Over-expression of YTHDC2 Contributes to Progression of Prostate Cancer and Predicts Poor Outcome in Patients with Prostate Cancer

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Abstract

Objective: YTHDC2, a member of N6-methyladenosine (m6A) readers, has been reported to be closely associated with multiple cancer types. However, very little is known about YTHDC2 gene and its involvement in prostate cancer.

Methods: YTHDC2 protein expression level was analyzed and correlated to clinical outcomes in prostate cancer patients who underwent prostatectomy in Guizhou Provinicial People's Hospital. YTHDC2 expression level was also detected in prostate cancer cell lines and an immortalized prostate epithelial cell line BPH-1 and RWPE1 by qRT-PCR. Furthermore, we established stable cell lines (DU145 and PC-3) transfected with either empty vector or the full-length YTHDC2 gene and conducted cell function assays in vitro. Fisher's exact test and Pearson $\chi 2$ test was employed, Kaplan-Meier method was used for the survival analysis.

Result: Of 32 patient samples who enrolled in this study, YTHDC2 was significantly upregulated in PCa patients with higher Gleason score and Serum PSA level. YTHDC2 expression was significantly elevated in all PCa cell lines compared to BPH-1 and RWPE1 (all $P<0.05$). Functionally, the enforced expression of YTHDC2 markedly promoted cell growth, migration and invasion efficacies in prostate cancer cells.

Conclusion: Our data indicate that YTHDC2 upregulation may be potentially associated with prognosis of prostate cancer patients.

Trial registration: Retrospectively registered.

Background

Prostate cancer (PCa) is the most common non-dermatologic malignancy and the second leading cause of cancer related death in American men. During 2021, an estimated 248,530 men will have PCa diagnosed, and 34,130 men will die of this disease in the United States[1]. Although early diagnosis provides an opportunity for curative surgery, up to 35% of men undergoing radical prostatectomy will develop a prostate-specific antigen (PSA) recurrence within 10 years after surgery, often as a result of micrometastatic disease present at the time of surgery[2-4]. The challenge is to identify those patients who are at high risk for relapse and who may benefit from secondary treatment that can negatively impact quality of life[5]. Several standard preoperative and postoperative clinical and pathological variables including tumor stage, Gleason score, and the serum PSA are used in routine clinical practice to stratify men into groups at low, intermediate, and high risk for tumor recurrence following local therapy[6-8]. However, the majority of patients who now undergo prostatectomy have similarly low to intermediate risk clinical features, and predicting outcomes differences among these patients remains difficult. A better understanding of molecular abnormalities that define those at high risk at relapse is needed.

In recent years, as the most abundant mRNA modification in eukaryotic cells[9, 10], m6A RNA modifications are widely regarded as essential epigenetic modifications in many biological processes through the dynamic regulation of gene expression and have received widespread attention. It is well known that m6A modifications are essential for a variety of pathological processes, including cancer development and progression.2-6 The current research found that m6A is a dynamic process, and its function is determined by the "Writer", "Eraser"

and "Reader". The function of m6A is recognized through the m6A binding protein, which is the "reader". To date, several m6A binding proteins have been identified such as the YTH (YT521-B homology) domain protein family, namely YTHDF1[11], YTHDF2[12], YTHDF3[13], YTHDC1[14], and YTHDC2[15], and the nuclear heterogeneous protein HNRNP family, namely HNRNPA2B1 and HNRNPC[15]. YTH domain containing 2 (YTHDC2) is the largest N6- Methyladenosine (m6A) binding protein of the YTH protein family and the only member containing ATP-dependent RNA helicase activity, and its biological function has not yet been fully elucidated[16, 17]. At present, YTHDC2 enhances translation efficiency and reduces the abundance of target mRNAs by preferentially binding m6A within the consensus motif[15, 17]. Kretschmer *et al.*[18] revealed that YTHDC2 is mainly enriched in the perinuclear region, and it can combine with ribosomes to act on small ribosomal subunits and improve translation efficiency. However, the precise association between YTHDC2 and cancer remains unclear. Upregulation of YTHDC2 has been reported to promote metastasis of colon cancer cells in vitro by promoting the translation of genes associated with cell migration. Down-regulation of YTHDC2 can inhibit HCC cell proliferation by reducing the translation of mRNAs involved in cell proliferation[19]. However, the role of YTHDC2 in other types of tumors, particularly prostate cancer, remains unresolved. To address this problem, in the current study, *YTHDC2* was analyzed and correlated to clinical outcomes in patients who underwent prostatectomy for clinically localized PCa between 2019 and 2020 in Guizhou Provinicial People's Hospital. *YTHDC2* expression level was also investigated in PCa cell lines and an immortalized prostate epithelial cell line by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). To further characterize *YTHDC2* functions in PCa cells, we established stable YTHDC2-overexpressing/knockdown PCa cell lines, and in vitro cell proliferation, cell cycle, cell migration and invasion assays were carried out.

Materials And Methods

Patients and Tissue Specimens

The retrospectively study was approved by the human study ethics committees at Guizhou Provinicial People's Hospital [No:(2020)394]. All specimens were handled and made anonymous according to the ethical and legal standards. Tissue samples (n =32) were obtained from consecutive patients who underwent RP surgery as part of their clinical care at Guizhou Provinicial People's Hospital from September 2019 to September 2020. Medical records were reviewed, and clinical data captured included demographic information, pre-surgery PSA, TNM stage, distant metastasis and Gleason score (Table 1). Meanwhile, a tissue microarray (TMA, n=116) including 58 prostate cancer tissues, 58 adjacent non-cancerous prostate tissues were obtained from Changhai Hospital of Second Military Medical University (Y.W.Y). The clinical features was exhibited in the Table 1.

The TCGA dataset (https://cancergenome.nih.gov/) is downloaded from the cBioPortal for Cancer Genomics (http://www.cbioportal.org/). In addition, the clinical information was downloaded. The median value of YTHDC2 expression levels in PCa tissues of TCGA dataset was used as a cutoff point. High expression group was defined when YTHDC2 expression was more than the median, while the rest was the low expression group.

Immunohistochemistry analysis

Specimens were fixed in 10% neutral buffered formalin and subsequently embedded in paraffin. Paraffin-embedded tissue was cut to 4 μm, then de-paraffined in xylene and rehydrated for further peroxidase immunohistochemical staining using the DAKO EnVision system (Dako Diagnostics, Switzerland). Following a brief proteolytic digestion and a peroxidase blocking of tissue slides, the slides were incubated overnight with the primary antibody against YTHDC2 (rabbit polyclonal antibody, ab220160, Abcam Co. Ltd., UK) at a dilution of 1: 500, at 4℃. After washing, peroxidase labeled polymer and substrate-chromogen were employed in order to visualize the staining of the interested protein. In each immunohistochemistry run, negative controls were carried out by omitting the primary antibody.

The intensity of immunostaining was scored separately by two independent experienced pathologists, who were blinded to the clinicopathological data and clinical outcomes of the patients. The scores of the two pathologists were compared and any discrepant scores were trained through reevaluated by a re-examination of the staining by both pathologists to achieve a consensus score. The immunolabeling of cancer cells was evaluated. The number of positive-staining cells in five representative fields at a 400-fold was counted and the percentage of positive cells was also calculated. According to the antibody specification sheet, cytoplasmic staining was regarded as positive signals. The semi-quantitative scoring of the expression intensity in each sample was performed according to a previous report and was based on the staining intensity and percentage. The staining intensity was visually scored and stratified according to the following criteria: no staining (0 points), mild staining (1 point), moderate staining (2 points), and strong staining (3points). The percentage scoring of immunoreactive tumor cells was defined as follows:<5% (0 points), 6-25% (1 point), 26-50% (2 points), 51-75% (3 points), and >75% (4 points). The final immunoreactivity scores (IRS) of each case were the addition of calculated by adding the two scores for the immunostaining intensity and immunostaining percentage.

Cell culture

Four human PCa cell lines (LNCaP, PC-3, DU-145 and 22Rv1) and two immortalized prostate epithelial cell line BPH-1/ RWPE1 were obtained from Guangzhou HYY Med.Co., Ltd and maintained in RPMI 1640 or DMEM supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) and penicillin (50 IU/ml)/streptomycin (50 μg/ml) (Thermo Fisher Scientific, Waltham, MA, USA).

YTHDC2-overexpressing/knockdown PCa cell model

To construct YTHDC2 overexpression/knockdown stable cells, two PCa cell lines DU145 and PC-3 were transfected with YTHDC2 human cDNA clone or pCMV6-neo vector according to the manufacturer's protocol (Origene, Rockville, MD, USA). 48 hours after transfection, cells were selected using G418 (1.2 mg/ml) (Cellgro, Manassas, VA, USA). Generate and maintain stable cell lines with DU145 knockout and PC-3 overexpressing YTHDC2 in intact RPMI 1640 containing G418 (1.2 mg/ml). Then the overexpression/knockdown of YTHDC2 cell lines were confirmed by real-time RT-PCR.

qRT-PCR

Expression levels of YTHDC2 mRNA in PCa cell lines and clinical PCa tissues were detected by qRT-PCR analysis according to the protocol of our previous studies [18-19]. The sequences of all the primers used in this study were as following: YTHDC2 (F:TGCCTTTGCTCAGGTCTTTC,R:CCAGCCATTTGATGCTTTAC); GAPDH (F:TGACTTCAACAGCGACACCCA, R:CACCCTGTTGCTGTAGCCAAA).

Cell viability assay

Cell proliferation rates were assessed by Cell Counting Kit 8 assay according to the supplier' s instructions. The effects of YTHDC2 on prostate cancer cell proliferation were measured by CCK-8 assay. The cells were plated in 96-well culture plates at a density of 1 × 104 cells/well and were counted at 1d, 2d,3d,4d and 5d after transfection.

Cell invasion and migration assays

For cell migration assay, 1 × 105 prostate cancer cells in 100 μL serum-free medium were added in the upper Transwell chamber (8.0-μm pore size; BD, San Jose, CA, USA). The upper chamber was coated with Matrigel for the invasion assay. The 10% fetal bovine serum was added to the bottom chambers. The cells were allowed to migrate or invade for 24h, respectively. And, the cells were allowed to migrate or invade for 24h, respectively. The migrating or invading cells at the lower surface were fixed with methanol and stained with 1% crystal violet. Cells were counted, and images were acquired using a Nikon TI-S microscope (Nikon, Japan) at magnification 10×.

Statistical analysis

The SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and R software version: 4.1.0 were used for statistical analysis. All experiments were repeated thrice, and data were expressed as mean ±SD. Statistical analysis was performed independently by two biostatisticians with Fisher's exact test for any 2×2 tables and Pearson χ2 test for non-2×2 tables. Kaplan-Meier method was used for the survival analysis. A probability level of $P<0.05$ was considered significant.

Results

YTHDC2 upregulation in human prostate cancer tissues

The expression level and localization of YTHDC2 protein in the our cohort and TMA were analyze. As shown in Figure 1A,B,C,D, YTHDC2 immunostainings occurred strongly in the cytoplasm in cancer cells of tissues, but weakly in adjacent tumor prostate tissues in our cohort. Among the 32 prostate samples, 17 (36.1%) samples exhibited low YTHDC2 expression, while 15 (63.9%) samples were highly stained for YTHDC2. Furthermore, the expression level of YTHDC2 in prostate adenocarcinoma tissues was significantly higher than that in adjacent normal prostate tissues (IRS: Prostate adenocarcinoma =2.00±0.65 vs. Normal= 1.30±0.42, $P< 0.05$) (Figure 1E). Meanwhile, YTHDC2 was closed correlated with PSA, TNM and Gleason (Table 2).

Then, we also validated YTHDC2 protein expression in TMA by Immunohistochemistry analysis (IHC) (Figure 2A). Similar findings were observed in the TMA cohort. Among the 116 samples, 2 samples were off-target during slicing process. Therefore, Of the 114 prostate adenocarcinoma samples, 55 (48.25%) demonstrated low YTHDC2 expression, while 59 (51.75%) were highly stained for YTHDC2. The level of YTHDC2 protein is higher in tumor tissues and lower in adjacent tumor samples (Figure 2B,C). As the Gleason score increases, the higher the expression of YTHDC2 protein (Figure 2D,E). Furthermore, the protein level of YTHDC2 in prostate adenocarcinoma tissues was significantly higher than that in adjacent tumor prostate tissues (IRS: Prostate adenocarcinoma = 1.32±0.72 vs. Prostate = 0.82±0.34, P< 0.001) (Figure 2F). Immunostaining results were analyzed using only the limited clinical information of the TMA. The results showed that the overexpression of YTHDC2 protein was significantly associated with serum PSA levels (P <0.05) and Gleason score (P<0.05). However, high YTHDC2 levels were not associated with patients' age and clinical stage (P > 0.05) (Table 2).

YTHDC2 upegulation predicts shorter BCR-free survival of PCa patients

To evaluate the prognostic value of YTHDC2 expression in PCa, the Kaplan-Meier method was performed to analyze the correlations between YHTDC2 expression with biochemical recurrence (BCR) -free survival of PCa patients in TCGA dataset. Pairwise comparisons showed a significant difference in the BCR-free survival between patients with high and low YTHDC2 expression based on TCGA cohort (P<0.05, Figure 3A). In the TCGA dataset, YTHDC2 expression was associated with the clinical T stage, nodal metastasis, Gleason score (Figure 3B,C,D). In addition, We detected the mRNA levels of YTHDC2 in castration-resistant prostate cancer (CRPC) with different metastasis sites using Human Cancer Metastasis Database (HCMDB) to further examine their expression in PCa. The expression of YTHDC2 was statistically significant at different CRPC metastasis sites (P < 0.05, Figure 3E). The relationship of the expression of YTHDC2 and AR was also assessed, and the results demonstrated that YTHDC2 was positively correlated with AR (P < 0.05, Figure 3F).

YTHDC2 overexpression promotes cell proliferation, invasion, migration and apoptosis of PCa cells in vitro

Firstly, we examined the expression of YTHDC2 at the nucleic acid level in prostate cancer cell lines (Lncap, PC3, DU145, 22RV1) and benign prostate cell lines (RWPE-1, BPH) by RT-PCR assay, and found that the expression of YTHDC2 was higher in prostate cancer cell lines (PC3, DU145) than in benign prostate cell lines (RWPE-1, BPH), and the difference was statistically significant (Figure 4A). To determine the tumor oncogene role of YTHDC2 in PCa, we first established stable cell line overexpressing/knockdown YTHDC2 after lentivectors transduction. qRT-PCR was used to confirmed that the YTHDC2-overexpressing PC-3 cells and YTHDC2-knockdown DU-145 cells were successfully established (Figure 4B,C).

CCK-8 assays indicated that the cellular proliferation of the YTHDC2-overexpressing PC-3 cells was significantly higher than those of control vectors-transfected cells (Figure 5A). YTHDC2-knockdown DU145-3 cells was significantly lower than those of control vectors-transfected cells (Figure 5B). Transwell assays clearly revealed that enforced expression of YTHDC2 significantly strengthened the invasive activities of PC-3

cells compared with those of control cells (Figure 5C). Vice versa, knockdown of YTHDC2 reduced the invasive activities of DU145 cells compared with those of control cells (Figure 5D). Wound-healing assays demonstrated that YTHDC2 upregulation markedly increased the migratory abilities of PC-3 cells and knockdown of YTHDC2 significantly reduced the migratory abilities of DU145 cells (Figure 5E). In contrast, the cell invasion of YTHDC2-transfected PC-3 cells were significantly higher than those of control cells (Figure 5F). Meanwhile, the cell invasion of YTHDC2-transfected PC-3 cells were significantly higher than those of control cells (Figure 5G). Vice versa, YTHDC2-transfected DU-145 cells were significantly lower than those of control cells (Figure 5H). The above results based on PC3/DU145 cells were in line with that of prostate cancer shown in Figure 1.

Discussion

Members of the YTH domain protein family act as key regulators of gene expression by specifically recognizing and binding m6A-containing RNAs. YTHDC1 has been shown to play an important regulatory role in precursor mRNA splicing in the nucleus, and YTHDF1, YTHDF2 and YTHDF3 have been found to act synergistically in promoting efficient translation and degradation of specific m6A-containing RNAs in the cytoplasm[20, 21]. Notably, the fifth member of the YTH protein family, YTHDC2, is found both in the cytoplasm and in the nucleus. As a m6A binding protein, YTHDC2 participates in a variety of biological processes in the body. Studies have shown that YTHDC2 plays an extremely important role in the process of spermatogenesis[15]. Other study demonstrated that YTHDC2, as a "reader" and 3'-5' RNA helicase for m6A in the cytoplasm, is essential for regulating m6A transcripts to ensure meiosis in the germline of mammals. The correctness of the gene expression program, which is essential for the fertility of male and female mice[16]. In addition to its important role in the reproductive system, YTHDC2 is also involved in the development of many tumors and other diseases. YTHDC2 has been implicated in the development of certain types of cancer, including HCC[19], lung cancer[22], head and neck cancer[23] and colon cancer[24]. However, very little is known about YTHDC2 gene and its involvement in PCa.

The treatment and survival of PCa patients has improved due to the widespread use of PSA screening, physical examination and tissue biopsy, and due to the innovation of surgical techniques and the reduced incidence of surgical complications, many local PCa patients who undergone radical prostatectomy surgery intervention have achieve a long-term survival[25]. But the accumulation of evidence suggests that the above method may still miss the malignant tumor for different reasons[26], once PCa patients get metastatic and invasive events, the tumor eventually progresses to a late stage leading to death[27]. There is considerable evidence that global PSA screening brings about over-diagnosis of PCa, which in turn leads to over-treatment of patients with inert disease[28]. Therefore, it is very necessary to identify new and more effective biomarkers to distinguish between indolent and aggressive PCa, so that patients with low risk of progression can better benefit from avoiding unnecessary treatments. In the present study, we found that YTHDC2 upregulation was significantly associated with shorter BCR-free survival in PCa patients in TCGA dataset, prompting us to identify a role for YTHDC2 in the malignant phenotype of PCa in vitro. In our in vitro studies, we found that PCa cell lines showed strong expression of YTHDC2 compared to BPH-1 and RWPE1 cells, suggesting that YTHDC2 expression levels may correlate with the malignancy of PCa cells. The mechanisms regulating YTHDC2 mRNA expression in normal and cancerous prostate epithelial cells remain unclear.

The expression of YTHDC2 at the protein level in human prostate cancer and non-cancerous prostate tissue was examined by immunohistochemical analysis, and a higher expression level of YTHDC2 was observed in the tumor samples. Moreover, the expression of YTHDC2 is significantly correlated with TNM stage, Gleason and PSA of prostate cancer. In the vitro study, YTHDC2 suppresses tumor growth and invasiveness. The regulatory mechanism of YTHDC2 mRNA expression in normal and cancerous prostate epithelial cells remains unclear. More and more studies have shown that N6-methyladenosine (m6A) plays a vital role in tumorigenesis and tumor development. As an m6A binding protein, YTHDC2 participates in a variety of biological processes in the body. Studies have shown that YTHDC2 plays an extremely important role in spermatogenesis[15]. Wojtas et al[16] suggest that YTHDC2, as a cytoplasmic m6A "reader" and 3'-5' RNA decapping enzyme, is essential for the regulation of m6A transcripts to ensure correct meiotic gene expression programs in the mammalian germline, which is critical for the fertility of male and female mice. In addition to its important role in the reproductive system, YTHDC2 is also involved in the development of many tumors and other diseases. Liu et al suggested that YTHDC2 may be associated with autism[29]. Fanale et al[30] reported that the YTHDC2 gene may be a potential susceptibility gene for pancreatic cancer, a useful marker for early detection of pancreatic cancer, and may also help in the development of new strategies for the treatment of pancreatic cancer. In addition, it has also been shown that YTHDC2 is highly expressed in some human cancer cells and that knockdown of YTHDC2 in hepatocellular carcinoma cells inhibits cell proliferation and reduces cell activity, suggesting that YTHDC2 plays an important role in the development of hepatocellular carcinoma[19]. Tanabe et al[24] demonstrated that the helicase function of YTHDC2 can help colon tumor metastasis by promoting the translation of HIF-1α. It is unknown whether chromosomal translocation of YTHDC2 occurs in PCa and whether this could affect YTHDC2 expression.

Conclusions

Our study suggests that enhanced YTHDC2 expression may be associated with PSA recurrence. In addition, our in vitro studies suggest that YTHDC2 promotes PCa cell growth and invasion. These findings suggest that YTHDC2 may play an oncogenic role in PCa. Our data also showed the possible post-transcriptional regulation of YTHDC2 expression.

Abbreviations

PCa: Prostate cancer; PSA: prostate-specific antigen; YTHDC2: YTH domain-containing protein 2; m6A: N6-Methyladenosine;qRT-PCR: quantitative real-time reverse transcription polymerase chain reaction; TMA: tissue microarray; IHC: immunohistochemistry analysis;BCR: biochemical recurrence; CRPC: castration-resistant prostate cancer; HCMDB: Human Cancer Metastasis Database.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Data availability could be obtained from TCGA website.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JKS; JGZ; WMC; GHZ wrote the main manuscript text;

JKS prepared Figures 1-5;

JKS, JGZ contributed to data analysis;

All authors reviewed the manuscript.

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Tables

Table 1

Clinical features of all patients.

Clinical features	Cohort	
	Our cohort	TMA
Tissue		
Prostate cancer samples	16	57
Cancer adjacent Prostate tissues	16	57
Age	56.80±11.16	61.04±6.81
<60	20	69
≥60	12	45
Gleason Score		
< 7	12	32
= 7	15	73
> 7	5	9
Clinical stage		
T2 <T3A	27	107
T3 ≥T3A	5	7
SerumPSA Levels (ng/ml)		
<4	4	38
≥4	28	76
Metastasis		
No	30	102
Yes	2	12

Table 2

Correlation of YTHDC2 expression with clinicopathologic characteristics in patients with prostate cancer.

Clinical features	Our cohort				TMA			
	Case	Low, n (%)	High, n (%)	*P*	Case	Low, n (%)	High, n (%)	*P*
Tissue								
Cancer	16	4(25.0)	12(75.0)	<0.001**	57	13(22.81)	44(77.19)	<0.001**
Adjacent-tumor	16	13(81.25)	3(18.75)		57	42(73.68)	15(26.32)	
Age								
<60	20	9(45.0)	11(55.0)	0.261	69	35(50.72)	34 (49.28)	0.462
≥60	12	8(66.67)	4(33.33)		45	20(44.44)	25 (55.56)	
Gleason Score								
< 7	12	8(66.67)	4(33.33)	0.237	32	17(53.13)	15(46.88)	0.03*
= 7	15	7(46.67)	8(53.33)		73	35(47.95)	38(52.5)	
> 7	5	2(40.0)	3(60.0)		9	3(33.33)	6(66.67)	
Clinical stage								
T2 <T3A	27	15(55.56)	12(44.44)	0.067	107	53(49.53)	54(50.47)	0.641
T3 ≥T3A	5	2(60.0)	3(40.0)		7	2(28.57)	5(71.43)	
Serum PSA Levels (ng/ml)								
<4	4	2(50.0)	2(50.0)	0.048*	38	22(57.89)	16(42.11)	0.03*
≥4	28	15(53.57)	13(46.43)		76	33(43.42)	43(56.58)	
Metastasis								
No	30	17(56.67)	13(43.33)	-	102	54(52.94)	48(47.06)	0.887
Yes	2	0(0.0)	2 (100.0)		12	1(8.33)	11(91.67)	

Note：The “-” means there are lack of relative information of patients in our cohort.

Figures

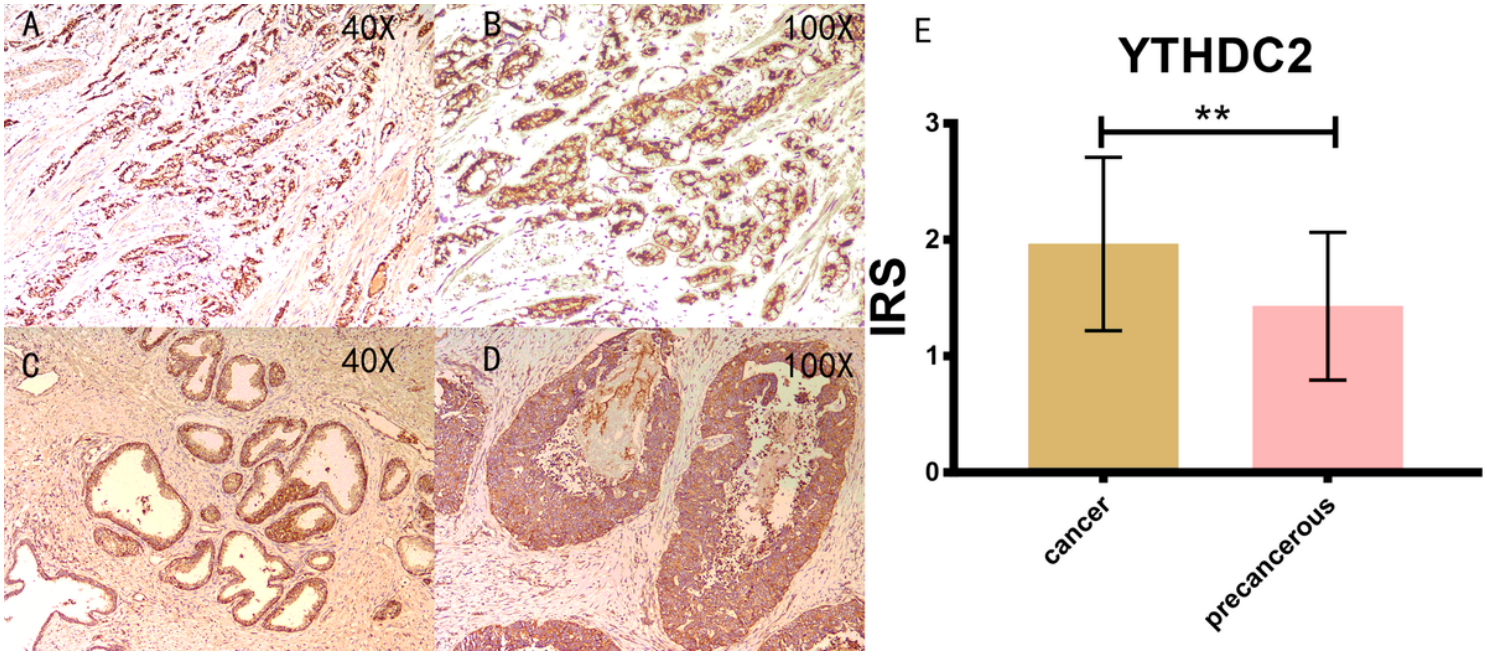

Figure 1

Immunohistochemical staining for YTHDC2 in prostate cancer and adjacent non-cancerous prostate tissues in our prostate cancer cohort. (A-D) Immunohistochemical staining indicated that YTHDC2 immunostaining occurred mainly in the cytoplasm in cancer cells from PCa tissues, however, weak or moderate stianing was observed in adjacent non-cancerous prostate tissues. (E) The expression level of YTHDC2 in the PCa tissues was signifcantly higher than that in the non-cancerous prostate tissues [immunoreactivity score (IRS): PCa, 2.00±0.65 vs. benign, 1.30±0.42; P<0.05] .

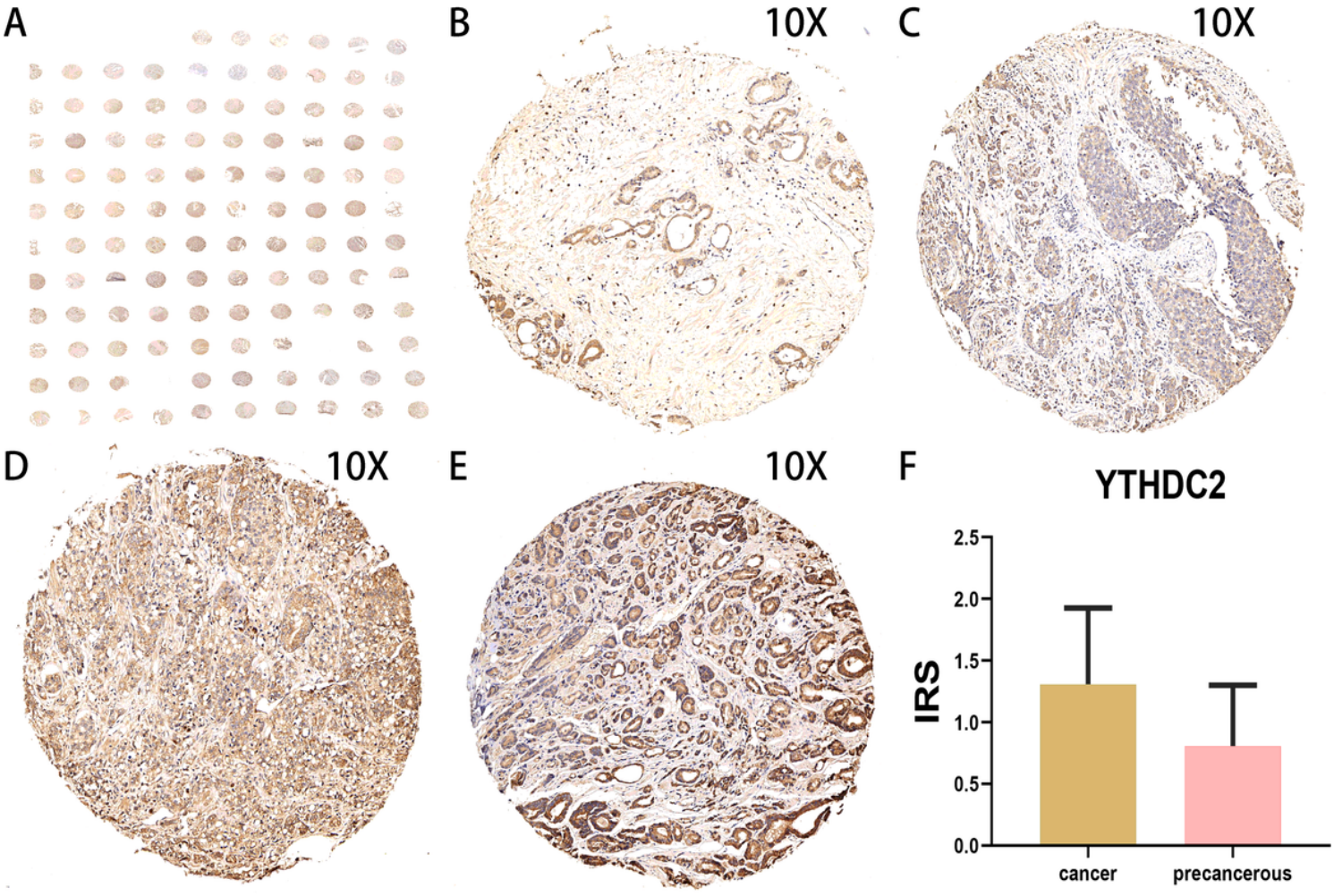

Figure 2

Immunohistochemical staining for YTHDC2 in prostate cancer, adjacent non-cancerous prostate, and normal prostate tissues in the tissue microarray (TMA) samples. (A) A panoramic view of TMA was exhibited. YTHDC2 immunostaining occurred in adjacent tumor prostate tissues (B) and PCa tissues (C). PCa patients with 6 Gleason score (D), and 7 Gleason score (E). The box plot showed the difference in the protein level of YTHDC2 between prostate cancer and adjacent non-cancerous prostate tissues (F).

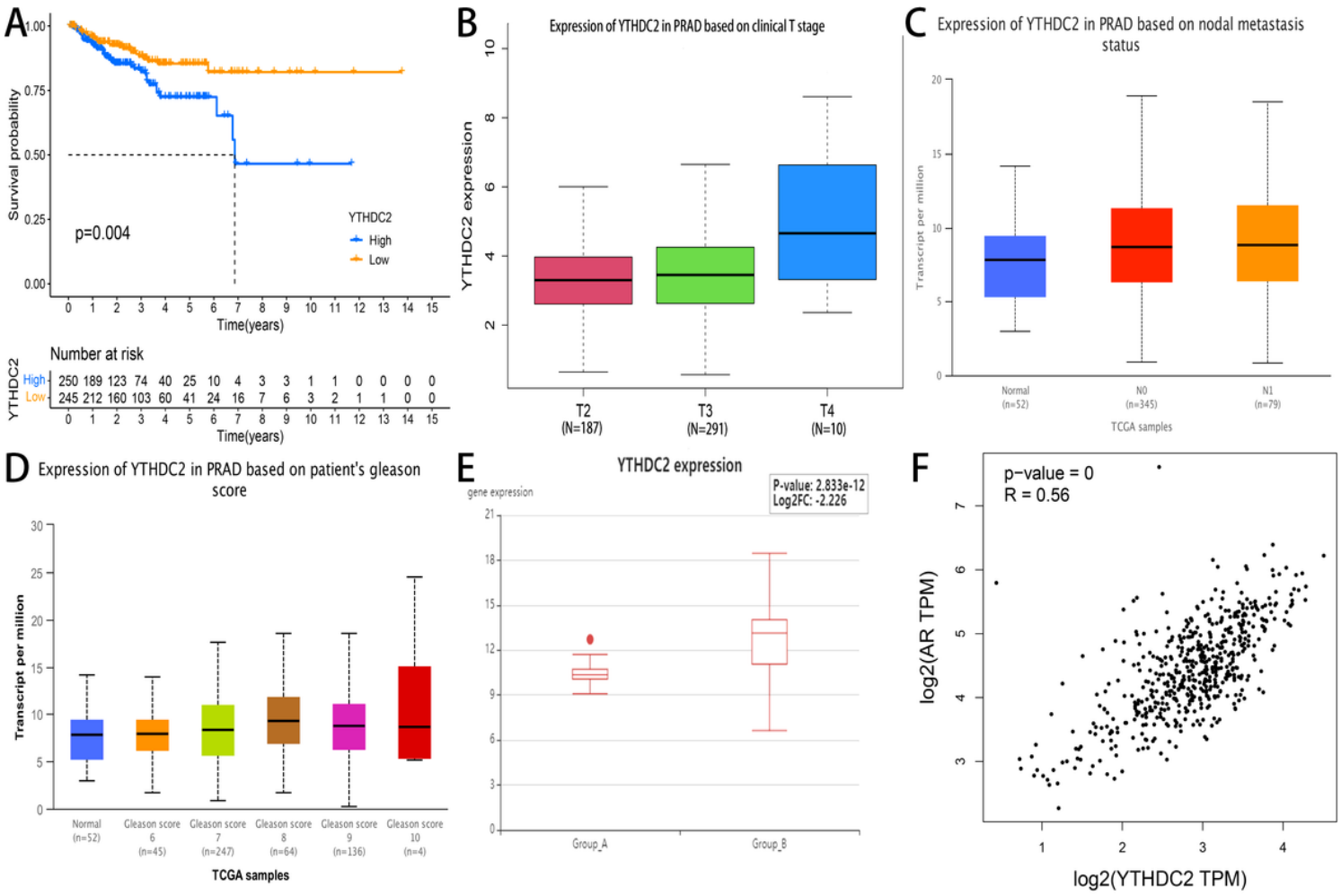

Figure 3

YTHDC2 upregulation predicts shorter RFS of PCa patients. (A) Kaplan-Meier analyses were conducted to assess the prognostic value of YTHDC2 in terms of RFS based on its expression in TCGA PCa cohort. YTHDC2 expression was associated with the clinical T stage (B), nodal metastasis (C), Gleason score (D). The expression of YTHDC2 was statistically significant at different CRPC metastasis sites (E). YTHDC2 was positively correlated with AR (F).

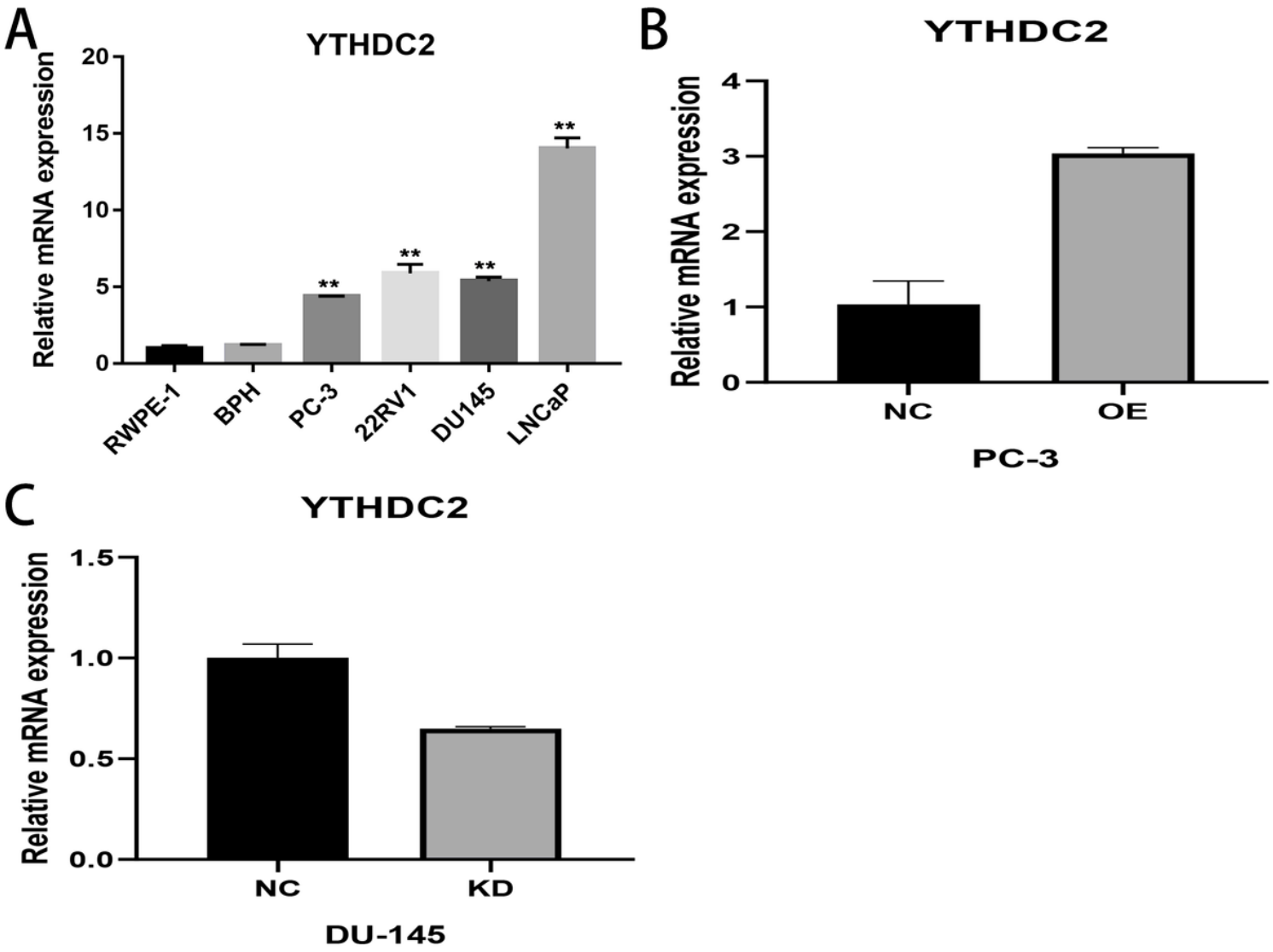

Figure 4

Construction of YTHDC2 overexpressing/knockdown prostate cancer cell lines. (A) qRT-PCR analysis of YTHDC2 expression in human normal prostate (RWPE-1 and BPH) and PCa cell lines (PC3, LNCap, 22RV1, and DU145). (B-C). Enforced expression of YTHDC2 in the YTHDC2-stably transfected PC3/DU145 cells verified by qRT-PCR.

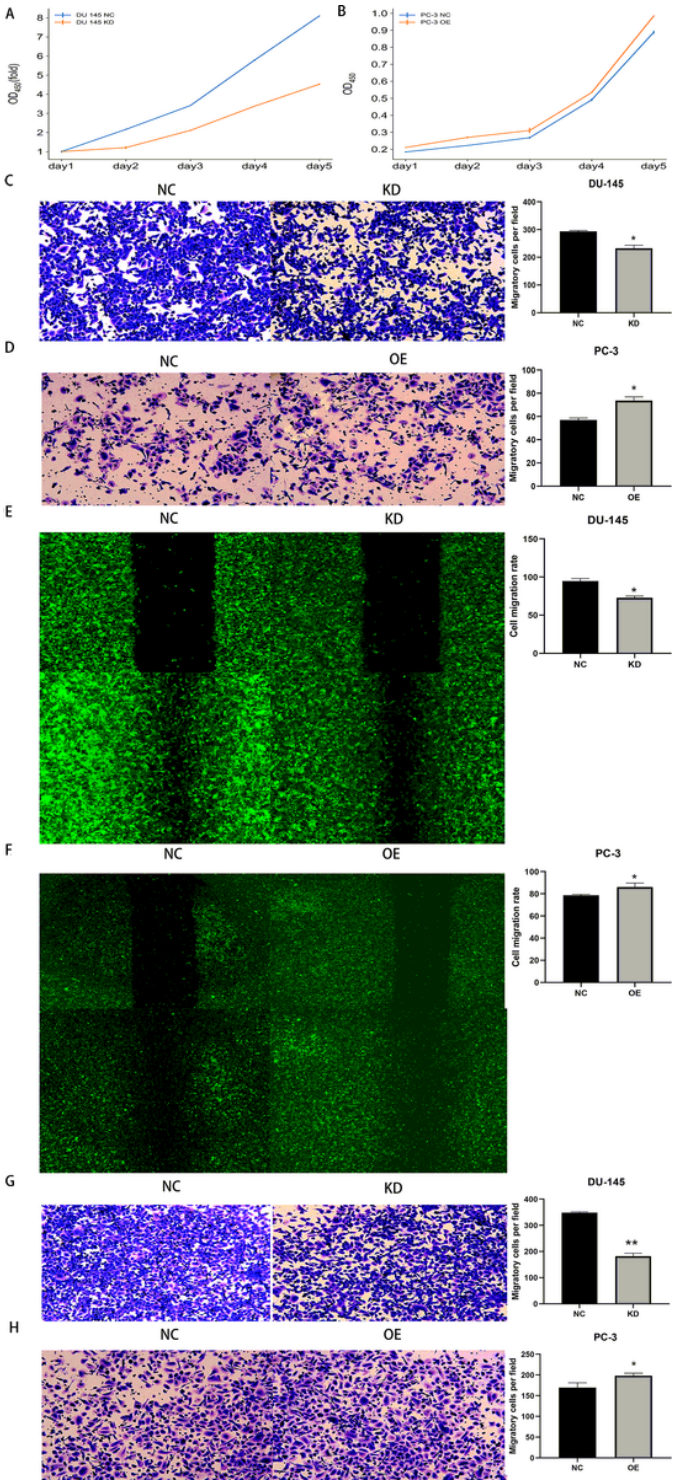

Figure 5

YTHDC2 overexpression promotes cell proliferation, invasion and migration in vitro. (A, B) CCK-8 assays demonstrated that YTHDC2 overexpression promotes proliferative activity of PC-3 cells. YTHDC2 knockdown suppressed proliferative activity of DU-145 cells. (C,D) Transwell analysis showed that YTHDC2 overexpression promotes invasive ability of PC-3 cells and YTHDC2 knockdown suppressed invasive ability of DU-145 cells. (E,F) Wound healing assays indicated that YTHDC2 upregulation promotes migration of PC-3 cells, YTHDC2 knockdown inhibits migration of DU-145 cells. (G,H) The cell invasion of YTHDC2-transfected PC-3 cells were significantly higher than those of control cells. Vise versa, YTHDC2-transfected DU-145 cells were significantly

lower than those of control cells. Data were presented as Mean ± SEM. *P<0.05, **P<0.01 compared with negative control.