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LncRNA TUG1 aggravates the progression of prostate cancer and predicts the poor prognosis

T. XU[1], C.-L. LIU[1], T. LI[2], Y.-H. ZHANG[1], Y.-H. ZHAO[1]

[1]Department of Urology, Qingdao Central Hospital, Qingdao, China
[2]Department of Ophthalmology, Qingdao Women and Children's Hospital, Qi[illegible]ao, Chi[illegible]

Abstract. – OBJECTIVE: To explore the correlation between plasma level of lncRNA TUG1 with PSA level, Gleason grading and tumor node metastasis (TNM) stage of prostate cancer (PCa) patients. This study aims to evaluate the potential diagnostic and prognostic values of TUG1 in PCa.

PATIENTS AND METHODS: Plasma level of TUG1 in 70 PCa patients and 70 healthy controls was determined using the quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Correlation between plasma level of TUG1 with PSA level, Gleason grading and TNM stage of PCa patients was analyzed. The potential diagnostic value of TUG1 in PCa was explored by introducing the receiver operating characteristic (ROC) curve. The survival analysis of PCa patients undergoing radical prostatectomy was conducted using the Kaplan-Meier method and the log-rank test. Finally, the regulatory effects of TUG1 on *in vitro* proliferative and migratory abilities of PCa cells were evaluated by the cell counting kit-8 (CCK-8) and transwell assay, respectively.

RESULTS: QRT-PCR revealed higher plasma level of TUG1 in PCa patients than in those of healthy controls. In particular, PCa patients with stage III+IV had a higher level of TUG1 relative to those with stage I-II. Moreover, the TUG1 level was higher in PCa patients with a higher PSA level (≥ 10 ng/mL), Gleason grading (≥ 7) and TNM stage (III+IV). There was no significant correlation between the plasma level of TUG1 and the age of PCa patients. The ROC and Kaplan-Meier curves indicated the diagnostic and prognostic value of TUG1 in PCa. The overexpression of TUG1 markedly accelerated PCa cells to proliferate and migrate.

CONCLUSIONS: The plasma level of TUG1 is upregulated in PCa patients and is correlated to PSA level, Gleason grading and TNM stage of PCa. TUG1 exerts certain diagnostic and prognostic values in PCa. The overexpression of TUG1 markedly accelerates PCa cells to proliferate and migrate.

Key Words:
PCa, TUG1, Biological marker, Proliferation, Migration.

Introduction

Prostate cancer (PCa) is a common malignancy of the urinary system in middle-aged and elderly males. It is the second leading cause of tumor death. About 75% of PCa cases are males over 65 years old. Early-stage PCa lacks obvious symptoms. Lower urinary tract symptoms (LUTS) occur when the tumor blocks the urethra or invades the bladder neck, manifesting an urinary frequency, obstruction, dysuria, and bladder irritation. Bone metastasis of the PCa leads to bone pain, pathological fracture, and anemia[2]. So far, conventional treatments for PCa include conservative treatment, radical prostatectomy, radiation therapy, endocrine therapy, chemotherapy, immunotherapy, etc.[3]. Despite the great strides already made in PCa treatment, PCa patients undergoing radical prostatectomy still experience tumor recurrence or distant metastasis within 10 years. The plasma level of PSA, Gleason grading, tumor grading and malignant degree of tumor margin are predictive indicators for the development and prognosis of PCa[4]. Nevertheless, there is no recognized clinical method to accurately predict the postoperative recurrence of PCa[4]. Hallmarks predicting the prognosis of PCa are widely explored, which may also serve as therapeutic targets for the individualized treatment of PCa[5]. Hence, it is of great significance to search for novel biological markers which effectively predict the risk and progression of PCa.

The Human Genome Project proposed that only about 1-2% of the genes in the entire genome are capable of encoding proteins and the remaining are non-coding sequences. With the maturity of the next-generation sequencing (NGS), the long noncoding RNA (lncRNA), which was originally thought to be transcriptional noise, gradually exerted its important functions[6]. LncRNA has a transcription of more than 200 bases in length, and it does not encode the protein or

Corresponding Author: Chunlei Liu, MM; e-mail: qdzxyymnwk@163.com

encodes only a short polypeptide (micropeptide). LncRNA presents a broad tissue expression profile, a strong tissue, and a cell expression specificity, which regulates gene expressions at multiple levels[7,8]. Accumulating researches[9-11] have demonstrated the crucial function of lncRNAs in accelerating or inhibiting tumor progression.

TUG1 was first discovered in the developing mouse retinal cells and is located on human chromosome 22q12.2, with a full length of 7.1 kb[12]. A growing number of clinical reports[13-15] have shown that the upregulation of TUG1 is closely associated with poor prognosis and high risk of tumor metastasis. However, the relationship between TUG1 and PCa has rarely been reported. This study mainly elucidated the potential role of TUG1 in mediating the progression of PCa, thus providing a novel directions to improve the clinical outcomes of the PCa patients.

Patients and Methods

Sample Collection

145 patients, diagnosed with PCa in our hospital from April 2016 to September 201[illegible] enrolled. Inclusive criteria were applied: PC[illegible]as diagnosed based on prostate biopsy or pathological diagnosis after electro-prostatectomy; tumor staging and grading were confirmed through the accessory examinations. PCa patients were accompanied with other malignant tumors, coronary heart disease and diabetes were excluded. 70 PCa patients with complete clinical data were enrolled in this study. 70 healthy male volunteers aged 55-75 years undergoing a physical examination in the hospital were enrolled as controls. After collecting 3 mL of peripheral venous blood from each subject, the blood sample was placed in the EDTA (ethylenediaminetetraacetic acid) anticoagulation tube, let stand for 30 min, and centrifuged at 3000 g/min for 15 min. The plasma samples were transferred into the RNase-free endotube (EP) tubes and stored at -80°C. Signed written informed consent was obtained from all participants before the study. This investigation was approved by the Ethics Committee of Qingdao Central Hospital.

Cell Culture

Human prostatic stromal immortalized cell line WPMY-1 and PCa cell lines (LNCap, DU145, PC-3 and CWR22Rv1) were provided by the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% of fetal bovine serum (FBS) and 1% of penicillin+streptomycin in a 5% CO_2 incubator at 37°C (Gibco, Rockville, MD, USA). The medium was replaced every other day.

Cell Transfection

TUG1 siRNA, TUG1 overexpression plasmid (TUG1-OE) and negative control (NC) were provided by GenePharma (Shanghai, China). Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). Transfected cells were harvested 48 h later for the following experiments.

RNA Extraction

0.5 mL of plasma was incubated with 1.5 mL of TRIzol reagent LS (Invitrogen, Carlsbad, CA, USA) and 0.4 mL of chloroform. Subsequently, the mixture was centrifuged at 4°C, 12000 rpm for 5 min. Isopropanol with the same volume of the supernatant was added for harvesting RNA precipitation by centrifugation. The extracted RNA was washed with ethanol, air dried for 10-15 min and diluted in diethyl pyrocarbonate (DEPC) water (Beyotime, Shanghai, China).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

RNA was subjected to reverse transcription on ice using the PrimeScript RT reagent Kit (TaKaRa, Code No. RR036A, Otsu, Shiga, Japan). The obtained cDNA was amplified by the qRT-PCR using the SYRB Premix Ex Taq Kit (TaKaRa, Otsu, Shiga, Japan). U6 was served as the internal reference. The relative level of the target gene was calculated using the $2^{-\Delta\Delta ct}$ method. The primer sequences were as follows: TUG1: F: TAGCAGTTCCCCAATCCTTG, R: CACAAATTCCCATCATTCCC; U6: F: CTCGCTTCGGCAGCACACATATA, R: AAATATGGAACGCTTCACGA.

Cell Counting kit-8 (CCK-8) Assay

Cells were seeded in the 96-well plate. At the appointed time points, 10 μL of CCK-8 reagent was applied in each well. Optical density (OD) at 450 nm was recorded 2 h later using the CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan).

Transwell Migration Assay

Cells were prepared for suspension in the serum-free medium at a density of 1×10^5/mL. 600 μL of medium containing 10% of FBS and 100

μL of suspension were applied on the bottom and upper chamber, respectively. After 24 h of incubation, cells were subjected to fixation for 30 min and violet crystal dying for 25 min. Five randomly selected fields were chosen for calculating the migratory cells.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) was used for data analyses. Data were expressed as mean ± standard deviation. Intergroup difference was analyzed by the *t*-test. Comparison between groups was done using the One-way ANOVA test followed by the Post-Hoc Test (Least Significant Difference). The Kaplan-Meier curve was introduced to analyze the survival of PCa patients, followed by the log-rank test. The receiver operating characteristic (ROC) curve was depicted to evaluate the prognostic value of TUG1. $p<0.05$ was considered statistically sig

Results

High Plasma Level of TUG1 in PCa Patients

The plasma level of TUG1 in PCa patients and healthy controls was measured by qRT-PCR. The data showed higher level of TUG1 in PCa patients relative to controls (p<0.05). Subsequently, the correlation between TUG1 level and

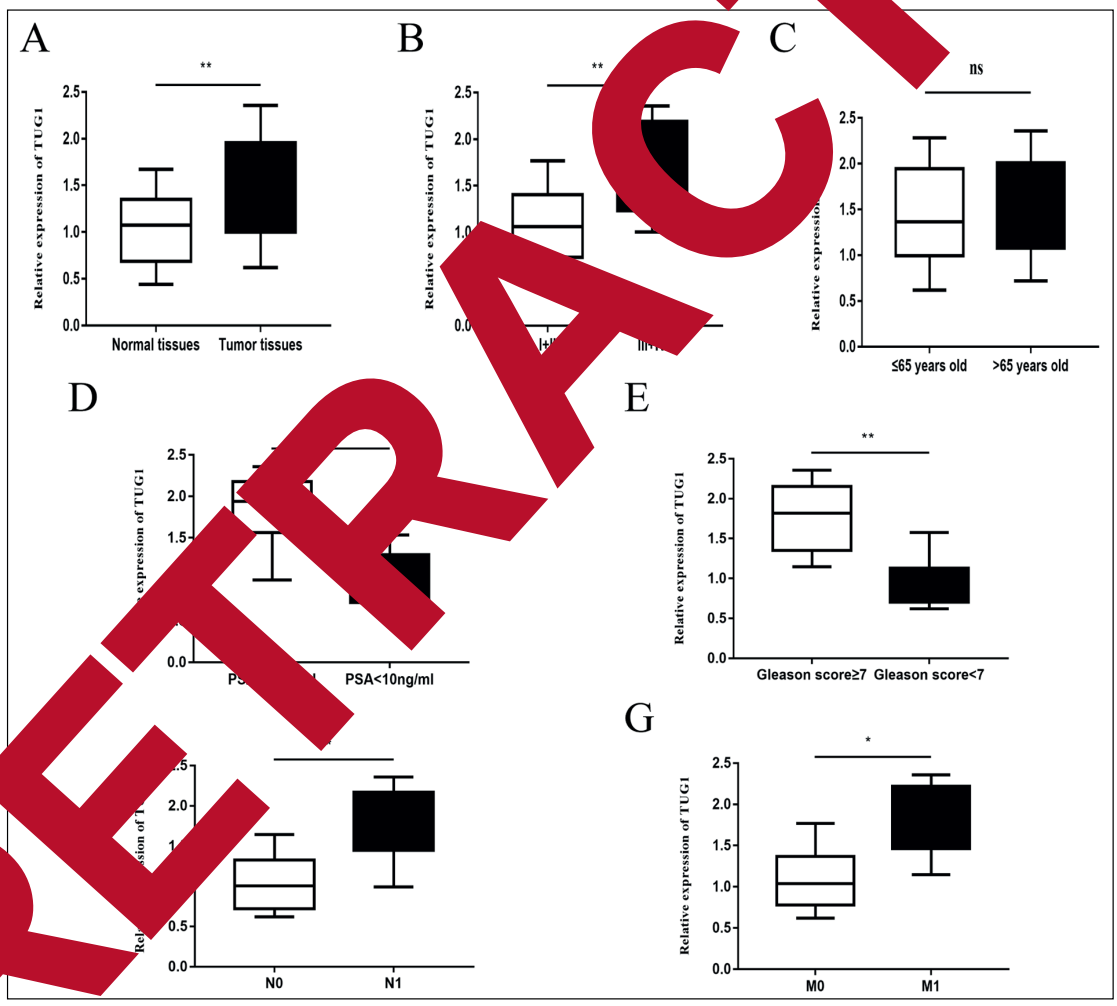

Figure 1. High plasma level of TUG1 in PCa patients. ***A,*** Plasma level of TUG1 in PCa patients and healthy controls determined by qRT-PCR. ***B,*** Plasma level of TUG1 in PCa patients with stage I+II and stage III+IV. ***C,*** Plasma level of TUG1 in PCa patients ≤ 65 years and those > 65 years. ***D,*** Plasma level of TUG1 in PCa patients with PSA ≥10 ng/mL and those with PSA < 10 ng/mL. ***E,*** Plasma level of TUG1 in PCa patients with Gleason score ≥ 7 and those with Gleason score < 7. ***F,*** Plasma level of TUG1 in PCa patients with N0 stage and N1 stage. ***G,*** Plasma level of TUG1 in PCa patients with M0 stage and M1 stage. *$p<0.05$; **$p<0.01$; ns: no significant difference.

pathological characteristics of PCa patients was analyzed. PCa patients with tumor stage of III+IV had higher plasma level of TUG1 compared with those of stage I+II (Figure 1B). No significant difference in plasma level of TUG1 was observed between PCa patients younger than 65 years and those over 65 years (Figure 1C). A higher level of TUG1 was observed in PCa patients with PSA ≥10 ng/ml or Gleason score ≥ 7 compared to those with PS A< 10 ng/ml or Gleason score < 7 (Figure 1D, 1E). Based on the TNM staging, PCa patients in N0 or M0 stage had a lower level of TUG1 than those in N1 or M1 stage (Figure 1F, 1G). The above data suggested the carcinogenic role of TUG1 in PCa.

Diagnostic Value of Plasma TUG1 in PCa

The ROC curve was introduced based on the plasma level of TUG1 in PCa patients and healthy controls. As Figure 2A depicted, the sensitivity and specificity of TUG1 were of 85.7% and 86.3%, respectively, demonstrating a potential diagnostic value of TUG1 in PCa (AUC 0.90, cut-off value=1.265). Subsequently, the PCa patients were divided into high-level and low-level group based on their TUG1 level. The Kaplan-Meier curve was depicted according to the follow-up data of PCa patients. Results showed that PCa patients with high-level TUG1 had a worse overall survival (OS) relative to those in low-level group (Figure 2B). Moreover, PCa patients with high Gleason score suffered worse OS than those with low Gleason score (Figure 2C). PCa patients with a stage higher than T2 experienced poorer prognosis compared to those with T1 stage (Figure 2D). These data indicated that plasma TUG1 may be a potential diagnostic and prognostic hallmark for PCa.

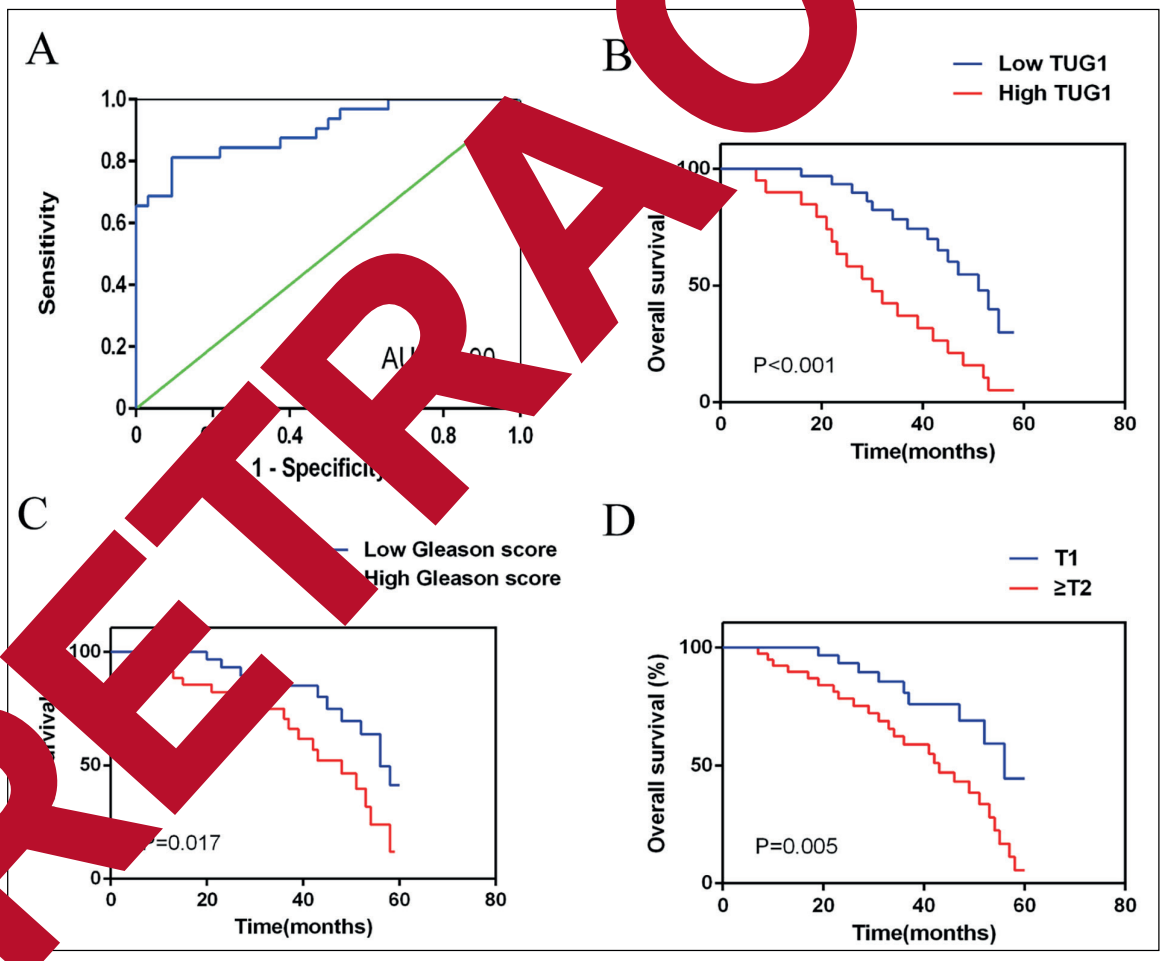

Figure 2. Diagnostic value of plasma TUG1 in PCa. ***A,*** ROC curve based on plasma level of TUG1 in PCa patients and healthy controls (AUC=0.90). ***B,*** Survival analysis based on PCa patients with high-level or low-level TUG1. ***C,*** Survival analysis based on PCa patients with higher Gleason score or low Gleason score. ***D,*** Survival analysis based on PCa patients with T1 stage or ≥ T2 stage.

Upregulated TUG1 in PCa Cells

Compared with the prostatic stromal immortalized cell line WPMY-1, TUG1 was highly expressed in PCa cell lines (Figure 3A). LNCap and PC-3 cells were selected for the following experiments. First of all, the transfection efficacy of TUG1 siRNA#1 and TUG1 siRNA#2 was verified in LNCap and PC-3 cells, both of which could effectively downregulate TUG1 level in PCa cells (Figure 3B, 3C). The transfection efficacy of TUG1 OE was verified in PCa cells as well, where the TUG1 level was greatly upregulated in the LNCap and PC-3 cells (Figure 3D, 3E).

Overexpression of TUG1 Accelerated PCa Cells to Proliferate and Migrate

The regulatory effects of TUG1 on proliferative and migratory abilities of PCa cells were examined using the CCK-8 and transwell assay, respectively. PCa cells overexpressing TUG1 showed an elevated viability, whereas those with TUG1 knockdown presented the inhibited proliferative ability (Figure 4A, 4B). The overexpression of TUG1 in PCa cells ma[illegible]ly improved the migratory ability, and [illegible] the knockdown of TUG1 inhibite[illegible]e migratory ability in PCa cells (Figur[illegible]4D). It is believed that TUG1 aggravated t[illegible]alignant progression of PCa *via* the [illegible]celera[illegible]f the proliferative and migrato[illegible]ilities.

Disc[illegible]

Although [illegible]esses hav[illegible]e in the screening,[illegible]is and rad[illegible]surgery of PCa, the [illegible]ecte[illegible]ients still suffer from a poor prognosis. The[illegible]ogy and pathogenesis of P[illegible]complex, i[illegible]ng multiple genetic[illegible]epigenetic change[illegible]. Previous studies [illegible]illustrated [illegible]hat age, ethnicity, regional

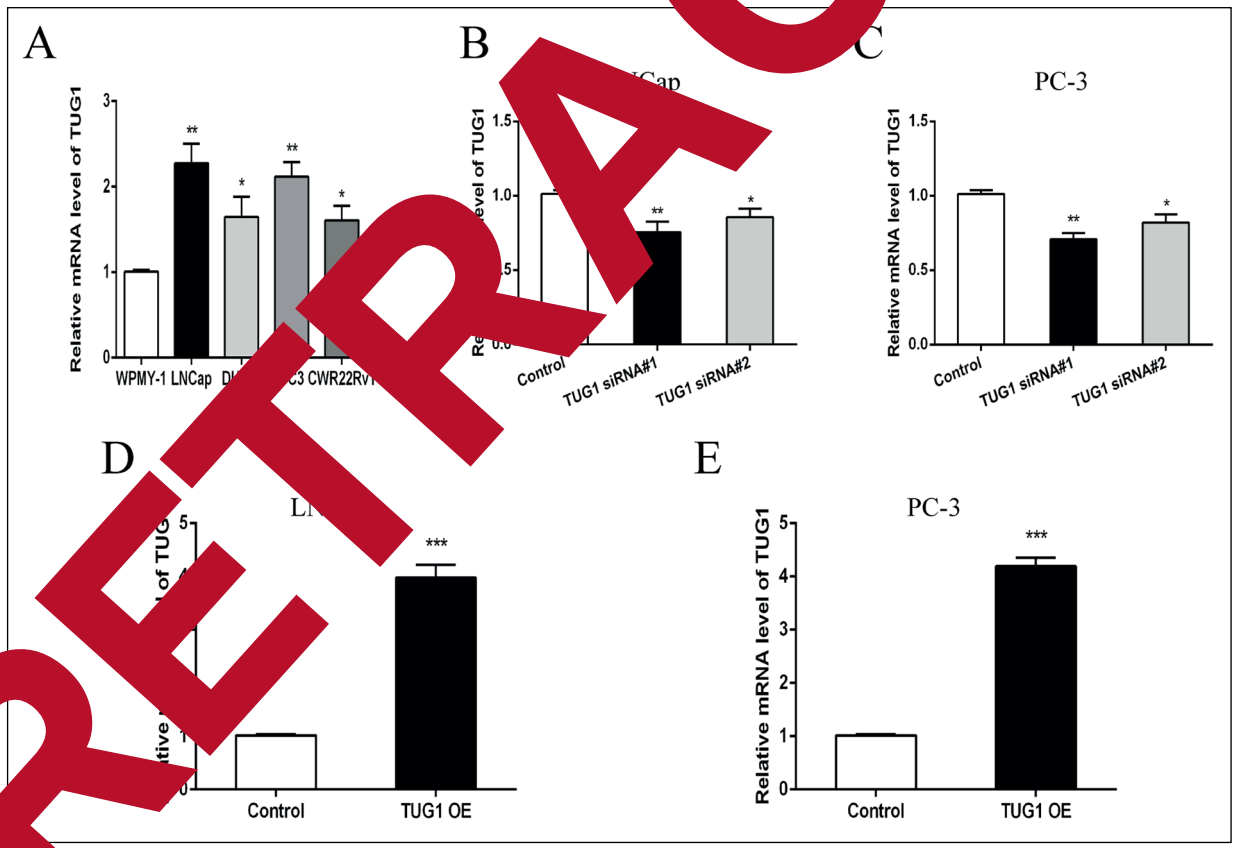

Figure 3. Upregulated TUG1 in PCa cells. ***A,*** The relative level of TUG1 in the prostatic stromal immortalized cell line WPMY-1 and PCa cell lines (LNCap, DU145, PC-3 and CWR22Rv1) determined by the qRT-PCR. ***B,*** Transfection efficacy of control, TUG1 siRNA#1 and TUG1 siRNA#2 in LNCap cells. ***C,*** Transfection efficacy of control, TUG1 siRNA#1 and TUG1 siRNA#2 in PC-3 cells. ***D,*** Transfection efficacy of control and TUG1 OE in LNCap cells. ***E,*** Transfection efficacy of control and TUG1 OE in PC-3 cells.

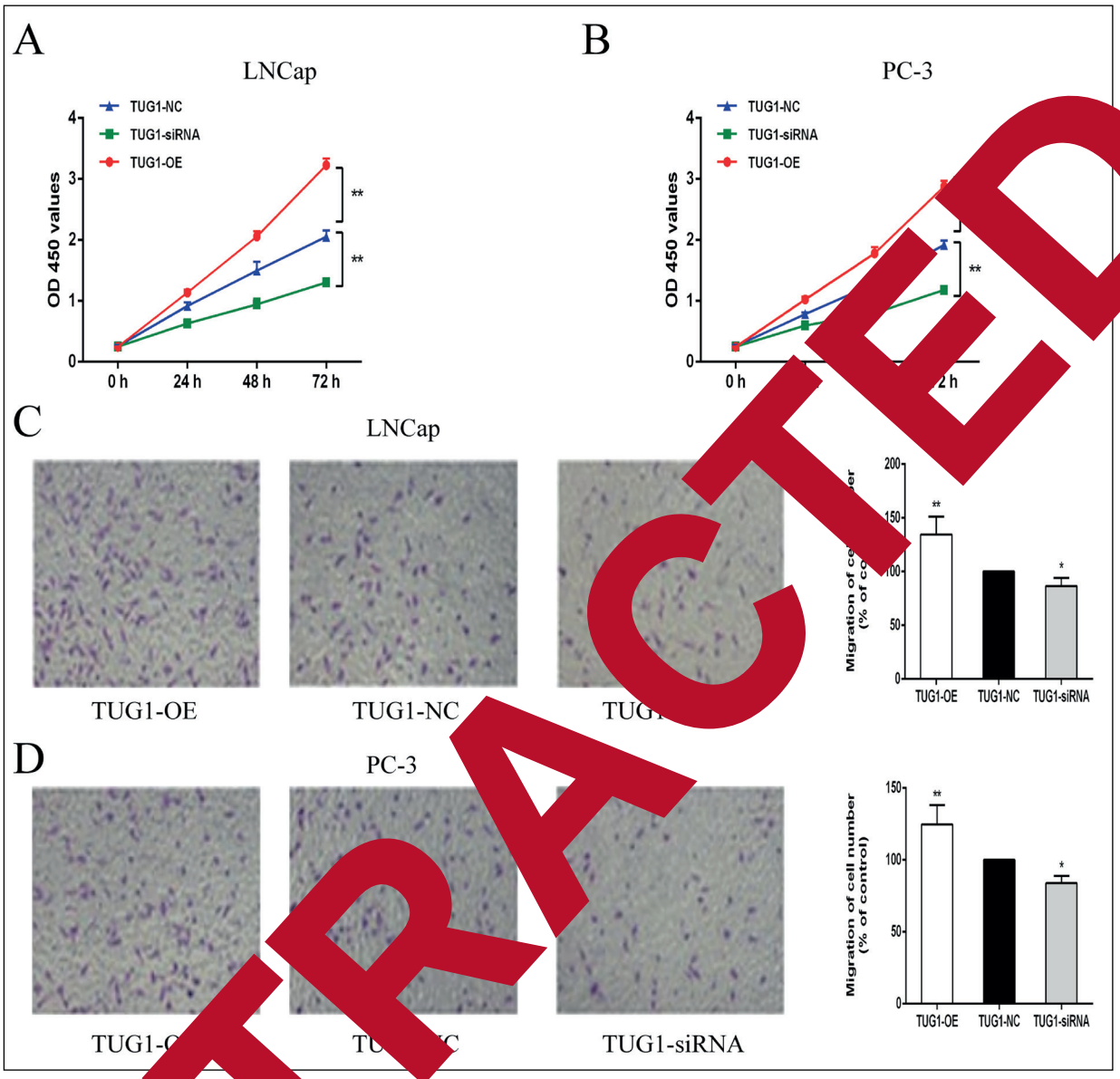

Figure 4. C[illegible]expression o[illegible] accelerated PCa cells to proliferate and migrate. ***A,*** The CCK-8 assay revealed viability in LNCap [illegible]transfected with [illegible]NC, TUG1-siRNA or TUG1-OE at 0, 24, 48 and 72 h. ***B,*** The CCK-8 assay revealed viability [illegible]C-3 cells transfected w[illegible]TUG1-NC, TUG1-siRNA or TUG1-OE at 0, 24, 48 and 72 h. ***C,*** The transwell assay reveal[illegible]gration [illegible]NCap cells transfected with TUG1-NC, TUG1-siRNA or TUG1-OE. ***D,*** The transwell assay revealed mi[illegible]in PC[illegible]s transfected with TUG1-NC, TUG1-siRNA or TUG1-OE. Magnification: ×20. *$p<0.05$; **$p<0.01$.

[illegible]nme[illegible]y history, and genetic alter[illegible]ns are c[illegible]in risk factors for PCa. Never[illegible]no definitive evidence has proved [illegible]esity, smoking, chemical exposure, prostatitis, and vasectomy could directly [illegible]nger the progression of PCa[17,18]. The exact pa[illegible]genesis of PCa still remains unclear, and relevant studies have been conducted to reveal the potential correlation between these risk factors and PCa.

Xiao et al[19] have indicated that lncRNAs can regulate gene expressions at epigenetic, transcriptional and post-transcriptional levels, thus participating in various physiological and pathological processes of the body. Some lncRNAs have been identified to influence the progression of PCa. For instance, lncRNA LOXL1-AS1 regulates proliferation and cell cycle progression of PCa through the mediation of miR-541-3p and CCND1[20]. LncRNA BDNF-AS exerts a func-

tional role in PCa and is correlated to the clinical outcome[21]. LncRNA NEAT1 serves as a carcinogenic gene to aggravate the progression of PCa through the SRC3/IGF1R/AKT pathway[22]. Plasma lncRNA is not sensitive to room temperature, nor to the repeated freezing and thawing conditions. It is stably present in the blood and it is resistant to RNase. Easy collection of blood samples, small invasive procedures, simple detection and high acceptability of patients allow lncRNAs to be the potential tumor hallmark[23]. Abnormally expressed lncRNAs are detected in the plasma of PCa patients. For example, PVT1 serves as a carcinogenic role in PCa and can be used as a potential diagnostic biomarker[24]. The upregulation of lncRNA FALEC promotes PCa cells to proliferate and migrate, predicting the prognosis of PCa patients[25]. LINC01296 is associated with the poor prognosis of PCa and accelerates the proliferation and metastasis of PCa cells[26].

This study mainly elucidated the relationship between the plasma level of TUG1 and the pathological characteristics of PCa patients. Our results may provide novel references for cl al treatment of PCa. First of all, we iden higher level of TUG1 in plasma samples o Ca patients than in those of healthy controls. Pla level of TUG1 was correlated with PSA le Gleason grading, and TNM st ther tha the age of PCa patients. It gg d that a high level of TUG1 could gravate malignant degree and migrati PCa. vital factor influenci th rogres l. Besides, the survi nalysis led a negative correlation h en the TU l and the postoperative PCa patient oreover, *in vitro* exper ents i ed the promotive effects of o pressed TU proliferative and migrat bilities of the PC s. Nevertheless, we d ot exp the molecular mechanism of TU me g the ellular behaviors of PCa cells, l be fu lucidated in the future.

onclusions

ed that plasma level of TUG1 was regulated in PCa patients, which was correlat- PSA level, Gleason grading, tumor grading, a NM stage of PCa. TUG1 could serve as diagnostic and prognostic hallmarks for PCa. The overexpression of TUG1 markedly accelerated PCa cells to proliferate and migrate.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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