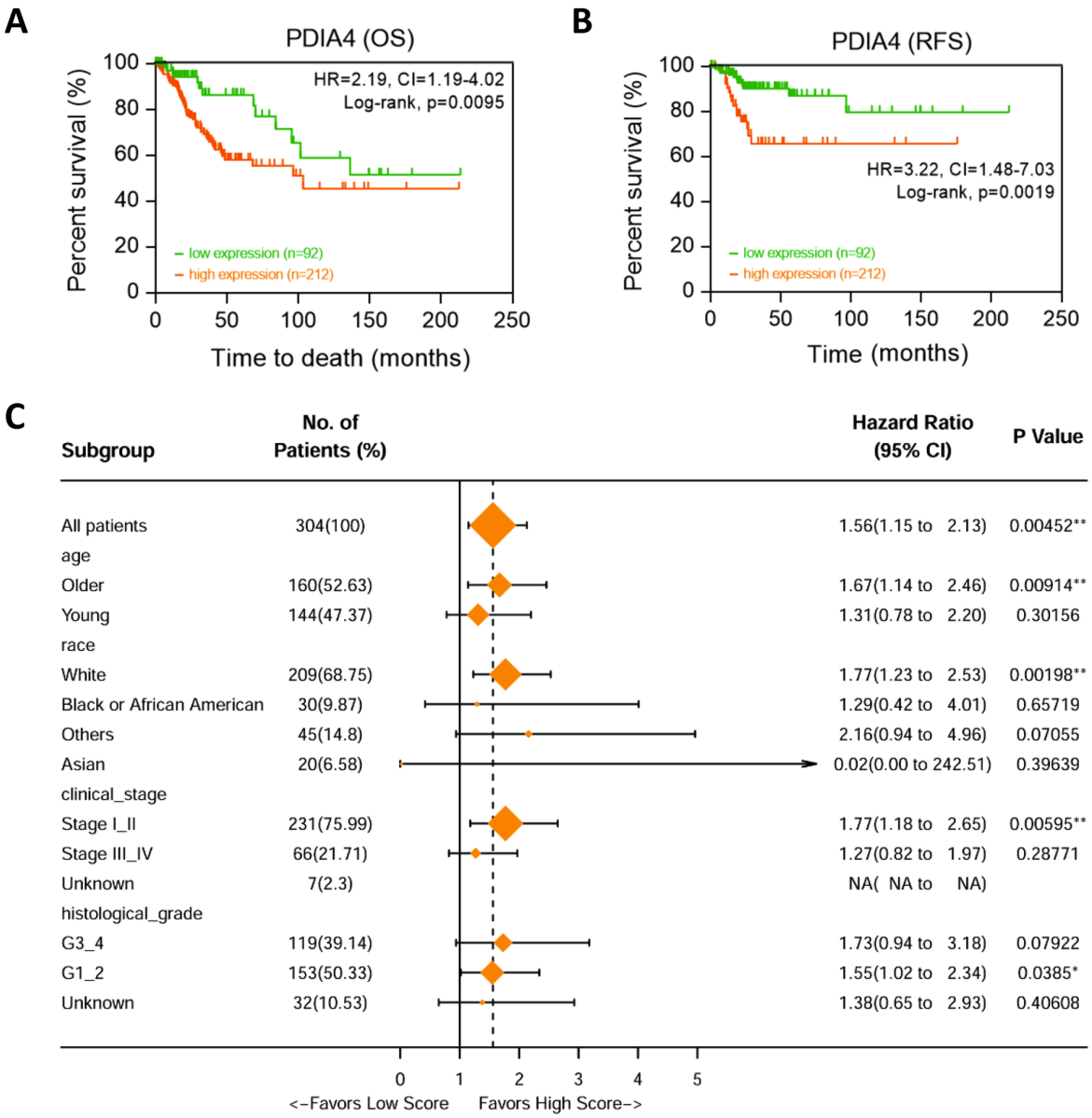


Figure 2. High expression of PDIA4 predicts worse survival in patients with cervical cancer. (A and B) PDIA4 predicted worse (A) OS and (B) RFS of patients with cervical cancer from Kaplan-Meier plotter (http://kmplot.com/analysis/). (C) A univariate Cox HR analysis showed that the expression of PDIA4 was statistically different in the subgroups classified by age, race, clinical stage and histological grade. *P<0.05 and **P<0.01. PDIA4, protein disulfide isomerase family A member 4; OS, overall survival; RFS, relapse-free survival; HR, hazard ratio; CI, confidence interval.

increased after knockdown of PDIA4 (Figs. 4C-G and S2B). These results suggested that PDIA4 functions as an oncogene in cervical cancer cells.

*Potential biological functions regulated by PDIA4 in cervical cancer.* As aforementioned, PDIA4 was overexpressed in cervical cancer and predicted worse survival. Then it was attempted to study biological functions which were potentially regulated by PDIA4. GSEA analysis showed that PDIA4 may be involved in several KEGG pathways, such as glycan biosynthesis, glycosaminoglycan degradation, protein processing in ER and protein export (Fig. 4A). Similarly, a variety of panther pathways (such as antigen biosynthesis, the Wnt signaling pathway, purine biosynthesis and the TGF-β signaling pathway) were associated with the mRNA expression of PDIA4 (Fig. 4B). GO biological process and molecular function associated with PDIA4 in cervical cancer were also explored. As demonstrated in Fig. S3, the mRNA expression of PDIA4 was associated with glycosylation, response to ER stress, glycoprotein metabolic process, protein folding and protein hydroxylation. Furthermore, GeneMANIA platform was used to construct a PDIA4-interaction network. Several PDIA4-associated genes were demonstrated in the interaction network, including SIRT7, LAMP1, ERO1A, PRDX4, LRRK2 and SHCBP1 (Fig. 4C). In addition, the BioGRID database also revealed a series of proteins that bind to PDIA4 (Fig. S4).

To verify these results, it was further showed that the mRNA expression of PDIA4 was associated with the expression levels of glycan biosynthesis-related genes (including ALG8, GANAB, B4GALT1, MGAT5, PLOD3, POFUT1 and GALNT10), glycosaminoglycan-related genes (such as FUT8,

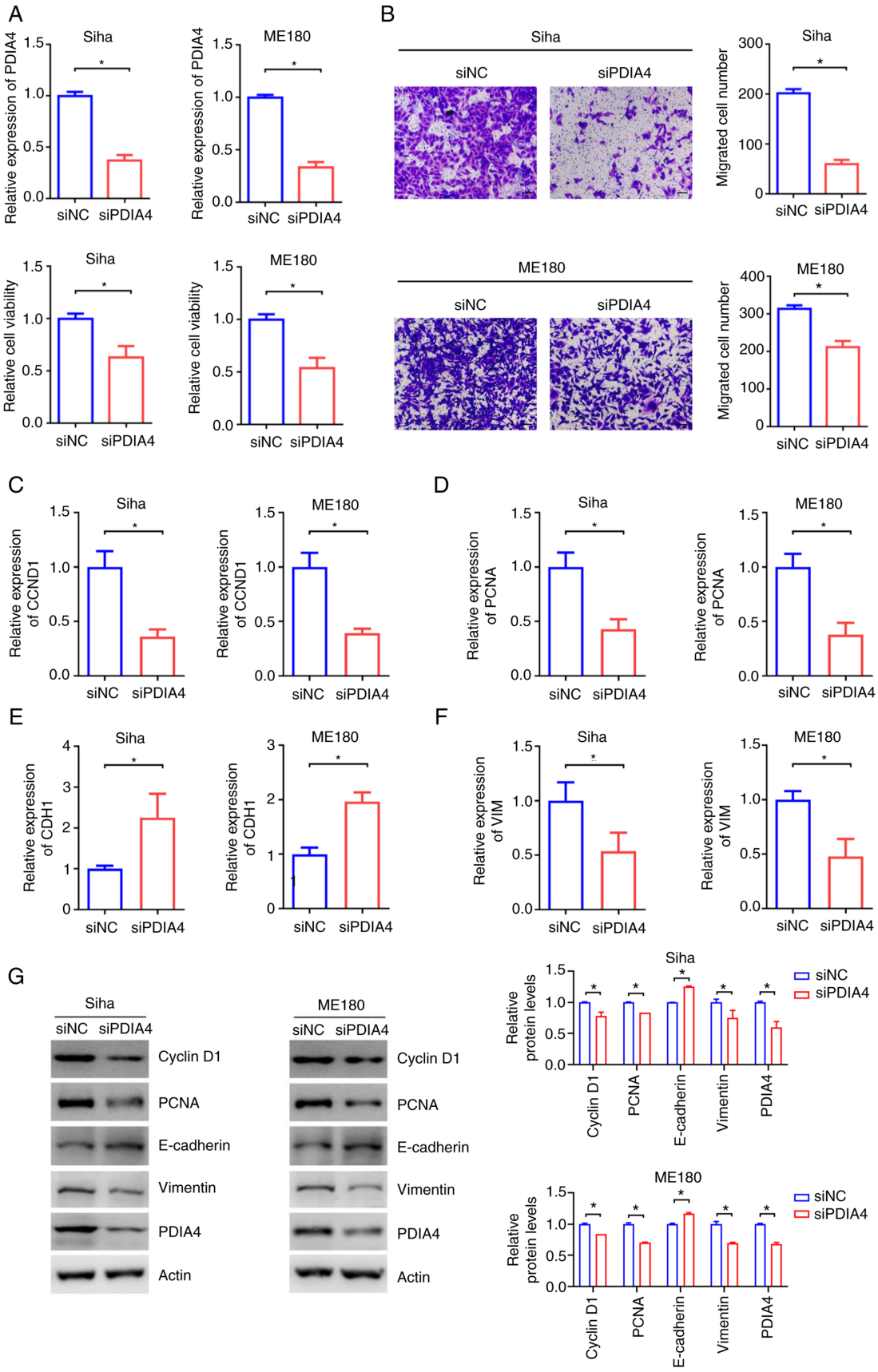


Figure 3. PDIA4 promotes proliferation and migration of cervical cancer cells. (A) RT-qPCR and Cell Counting Kit-8 assay detected PDIA4 mRNA expression and cell growth of Siha and Me180 cells after knockdown of PDIA4. (B) Transwell assay after knockdown of PDIA4 in Siha and Me180 cells (Scale bar=50 $\mu$m). These experiments were performed three times. (C-F) The mRNA expression of (C) CCND1, (D) PCNA (E) CDH1 and (F) VIM was detected using RT-qPCR after treatment of control or PDIA4 siRNA in Siha and ME180 cells. (G) The protein levels of CCND1, PCNA, E-cadherin and Vimentin were detected using western blotting after silencing of PDIA4 in Siha and ME180 cells. These experiments were performed three times. $^{*}$P<0.05. PDIA4, protein disulfide isomerase family A member 4; RT-qPCR, reverse transcription-quantitative PCR; si-, small interfering; CCND1, cyclin D1; PCNA, proliferating cell nuclear antigen; CDH1, E-cadherin; VIM, vimentin; NC, negative control.

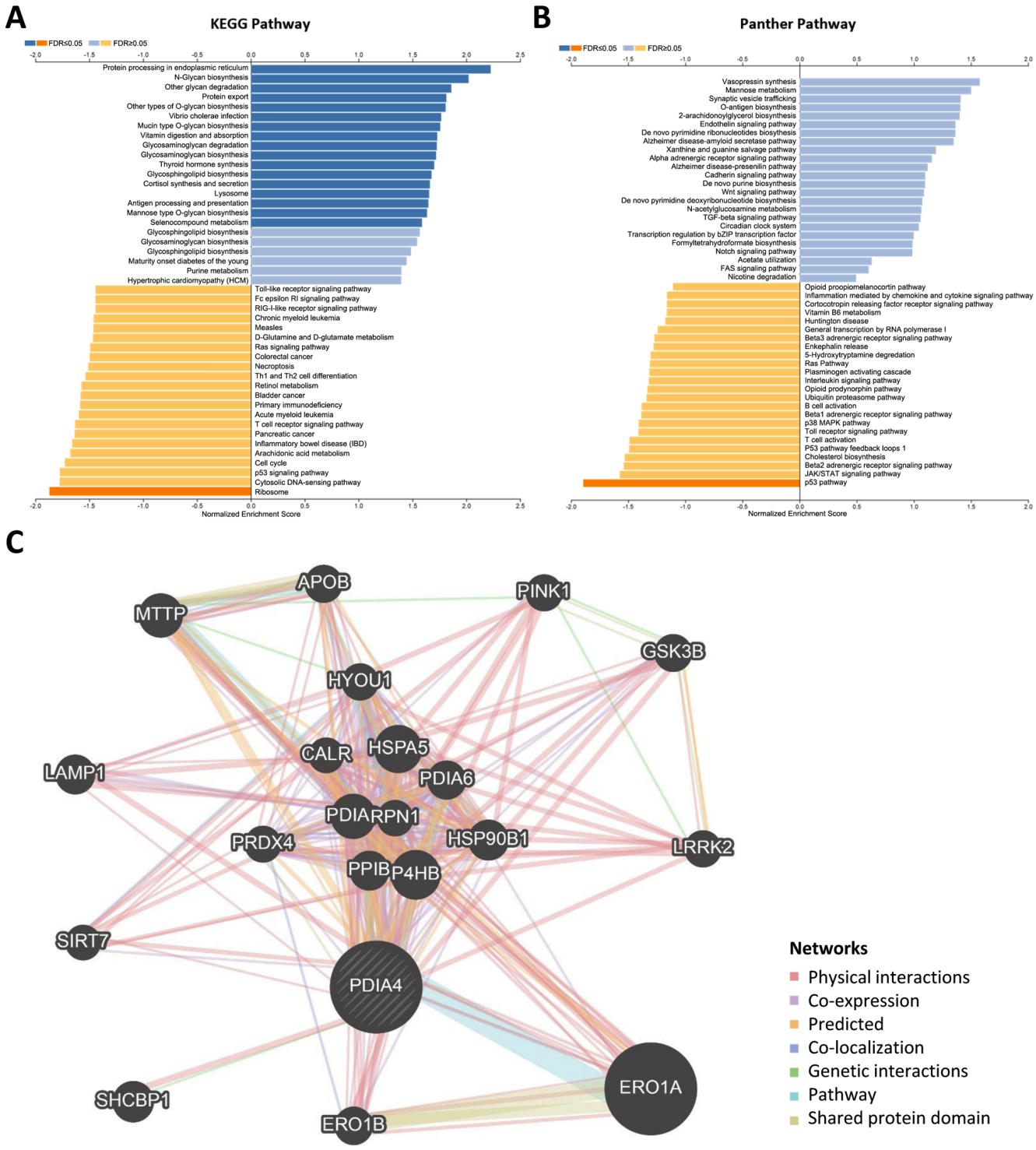


Figure 4. Potential biological functions regulated by PDIA4 in cervical cancer. (A) The KEGG pathways potentially regulated by PDIA4 deposited in the GSEA via LinkedOmics website (http://linkedomics.org/login.php). (B) The Panther pathways potentially regulated by PDIA4 deposited in the GSEA via LinkedOmics website. (C) The gene interaction network of PDIA4 constructed by the GeneMANIA (http://genemania.org/). PDIA4, protein disulfide isomerase family A member 4; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene set enrichment analysis.

CHST4, GLB1 and GNS) and protein export-related genes (such as SCE63, SEC11A, SPCS1 and SPCS3) (Fig. 5). Moreover, Kaplan-Meier analysis revealed that PDIA4$^{high}$PLOD3$^{high}$ group was associated with poor prognosis, compared with PDIA4$^{low}$PLOD3$^{low}$ group in patients with cervical cancer (P<0.0001; HR=3.68; 95% CI, 2.23-7.60) (Fig. 6A). Similar results were also observed in the co-expression of PDIA4 combined with GALNT10, GLB1, MOGS, POFUT1 or SEC63 (Fig. 6B-F).

## Discussion

PDI family member PDIA4 has been shown to play roles in the pathogenesis of various diseases (4). Previous studies have

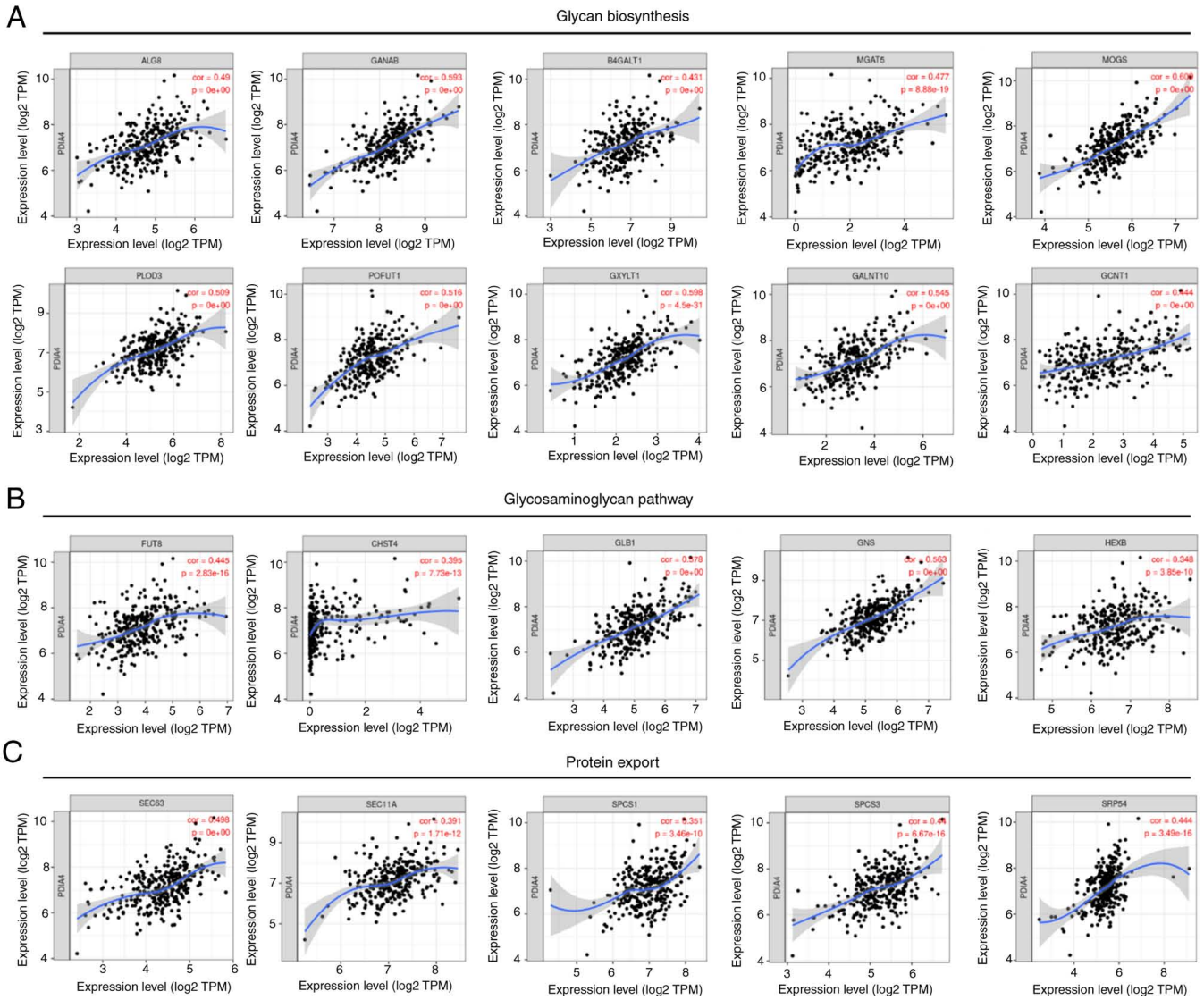


Figure 5. Association of PDIA4 and glycan biosynthesis, glycosaminoglycan or protein export related genes. (A-C) Association between the expression of PDIA4 and (A) glycan biosynthesis-related genes, (B) glycosaminoglycan-related genes and (C) protein export-related genes via TIMER platform (https://cistrome.shinyapps.io/timer/) PDIA4, protein disulfide isomerase family A member 4.

uncovered the overexpression and cancer-promoting activity of PDIA4 in several types of cancer (6,7,9). In the present study, the expression pattern and biological roles of PDIA4 in cervical cancer were investigated.

The PDI gene family, which is a group of multifunctional ER enzymes, plays key roles in the correct folding of polypeptide chains in the ER and the maintenance of several cellular functions including gluconeogenesis, lipogenesis and organelle biogenesis (23,24). Gene Expression Atlas datasets have showed that PDIA1, PDIA3, PDIA4 and PDIA6 are increased in various types of cancer compared with normal tissue (25). Moreover, the expression of PDIA1 is significantly higher in the metastatic lymph node breast tumor than in primary breast tumors (23), and PDI family genes can also activate membrane proteins such as matrix metallopeptidases (26), indicating that PDI is correlated with the promotion of metastasis. Additionally, several studies have shown that small molecule irreversible PDI inhibitor propionic acid carbamoyl methyl amides could play a cytotoxic role in a wide range of cancer cells (27,28). These results suggested that targeting key PDI proteins may provide more effective and personalized treatment strategies in a specific type of cancer.

Previous studies revealed that PDIA4 was abnormally expressed in several types of cancer. For instance, PDIA4 overexpression was reported in a variety of cancer cell lines, lung adenocarcinoma tissues and esophageal squamous cell carcinoma samples (6,8). PDIA4 exhibited a dramatic upregulation in docetaxel-resistant prostate cancer cells (9). Moreover, PDIA4 was an independent risk factor for disease-free survival and OS in ovarian cancer (29) and was associated with poor prognosis in patients with glioma (7,30). Recently, Wang *et al* (31) reported that PDIA4 could promote glioblastoma progression via the PI3K/AKT/mTOR pathway. Consistently, the present results showed that both public database and IHC analysis in cervical samples revealed the aberrant overexpression of PDIA4 in cervical cancer tissues. Moreover, Kaplan-Meier

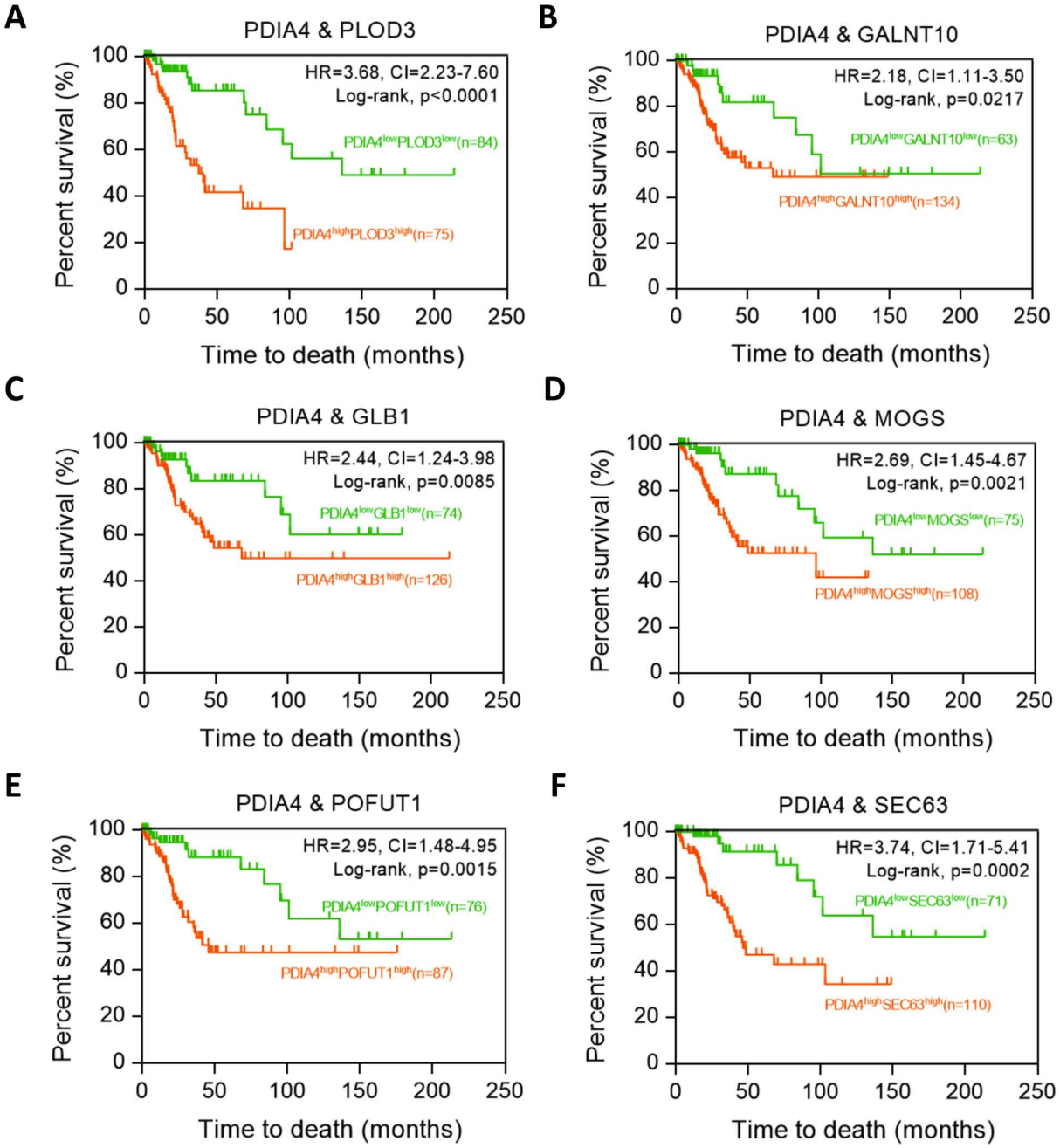


Figure 6. Impact of PDIA4 on the prognostic assessment of glycan biosynthesis, glycosaminoglycan or protein export-related genes. (A-F) Kaplan-Meier analysis of overall survival stratified according to the combination of PDIA4 and (A) PLOD3, (B) GALNT10, (C) GLB1, (D) MOGS, (E) POFUT1 or (F) SEC63 in patients with cervical cancer from Kaplan-Meier plotter. PDIA4, protein disulfide isomerase family A member 4.

survival analysis demonstrated that cervical cancer patients with higher PDIA4 expression had a poorer clinical outcome. In addition, high expression of PDIA4 expression predicted worse survival of patients in other types of cancer. Functionally, knockdown of PDIA4 significantly impaired cervical cancer cell proliferation and migration. Moreover, PDIA4 also affected the expression of proliferation-related genes and migration-related genes. Therefore, it was indicated that PDIA4 may be used as an independently prognostic biomarker of cervical cancer.

The potential biological roles associated with PDIA4 in cervical cancer were revealed by GSEA analysis. Similar to its role in ER and protein folding, cluster analysis of sequencing data of TCGA cervical cancer samples revealed that high expression of PDIA4 was closely related to protein processing in ER, glycoprotein metabolic process, protein export, folding and hydroxylation. It is worth noting that the expression of PDIA4 was associated with the expression levels of glycan biosynthesis-related genes, glycosaminoglycan-related genes and protein export-related genes. Additionally, several PDIA4-interacting proteins were also demonstrated. These results indicated the role of PDIA4 in cervical cancer. However, the specific molecular mechanism of PDIA4 on cervical tumorigenesis remains to be explored. Moreover, there are available effective pan-inhibitors of the PDI family. In future studies, it is also necessary to carry out relevant *in vivo* and

*in vitro* experiments to confirm the roles of PDI in the treatment of cervical cancer.

Taken together, the present study revealed the expression features and prognostic values of PDIA4 in cervical cancers. Silencing PDIA4 could also inhibit the proliferation and migration of cervical cancer cells. Moreover, potential biological functions regulated by PDIA4 were demonstrated by functional cluster analysis and protein-protein interaction map. These observations provided an understanding of the pathological role of PDIA4 in cervical cancer, which may help to represent a novel anti-tumor therapeutic option for cervical cancer.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Authors' contributions**

ZPC and ZJS developed the project and wrote the manuscript. FX and ZJS performed the experiments and collected data. FX performed data analysis and contributed to manuscript writing. FX and ZJS confirm the authenticity of all the raw data. The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final version of the manuscript.

**Ethics approval and consent to participate**

The present study was approved (approval no. 2021KN81) by the Institutional Ethics Committee of Shanghai Tenth People's Hospital (Shanghai, China).

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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