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Original Article

WDR3 drives pancreatic cancer metastasis by enhancing TGF-α mRNA stability through YTHDC1

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Abstract: Pancreatic cancer is a highly aggressive malignancy associated with poor prognosis and early metastasis. However, the molecular mechanisms underlying its invasive behavior remain incompletely understood. Here, we identified WD repeat domain 3 (WDR3) as a key driver of pancreatic cancer cell invasion. WDR3 expression is significantly elevated in liver metastatic lesions and is correlated with disease progression. Functional assays revealed that WDR3 promotes cell migration and invasion by upregulating transforming growth factor-α (TGF-α). Mechanistically, WDR3 interacts with the m6A reader YTH domain-containing protein 1 (YTHDC1) and facilitates its K63-linked ubiquitination, resulting in increased cytoplasmic localization of YTHDC1. This modification enhances the stability of TGF-α mRNA, thereby promoting its expression. Knockdown of either WDR3 or YTHDC1 impairs TGF-α expression and suppresses cancer cell invasiveness, whereas YTHDC1 overexpression restores the metastatic phenotype in WDR3-deficient cells. Our findings reveal a novel WDR3-YTHDC1-TGF-α axis that drives pancreatic cancer progression and suggest that targeting WDR3 may be a promising therapeutic strategy.

Keywords: Pancreatic cancer, metastasis, WDR3, YTHDC1, TGF-α

Introduction

Pancreatic cancer is a highly malignant gastrointestinal tumor with a Median survival is approximately 4 months with a 5-year survival of 13% [1-3]. Owing to its aggressive nature and early metastatic potential, radical surgical resection remains the most effective strategy for prolonging survival. However, 85-90% of patients are diagnosed at an advanced stage with distant metastases, rendering them ineligible for surgery [4, 5]. Autopsy-based studies on metastatic patterns have shown that liver metastasis occurs in 76%-80% of patients, with other common metastatic sites including the peritoneum (48%) and lungs (45%) [6].

A growing body of research has revealed the critical role of posttranslational modifications in driving pancreatic cancer metastasis. Among these modifications, ubiquitination has emerged as a key regulatory mechanism. For example, tripartite motif-containing protein 15 (TRIM15) has been shown to promote pancreatic cancer invasion and metastasis by inducing the ubiquitin-mediated degradation of apolipoprotein A1 (APOA1) and altering lipid metabolism [7]. Ubiquitin-specific protease 44 (USP44) enhances gemcitabine sensitivity by deubiquitinating fructose-1,6-bisphosphatase 1 (FBP1), thereby inhibiting tumor progression [8]; FBP1 also interacts with the bromodomain of bromodomain-containing protein 4 (BRD4) to suppress pancreatic cancer progression [9]; Neuron-derived neurotensin (NTS) promotes pancreatic cancer invasiveness and gemcitabine resistance via the NTSR1/Akt pathway [10]. Furthermore, cyclin-dependent kinase 5 (CDK5)/F-box and WD repeat domain-containing 7 (FBW7)-dependent ubiquitin-mediated degradation of enhancer of zeste homolog 2 (EZH2) has been reported to inhibit pancreatic cancer cell migration and invasion [11].

However, the complex and multipathway nature of pancreatic cancer metastasis, along with the

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intricate crosstalk among these pathways, remains incompletely understood. This complexity significantly limits the development of effective combination therapies targeting metastatic disease. Therefore, deeper insights into the biology of pancreatic cancer invasion and metastasis are essential for improving patients' responses to chemotherapy and targeted therapies.

The WD-repeat protein family is characterized by multiple WD-repeat motifs. WDR3, a member of this family, encodes a nuclear protein composed of 943 amino acids, including 10 WD repeat domains [12-14]. In addition to its known role in the biogenesis of the 40S ribosomal subunit, WDR3 functions as a crucial deubiquitinating enzyme, contributing to tumor proliferation and invasion by maintaining genome stability, regulating p53 expression, and facilitating DNA damage repair [12-14]. Elevated expression of WDR3 has been associated with an increased risk of thyroid cancer in specific populations [15], and it has been shown to promote thyroid cancer progression by enhancing genomic instability [16]. Recent studies have shown that downregulation of WDR3 significantly inhibited the malignant progression of osteosarcoma [17]. Additionally, McMahon et al. reported that the suppression of WDR3 expression inhibits breast cancer cell proliferation [13]. These findings suggest that WDR3 may be a key regulator of pancreatic cancer invasion.

In the present study, we demonstrated that overexpression of WDR3 is correlated with invasive characteristics in pancreatic cancer patients. Silencing of WDR3 significantly reduced the proliferation and invasiveness of pancreatic cancer cells, an effect that was mediated by the inhibition of TGF-α and dependent on the deubiquitinating function of WDR3. Collectively, our findings highlight a critical role for WDR3 in mediating pancreatic cancer invasion and suggest that it may serve as a promising therapeutic target for clinical intervention.

Methods and material

Cell culture

The human pancreatic cancer cell lines PANC-1 and BxPC-3 were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). PANC-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; #30030, Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS; #10099141, Thermo Fisher Scientific) and 1% penicillin - streptomycin. BxPC-3 cells were maintained in RPMI-1640 medium (#88365, Thermo Fisher Scientific) supplemented with 10% FBS and 1% penicillin - streptomycin. All the cell lines were incubated in a humidified atmosphere with 5% CO_2 at 37°C.

Antibodies and chemicals

The primary antibodies used in this study included anti-WDR3 (#ab176817, 1:1000, Abcam), anti-TGF-α (#ab208156, 1:1000, Abcam), anti-YTHDC1 (#14392-1-AP, 1:1000, Proteintech), and anti-β-actin (#66009-1-Ig, 1:20000, Proteintech) antibodies. Protein A+G agarose (#P2029) and the IgG control (#A7007) were purchased from Beyotime Biotechnology (China).

Coimmunoprecipitation and Western blot analysis

The cells were lysed in RIPA buffer (#P0013, Beyotime) supplemented with protease inhibitors and incubated with the indicated antibodies and Protein A+G agarose beads overnight at 4°C. The beads were washed six times with RIPA buffer, and the immunoprecipitated proteins were eluted for Western blotting. Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. After blocking, the membranes were incubated with primary antibodies followed by HRP-conjugated secondary antibodies. Protein signals were visualized using an Amersham Imager 600 system (GE Healthcare, USA).

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

To identify WDR3-interacting proteins, Flag-tagged WDR3 was overexpressed in pancreatic cancer cells and immunoprecipitated with an anti-WDR3 antibody and protein A+G agarose beads (#P2012, Beyotime). The eluted proteins were analyzed by LC-MS/MS at Bioprofile Technology Co., Ltd. (Shanghai, China) using a Thermo Scientific Ultimate 3000 UHPLC system coupled with a Q Exactive Plus mass spec-

trometer. The raw data were processed with MaxQuant software (v1.6.6) utilizing the Andromeda search engine against the UniProt human protein database. Peptides and proteins were filtered at a false discovery rate (FDR) of 1%.

Real-time RT-PCR analysis

Total RNA was extracted using TRIzol reagent (#AG21102, Accurate Biotechnology, China). cDNA was synthesized using a reverse transcription kit (#AG11728, Accurate Biotechnology), and qRT-PCR was performed using a SYBR Green PCR kit (#AG11701, Precision Biotechnology, China) according to the manufacturers' protocols. Gene expression was normalized to that of GAPDH, and fold changes in expression were calculated using the 2^-ΔΔCt method. The primer sequences are listed in Supplementary Table 2.

RNA interference

shRNA constructs targeting WDR3, YTHDC1, and TGFA and the corresponding control vectors were obtained from the MiaoLing Plasmid Platform (China). The shRNA sequences are listed in Supplementary Table 1. Transfections were performed using Lipofectamine 2000 (#11668019, Thermo Fisher Scientific) according to the manufacturer's protocol. For knockdown experiments, cells were harvested after 48 h; for Flag-tagged RNA expression, cells were collected at 24 h post-transfection.

Preparation of nuclear and cytoplasmic extracts

The cells were harvested and resuspended in Buffer A (10 mM HEPES-KOH, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.1% NP-40) and incubated on ice for 10 min. The nuclear fraction was pelleted by centrifugation at 6500 rpm for 3 min at 4°C, washed with Buffer A, and recentrifuged at 3500 rpm for 5 min. The nuclear pellet was subsequently lysed in IP buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, and protease inhibitor cocktail) for 30 min on ice. Protein concentrations were determined using a BCA assay kit (Beyotime, China).

Cell invasion assay

Cell invasion assays were conducted using 24-well Transwell chambers with 8 μm pore membranes (Millipore) precoated with Matrigel (BD Biosciences, USA). Briefly, 1×10^5 transfected cells suspended in 200 μl of serum-free medium were seeded into the upper chamber, while 600 μl of complete medium containing 30% FBS was added to the lower chamber. After 12-24 h of incubation, the cells remaining on the upper surface were removed, and the invading cells on the lower surface were fixed with methanol and stained with 0.4% crystal violet. Invaded cells were imaged and counted in five random fields under a microscope. All the experiments were repeated in triplicate.

RNA immunoprecipitation (RIP) and Methylated (Me) RIP-qPCR

Total cell lysates were prepared by lysing cells with 1× RIPA buffer (Beyotime Biotechnology, China). Ten percent of the lysate was collected and reserved as input. Subsequently, primary antibody or IgG, along with Protein A+G beads (#P2029, Beyotime, China), was added to the remaining cell lysate and incubated at 4°C for 12 hours. The beads were then washed and resuspended in Proteinase K buffer, followed by shaking incubation at 55°C for 30 minutes in RNAiso Plus (Takara, 9109). Experiments were performed in accordance with the manufacturer's instructions using reverse transcription kits and PCR kits (#RR037A, PrimeScript™ RT Kit; #RR430A, TB Green™ Fast qPCR Mix; Takara Bio Inc., Shingo, Japan). Primer sequences are listed in Supplementary Table 3. The Magna m6A MeRIP Kit (#A-17-10499, A&D Technology, Beijing, China) was used for MeRIP-qPCR following the manufacturer's protocol, and the corresponding primer sequences are provided in Supplementary Table 4.

Bioinformatic mining

The correlation between WDR3 and TGF-α mRNA expression levels was assessed using the GEPIA2 database (http://gepia2.cancer-pku.cn/), which integrates data from the TCGA and GTEx projects.

Statistical analysis

All experimental data are presented as the mean ± standard deviation (SD) from at least three independent experiments. Differences between two groups were assessed using Student's t test. For multiple comparisons, one-way or two-way ANOVA followed by the

Bonferroni post hoc correction was employed. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software Inc., USA).

Results

WDR3 overexpression promotes pancreatic cancer cell migration and invasion

To elucidate the potential role of WDR3 in pancreatic cancer progression, we analyzed a GEO dataset (GSE71729) and observed that WDR3 expression was significantly elevated in liver metastatic lesions compared with primary pancreatic tumors. Furthermore, its expression progressively increased with tumor stage (**Figure 1A**, **1B**), suggesting the possible involvement of WDR3 in tumor metastasis. Functionally, we performed invasion assays in two distinct human pancreatic ductal adenocarcinoma (PDAC) cell lines: PANC-1 and BxPC-3, by silencing WDR3 in both BxPC-3 cells and PANC-1 cells significantly suppressed invasion, whereas WDR3 overexpression enhanced invasion (**Figure 1C-J**).

WDR3 promotes pancreatic cancer cell migration via TGF-α regulation

Transcriptomic profiling revealed that knockdown of WDR3 resulted in marked downregulation of TGF-α (**Figure 2A**). Correlation analysis using the GEPIA2 database further confirmed a strong positive association between WDR3 and TGF-α expression (**Figure 2B**). Consistent with this, silencing WDR3 in PANC-1 and BxPC-3 cells significantly reduced TGF-α expression, whereas WDR3 overexpression restored TGF-α levels (**Figure 2C-F**). To determine whether TGF-α mediates WDR3-driven migration, we constructed a dual-interference model in which TGFA was silenced and WDR3 was overexpressed. Transwell assays demonstrated that TGFA silencing partially abrogated the promigratory effect induced by WDR3 overexpression (**Figure 2G**, **2I** and Supplementary Figure 1). Moreover, cosilencing of TGFA and WDR3 resulted in more pronounced suppression of invasive capacity (**Figure 2H**, **2J** and Supplementary Figure 1).

WDR3 interacts with YTHDC1 and facilitates its K63-linked ubiquitination in pancreatic cancer cells

To investigate the mechanistic basis of WDR3-mediated signaling, we performed coimmunoprecipitation followed by LC-MS/MS analysis in PANC-1 cells. YTHDC1 emerged as the most prominent protein that interacts with WDR3 (**Figure 3A**). Notably, GATA binding protein 4 (GATA4), which was previously identified as a WDR3 interactor - validating the reliability of the mass spectrometry data. Coimmunoprecipitation assays confirmed the physical interaction between WDR3 and YTHDC1 (**Figure 3B**, **3C**). Given that WDR3 functions as an E3 ubiquitin ligase, we next assessed whether WDR3 could modulate YTHDC1 ubiquitination. Mutational analysis revealed that WDR3 specifically promoted the K63-linked ubiquitination of YTHDC1 (**Figure 3D-F**). Subcellular fractionation further revealed that WDR3 knockdown led to the cytoplasmic accumulation of YTHDC1, while overexpression promoted its nuclear redistribution (**Figure 3G-J**).

YTHDC1 regulates TGF-α mRNA stability and mediates WDR3-induced migration

YTHDC1 is a known m6A reader protein that modulates mRNA fate through m6A recognition. RIP-qPCR analysis demonstrated that YTHDC1 binds directly to TGF-α mRNA in both PANC-1 and BxPC-3 cells (**Figure 4A**). In parallel, data mining from the ENCORI database revealed the presence of YTHDC1 binding sites within the 3'UTRs of the TGF-α transcripts, which was further confirmed by m6A-RIP enrichment analysis (**Figure 4B**). Functional experiments demonstrated that YTHDC1 enhances TGF-α expression and stabilizes its mRNA, although it does not alter the subcellular distribution of TGF-α mRNA (**Figure 4C-J**). In contrast, we found that a YTHDC1 mutant specifically lacking the m6A-binding domain failed to exert such regulatory effects, underscoring the dependence on m6A recognition.

WDR3 promotes pancreatic cancer metastasis by stabilizing TGF-α mRNA via YTHDC1 ubiquitination

To determine whether WDR3 plays a pro-metastatic role through YTHDC1-mediated

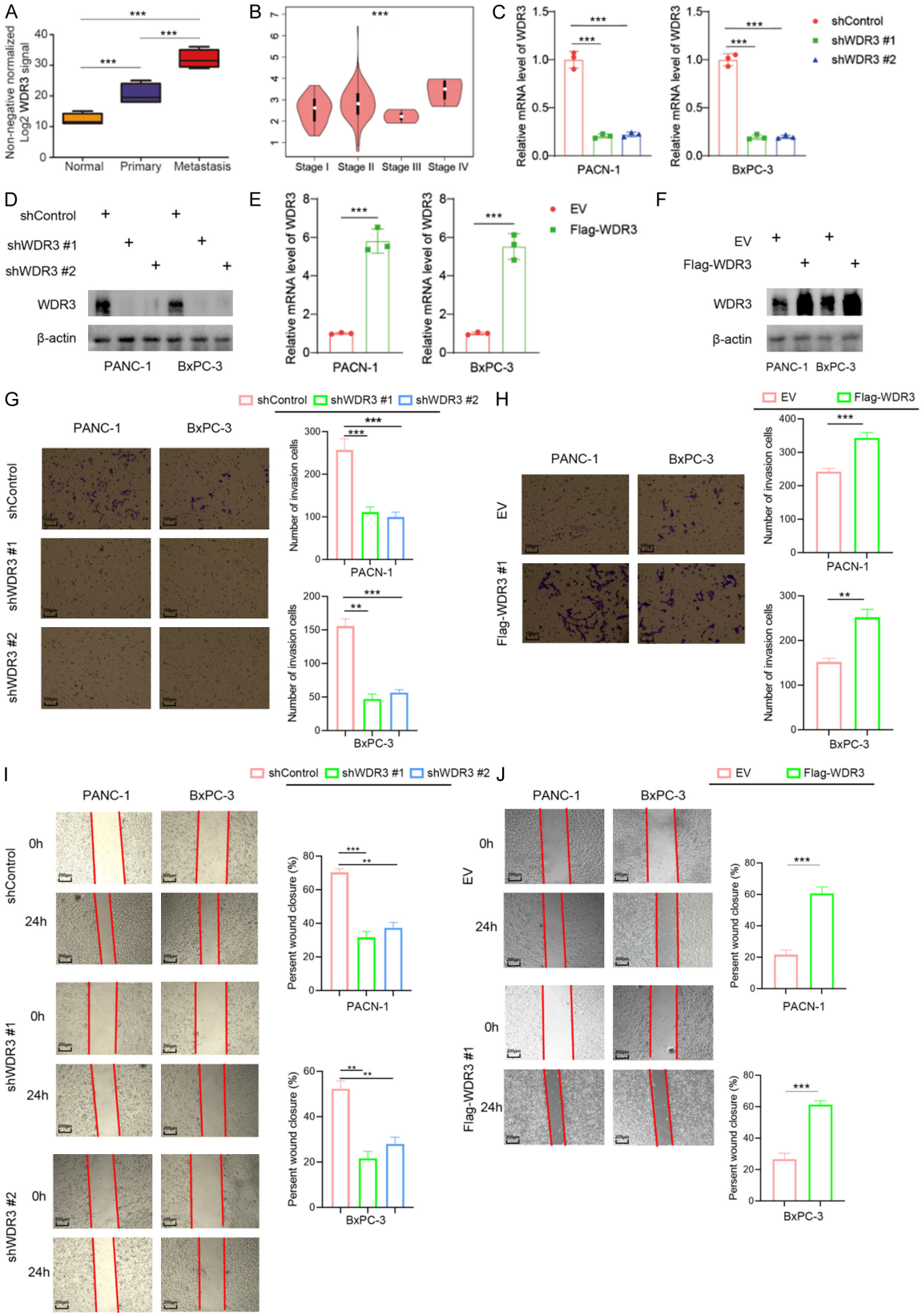

Figure 1. WDR3 promotes pancreatic cancer cell invasion and migration. A, B. Boxplot and violin plot show the expression levels of WDR3 in primary and liver metastatic pancreatic cancer tissues, based on the GSE71729 dataset. C-F. PANC-1 and BxPC-3 cells were transfected with control shRNA, WDR3-targeting shRNA, or Flag-WDR3 con-

structs. Cells were harvested after 72 h, and WDR3 expression was assessed by RT-qPCR. G, H. Transfected PANC-1 and BxPC-3 cells were seeded in Matrigel-coated transwell chambers. After 48 h, invaded cells were fixed, stained, and quantified. I, J. Wound-healing assay showing migration ability of PANC-1 and BxPC-3 cells after transfection with shWDR3#1/#2 or Flag-WDR3. Images were captured at 0 and 24 h post-scratch under serum-free conditions. ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

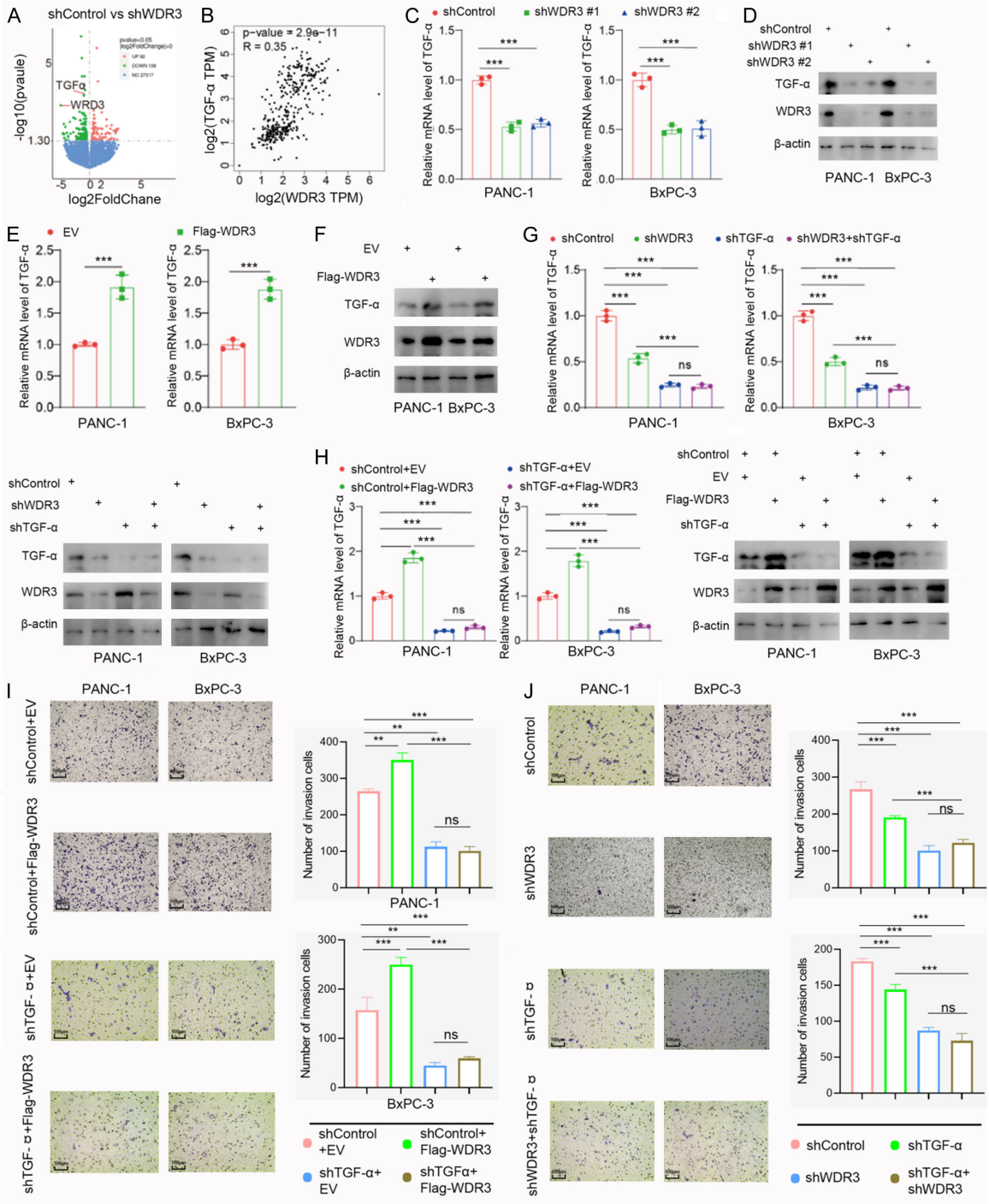

Figure 2. WDR3 regulates TGF-α expression and function in pancreatic cancer. A. RNA-seq analysis of PANC-1 cells following WDR3 knockdown. B. Correlation analysis of WDR3 and TGF-α mRNA expression in pancreatic cancer using the GEPIA2 online database. C-F. RT-qPCR analysis of TGF-α expression in PANC-1 and BxPC-3 cells transfected with WDR3 shRNAs or Flag-WDR3. G, H. RT-qPCR detection of TGFA mRNA levels in cells transfected as above. I, J. Invasion assays in cells with indicated genetic modifications. Cells were cultured in Matrigel-coated chambers for 48 h, and invaded cells were stained and counted. ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

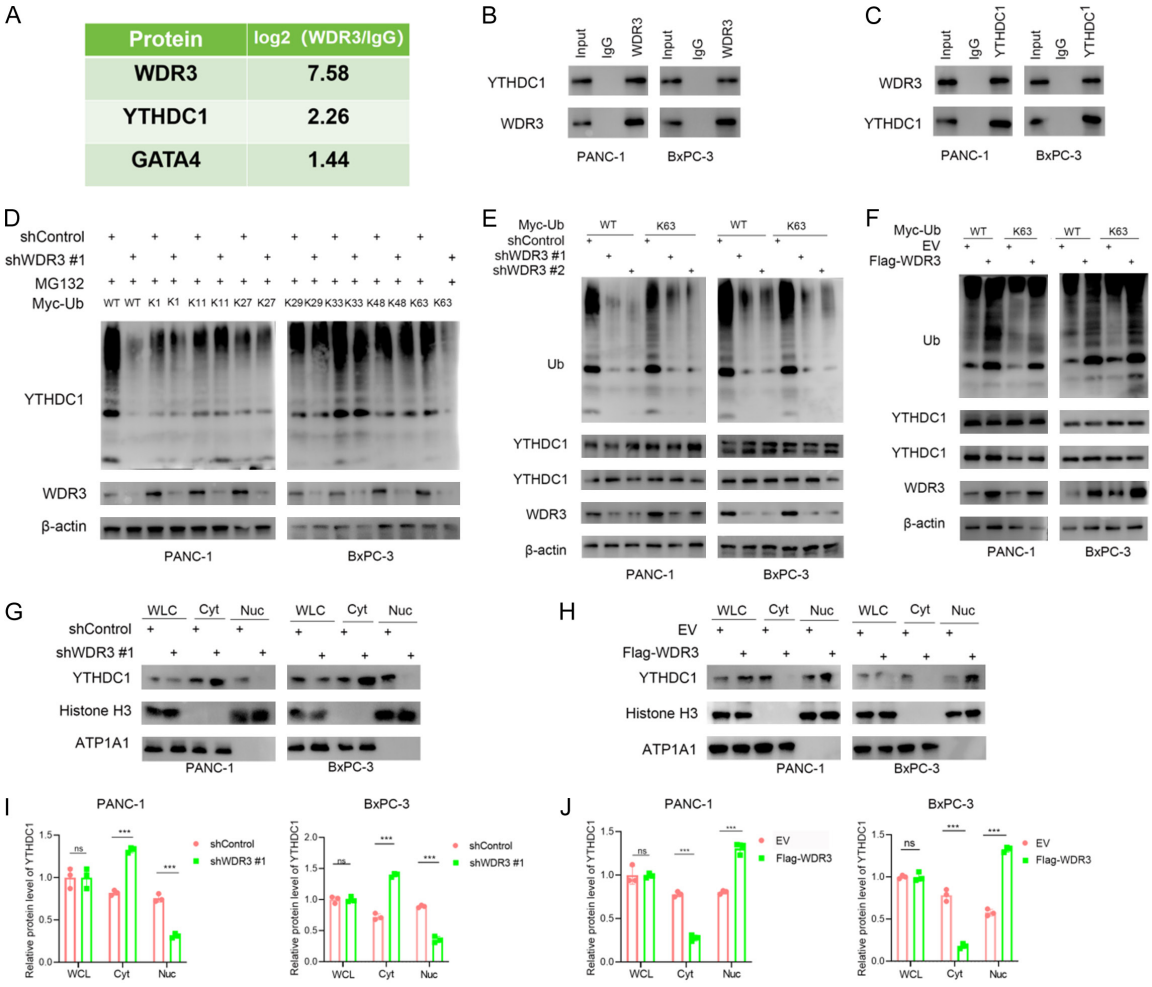

Figure 3. WDR3 interacts with and ubiquitinates YTHDC1 in pancreatic cancer cells. A. Flag-WDR3 was overexpressed in PANC-1 cells. After 24 h, cells were harvested for co-immunoprecipitation with anti-Flag antibody and subjected to LC-MS/MS analysis. B, C. Co-immunoprecipitation of endogenous WDR3 and YTHDC1 in PANC-1 and BxPC-3 cells. D. Site-directed mutagenesis was used to generate ubiquitination-deficient YTHDC1 mutants. Ubiquitination levels were detected by Western blotting. E, F. K63-specific YTHDC1 mutants were transfected along with WDR3 shRNA or Flag-WDR3. Ubiquitination of YTHDC1 was examined by Western blot. G-J. Cytoplasmic and nuclear fractions were extracted from PANC-1 and BxPC-3 cells after transfection with the indicated constructs. YTHDC1 distribution was analyzed by Western blot. ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

mRNA stabilization, we further knocked down WDR3 in YTHDC1-deficient cells. The results revealed a further reduction in TGF-α expression and mRNA stability (**Figure 5A-C**). Conversely, overexpression of YTHDC1 in WDR3-depleted cells restored both the stability of TGF-α mRNA and the invasive capacity of pancreatic cancer cells (**Figure 5D-H**). These findings collectively indicate that WDR3 stabilizes TGF-α transcripts by enhancing YTHDC1 cytoplasmic localization via K63-linked ubiquitination, thereby promoting pancreatic cancer cell invasion and migration.

Discussion

Pancreatic cancer, a highly malignant tumor of the digestive system, is characterized by its aggressive and metastatic nature, which often results in missed therapeutic windows at diagnosis. In our previous studies, we demonstrated that WDR3 is overexpressed in pancreatic cancer and is positively associated with poor patient survival. Similarly, Akdi et al. reported that WDR3 overexpression represents a significant risk factor for thyroid cancer [15]. Liu W et al. reported that overexpression of WDR3

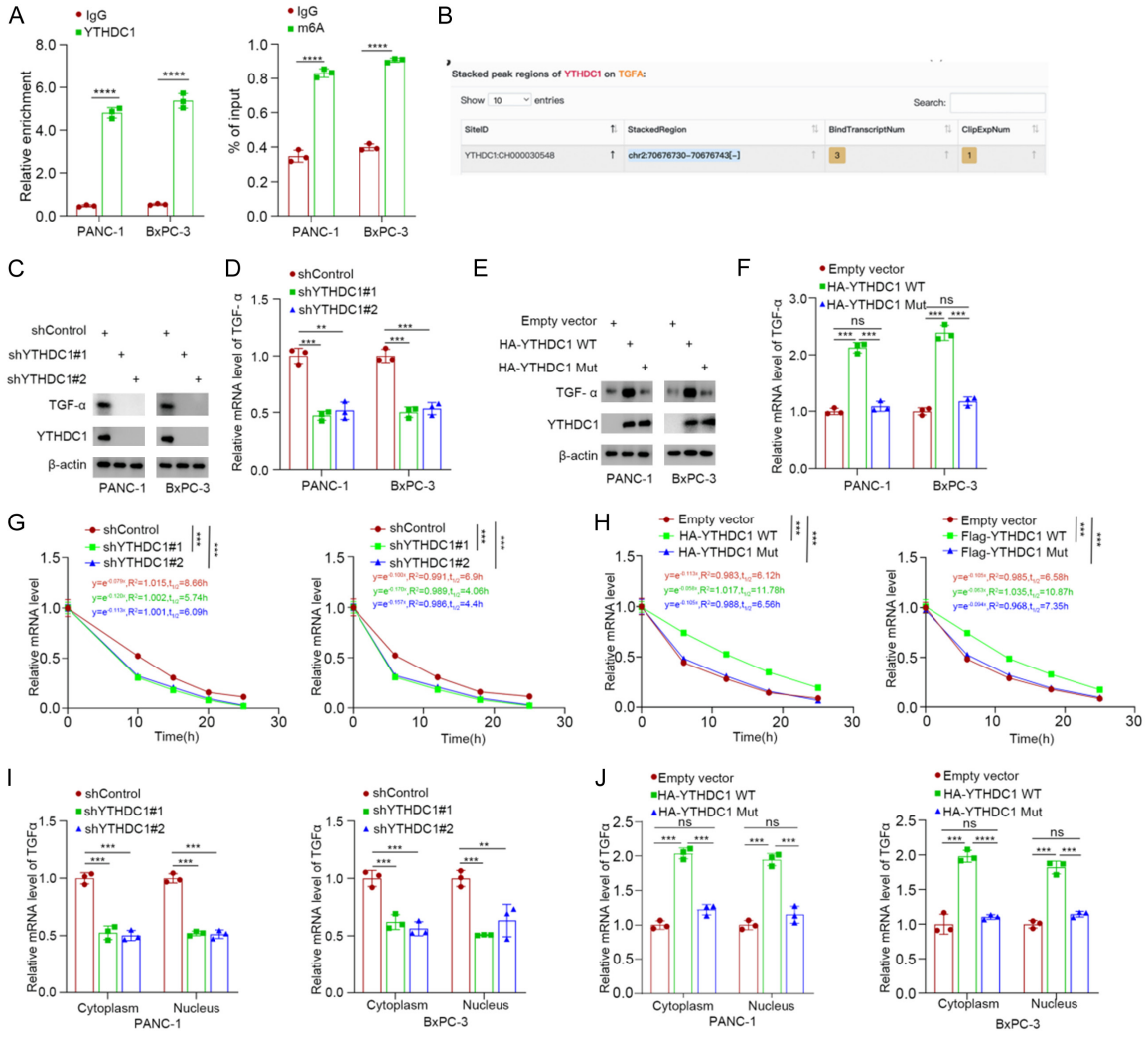

Figure 4. YTHDC1 binds to and stabilizes TGF-α mRNA. A. RIP assays were performed using anti-YTHDC1 or IgG antibodies in PANC-1 and BxPC-3 cells. MeRIP-qPCR confirmed m6A modification of TGF-α mRNA in both cell lines. B. ENCORI database analysis revealed putative YTHDC1 binding sites in the 3'UTR of TGFA mRNA. C-F. RT-qPCR and Western blot analyses of TGF-α expression after knockdown or overexpression of YTHDC1. G, H. mRNA stability assays showed that YTHDC1 knockdown reduced, while wild-type overexpression enhanced, TGFA mRNA half-life. I, J. RT-qPCR detection of cytoplasmic and nuclear TGFA mRNA levels after YTHDC1 knockdown or overexpression. ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

enhanced PCa cell proliferation, decreased cell apoptosis rate, increased spherical cell number and increased indicators of stem cell-like properties [18]. Furthermore, several research groups have implicated WDR3 in the regulation of genomic stability [16], increased cancer susceptibility [19], and cell proliferation by promoting G1 phase cell cycle arrest [13]. In the present study, we further revealed that WDR3 overexpression enhances the invasive capacity of pancreatic cancer cells. However, the underlying biological mechanisms by which WDR3 promotes pancreatic cancer invasiveness remain to be fully elucidated.

TGF-α, the second identified ligand of epidermal growth factor receptor (EGFR) following EGF, has been recognized as a critical mediator of tumor invasion and metastasis. In recent years, numerous studies have demonstrated that TGF-α stimulates EGFR activation and drives SMAD signaling, thereby promoting epithelial - mesenchymal transition (EMT) and spontaneous metastasis in lung cancer [20]. In addition, TGF-α has been shown to activate the NF-κB signaling axis and induce EMT in colorectal cancer [21]. However, the role of TGF-α in pancreatic cancer invasion remains largely unexplored.

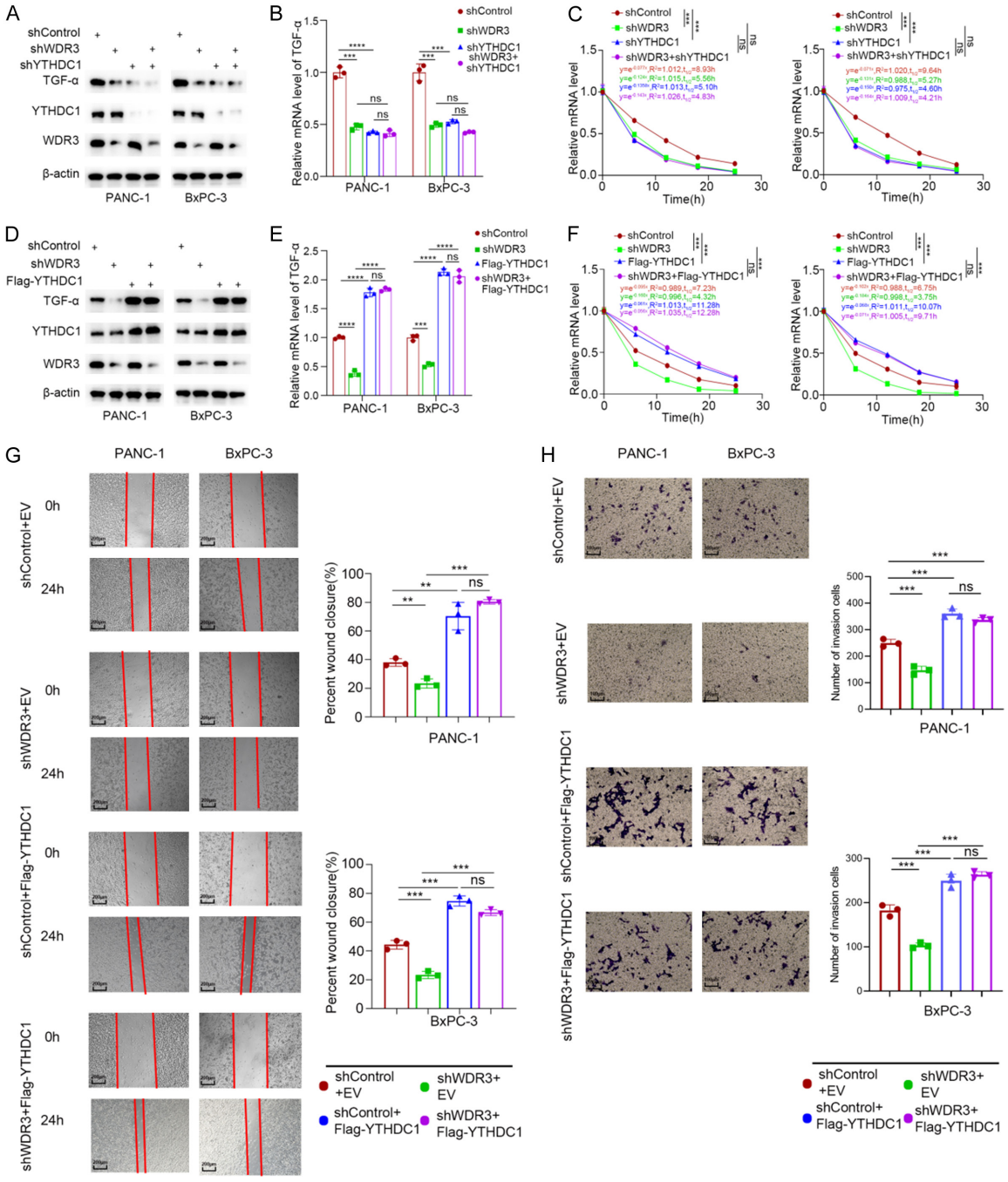

Figure 5. WDR3 stabilizes TGF-α mRNA through YTHDC1 ubiquitination. A, B, D, E. PANC-1 and BxPC-3 cells were transfected with YTHDC1 or WDR3 shRNAs, alone or in combination with Flag-YTHDC1. TGF-α expression was analyzed by RT-qPCR and Western blot. C, F. mRNA stability assays showing TGFA half-life changes in cells with YTHDC1 or WDR3 modulation. G. PANC-1 and BxPC-3 cells were transfected with shRNAs targeting specific genes (WDR3, YTHDC1), Flag-tagged constructs, shControl, or EV. Scratch assays were performed after confluence in serum-free medium, and wound closure was evaluated at 0 h and 24 h. H. Transfected PANC-1 and BxPC-3 cells were seeded into Matrigel-coated transwell chambers. After 48 h, invaded cells were fixed, stained, and counted under a microscope. ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

Our study identified TGF-α as a key downstream effector of WDR3, which indicates that WDR3 promotes pancreatic cancer metastasis through the upregulation of TGF-α. Importantly, we further identified YT521-B homology domain-containing protein 1 (YTHDC1), a

known N6-methyladenosine (m6A) reader protein, as a critical mediator of WDR3-induced TGF-α upregulation. As an m6A-binding protein, YTHDC1 can recognize and bind to m6A-modified mRNAs, thereby regulating various post-transcriptional processes, including mRNA nuclear export [22], alternative splicing [23], and mRNA stability [24].

Conclusion

In summary, we found that WDR3, YTHDC1, and TGF-α form a regulatory axis promoting pancreatic cancer metastasis. WDR3 overexpression is associated with disease progression, particularly in liver metastasis. WDR3 promotes the ubiquitination of YTHDC1, enhances its binding and stabilization of TGF-α mRNA, up-regulates TGF-α expression, and promotes cancer cell invasion and migration. Silencing WDR3 or YTHDC1 inhibits metastasis, while YTHDC1 overexpression can restore the phenotype of WDR3 deficiency. Targeting the WDR3-YTHDC1-TGF-α axis may provide a new strategy for improving the clinical outcome of patients with pancreatic cancer.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. The shRNA sequences

ShWDR3 #1	CCGGAGGACAAGCAGAATCACTATTCTCGAGAATAGTGATTCTGCTTGTCCTTTTTTTG
ShWDR3 #2	CCGGCCTGGAATACAAGATACTCTTCTCGAGAAGAGTATCTTGTATTCCAGGTTTTTTG
Sh-Control	Provided by RIBOBIO
ShYTHDC1 #1	GATCTGGATTTGCAGGCGTGAATTACTCGAGTAATTCACGCCTGCAAATCCATTTTTG
ShYTHDC1 #2	GATCTGCCTCCAGAGAACCTTATAACTCGAGTTATAAGGTTCTCTGGAGGCATTTTTG
ShTGFα #1	GAAGAATATAACACCAGCAAT
ShTGFα #2	CTTCTACTGCTACCGCGTTAA

Supplementary Table 2. The primer sequences for RT-qPCR

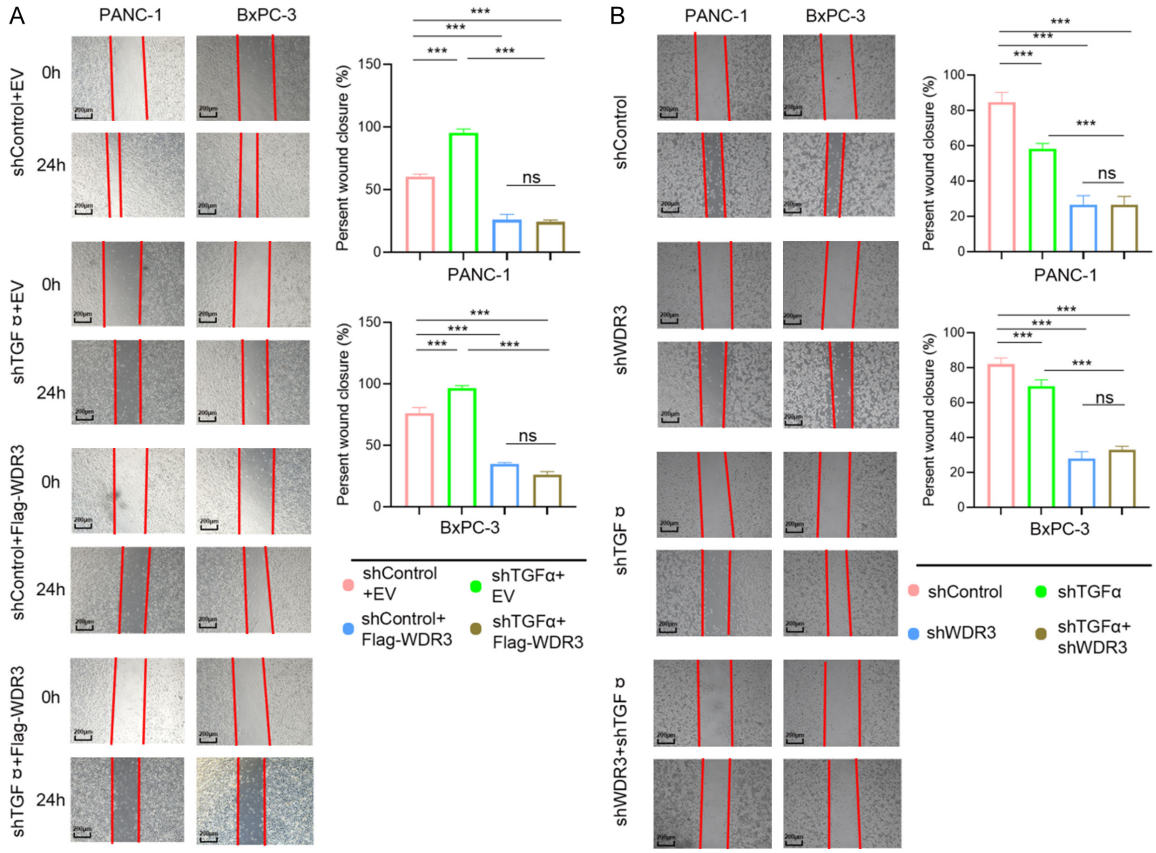
Gene (Human)	Forward primer (5'-3')	Reverse primer (5'-3')
β-actin	CTTCGCGGGCGACGAT	CCACATAGGAATCCTTCTGACC
WDR3	ACCAAGCAGTACCTACGCTAT	TTCTCACCACGAAGTGTCACA
YTHDC1	ATCTTCCGTTCGTGCTGTCC	GGACCATACACCCTTCGCTT
TGFα	CCGTAAAATGGTCCCCTCGG	TGATGGCCTGCTTCTTCTGG

Supplementary Table 3. The primer for RIP-PCR

Gene (Human)	Forward primer (5'-3')	Reverse primer (5'-3')
TGF-α	CCGTAAAATGGTCCCCTCGG	TGATGGCCTGCTTCTTCTGG

Supplementary Table 4. The primer for MeRIP-qPCR

Gene (Human)	Forward primer (5'-3')	Reverse primer (5'-3')
TGF-α	CCGTAAAATGGTCCCCTCGG	TGATGGCCTGCTTCTTCTGG



Supplementary Figure 1. WDR3 promotes pancreatic cancer cell migration via TGF-α regulation. WDR3 modulates pancreatic cancer cell migration in wound-healing assays. A, B. PANC-1 and BxPC-3 cells were transfected with control shRNA, empty vector (EV), WDR3-targeting shRNA#1 or shRNA#2, or Flag-WDR3. Upon reaching confluence, scratch wounds were introduced and cells were cultured in serum-free medium. Images were taken at 0 h and 24 h to assess migration ability. ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.