Eukaryotic translation initiation factor 5A2 is highly expressed in prostate cancer and predicts poor prognosis

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Abstract. Eukaryotic translation initiation factor (EIF) 5A2 exerts important functions that regulate the development and progression of cancers. The present study aimed to investigate the expression of EIF5A2 in prostate cancer (PCa) and its association with biological and prognostic significance. EIF5A2 mRNA and protein levels were analyzed in three paired samples of freshly resected PCa and adjacent non-tumor tissues. Immunohistochemical staining was used to detect the expression of EIF5A2 protein levels in 72 paraffin-embedded PCa tumor specimens. Subsequently, the association between EIF5A2 protein expression and clinicopathological parameters was assessed. Semi-quantitative reverse transcription-polymerase chain reaction and western blot analyses showed both EIF5A2 mRNA and protein levels were elevated in PCa compared with adjacent non-tumor tissues. Elevated EIF5A2 protein levels were observed in 73.6% (53/72) of the clinical PCa tissues using immunohistochemical staining. EIF5A2 expression was significantly associated with tumor stage (P=0.011) and biochemical recurrence status (P=0.032). Additionally, high levels of EIF5A2 predicted worse progression-free survival (P=0.007). Multivariate Cox regression analysis indicated that high expression of EIF5A2 was an independent prognostic factor for poor progression-free survival (hazard ratios, 0.366; 95% confidence interval, 0.349-0.460; P=0.021). The present study demonstrated that EIF5A2 is overexpressed in prostate cancer and may be a potential predictor and therapeutic target in PCa patients.

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Introduction

Prostate cancer (PCa) is the leading cause of cancer-associated mortality in men worldwide (1). Approximately 60,300 new PCa cases are diagnosed annually, and ~26,600 die of aggressive PCa in China annually (2). Although many approaches have been explored to improve the diagnosis and treatment of PCa, the incidence and mortality of PCa has still increased in recent years (1,3). Furthermore, the morphological features and grading system evaluated by pathologists are still the most valuable diagnostic criteria and predictors for PCa patients (4). Hence, novel biomarkers contributing to the diagnosis and prognosis predictions of PCa patients need to be identified.

Eukaryotic translation initiation factor (EIF) 5A2 is one of two isoforms in the EIF5A family that mainly acts as an elongation factor during the mRNA translation step (5). The EIF5A2 gene is located on the human chromosome at 3q26, where many other candidate oncogenes exist (6,7). EIF5A2 is only found in testes, brain and tumor tissues (8). A series of studies have shown that overexpression of EIF5A2 is closely associated with tumor growth, metastasis and chemoresistance in diverse cancers and may serve as a potential prognostic marker (9,10). Our previous study also indicated that EIF5A2 overexpression predicts tumor metastatic potential in patients with localized invasive bladder cancer treated with radical cystectomy (11). Previous studies have shown that inhibition of EIF5A2 activity by N1-guanyl-1,7-diaminoheptane (GC7) has strong anti-tumor effects on human cancer cells (12,13), indicating that EIF5A2 could be a potential target for anticancer therapy. However, the expression pattern and clinical significance of EIF5A2 in PCa have not yet been elucidated.

The present study investigated EIF5A2 expression and characterized its clinicopathological significance in a large cohort of PCa tissues. It was identified in the present that EIF5A2 expression was upregulated at both mRNA and protein levels in PCa tumor compared with non-tumor tissues. EIF5A2 overexpression was associated with aggressive clinicopathological features. Notably, elevated expression of EIF5A2 predicted

unfavorable progression-free survival and could be an independent prognostic biomarker for PCa patients.

Materials and methods

Human tissue specimens and patients. Three clinical specimens including cancer and corresponding adjacent non-tumor tissues, were collected during radical resection to detect the mRNA and protein levels of EIF5A2. In addition, 72 formalin-fixed, paraffin-embedded PCa tumor specimens, and 20 matched adjacent non-tumor tissue specimens were collected to perform immunohistochemical staining. The median age of the patients was 65 years (range, 48-87 years). The samples in the present study were collected from The First Affiliated Hospital, Sun Yat-sen University, Guangdong, China, Jiangmen Central Hospital, Guangdong, China and Affiliated Yantai Yuhuangding Hospital, Qingdao University, Shandong, during January 2005 to December 2008, after a distinctive pathological diagnosis of prostate adenocarcinoma. Most of the patients (69/72) received radical prostatectomy, while only few of them (3/72) underwent transurethral resection prostate as they were diagnosed with benign prostate hyperplasia before surgery. None of the patients received immunotherapy or radiotherapy before surgical treatment. All the patients were staged using the classification of the American Joint Committee on Cancer staging system for prostate cancer (14). Biochemical recurrence was defined as Prostate specific antigen (PSA) levels >0.4 ng/ml. The last follow-up update was June 2017, and the median follow-up time was 100 months. All the samples were collected with the patient's written informed consent after approval from the Institute Research Medical Ethics Committee of the Hospitals.

RNA extraction and semi-quantitative reverse transcription polymerase chain reaction (SqRT-PCR). Total RNA was isolated from three resected fresh tumors and matched with adjacent on-tumor tissues using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). RNA purity and concentration were determined by a standard ultraviolet spectrophotometric assay. RT-PCR was performed using PrimeScript RT reagent kit (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocol. GAPDH was used as an internal control. The primers used were as follows: EIF5A2 forward, 5'-AAGATGGTTACCTTTCCCTG-3' and reverse, 5'-TACAGCATATTCTTCACTCATTG-3'; GAPDH forward, (5'-TGCACCACCAACTGCTTAGC-3' and reverse, 5'-GGCATGGACTGTGGTCATGAG-3'. The thermocycling conditions were as follows: Initial denaturation at 95˚C for 5 min, followed by 35 cycles of denaturation at 95˚C for 1 min, annealing at 55˚C for 1 min, extension at 72˚C for 1 min and elongation for 7 min at 72˚C. Samples were then resolved on a 2% agarose gel, stained with ethidium bromide for 30 min at room temperature and visualized using a high-performance ultraviolet transilluminator (Analytik Jena US LLC; Upland, CA, USA).

Western Blot analysis. Resected fresh tumor tissue and matched adjacent non-tumor tissue were homogenized and lysed in radioimmunoprecipitation assay buffer on ice. Protein concentrations were determined using a BCA Protein Assay kit (Pierce; Thermo Fisher Scientific, Inc.) standardized with bovine serum albumin (Invitrogen; Thermo Fisher Scientific, Inc.). Different proteins were separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% (W/V) nonfat-dry milk in TBST (25 mM Tris HCl, pH 7.5; 150 mM NaCl; 0.05% Tween-20) for 1 h at room temperature followed by probing with primary antibodies against EIF5A2 (1:1,000; cat. no. ab150439; Abcam, Cambridge) or GAPDH (1:1,000; cat. no. ab181602; Abcam, Cambridge, UK) for 2 h at room temperature and then washed 3 times with TBST, followed by incubating for 1 h at room temperature with horse radish peroxidase-conjugated secondary antibodies (1:2,000; cat. no. ab150077; Abcam). Immunoreactive protein was detected using enhanced chemiluminescence detection reagents (GE Healthcare, Chicago, IL, USA) according to the manufacturer's protocol.

Immunohistochemical staining and evaluation. A total of 72 formalin-fixed paraffin-embedded tumor tissue samples and 20 corresponding samples of non-tumor tissues were used for EIF5A2 immunohistochemical staining. The experiment was performed using a standard streptavidin-biotin-peroxidase complex protocol as described previously (11). The slides were incubated at 4˚C in a moist chamber overnight with a primary antibody against human EIF5A2 (1:200; cat. no. ab150439; Abcam). Staining with PBS instead of the primary antibody against EIF5A2 was used as the negative control; ovarian tumor tissue with positive EIF5A2 expression was used as the positive control.

For evaluation of EIF5A2 in different prostate tissues, a semiquantitative scoring method was used, according to our previous study (11). Briefly, a staining index (values 0-12) was obtained as the intensity of EIF5A2 staining (negative=0, weak=1, moderate=2, or strong=3 scores) and the proportion of immunopositive cells of interest (<25%=1, 25-50%=2, >50% to <75%=3, and ≥75%=4 scores) was calculated. The results were observed and assessed by two independent experienced researchers in a blinded manner.

Statistical analysis. SPSS package (v20.0; IBM Corp., Armonk, NY, USA) was used for statistical analysis. Image J (v1.8.0; National Institutes of Health, Bethesda, MD, USA) software was used to calculate the intensity of western blot bands and SqRT-PCR results. Student's t-test was employed to compare EIF5A2 expression between the tumor and adjacent non-tumor tissue. Pearson χ^2 tests were performed to determine the association between EIF5A2 protein expression and clinicopathologic parameters. Kaplan-Meier analysis was used for univariate survival analysis and the log-rank test was applied to compare different survival curves. The multivariate Cox regression model was used to evaluate the potential independent prognostic factors and 95% confidence intervals (CI) of the hazard ratio (HR). $P<0.05$ was considered to indicate a statistically significant difference.

Results

EIF5A2 mRNA and protein levels are highly expressed in freshly resected PCa specimens. To detect the EIF5A2 mRNA

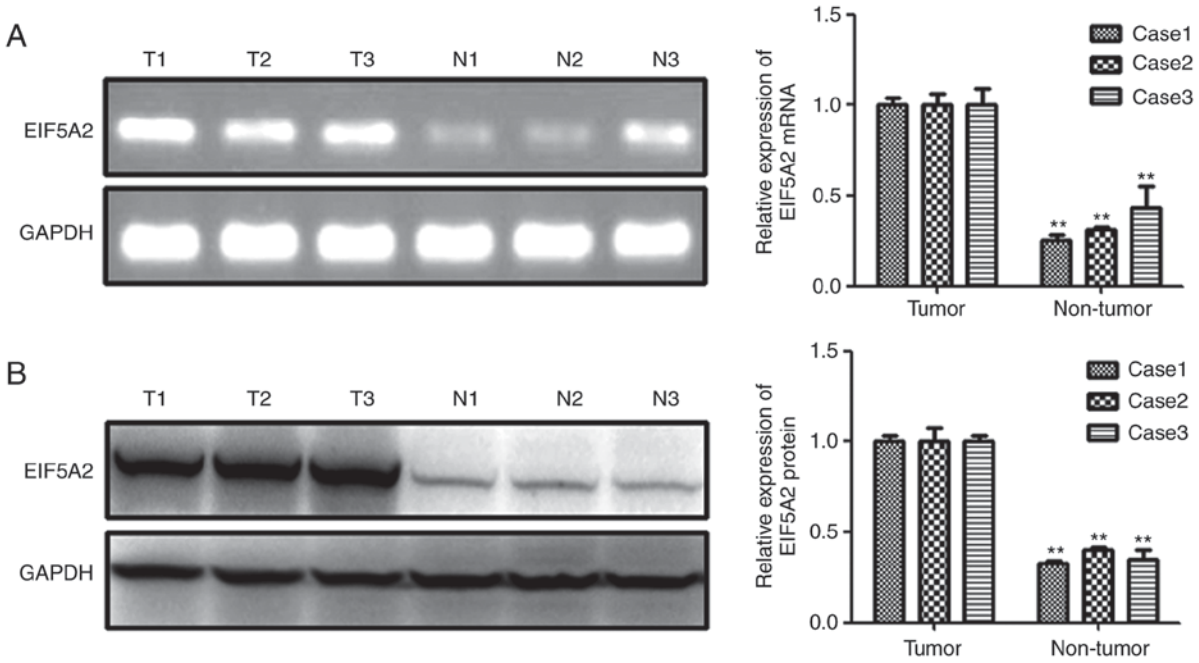

Figure 1. EIF5A2 mRNA and protein levels are highly expressed in freshly resected PCa specimens. (A) Semi quantitative reverse transcription polymerase chain reaction showed that EIF5A2 mRNA levels were significantly higher in PCa tissue than in adjacent non-tumor tissue in all 3 sets of paired, fresh specimens. **P<0.01 (B) Western blot analysis indicated that EIF5A2 protein was highly expressed in PCa tissues. EIF5A2, eukaryotic translation initiation factor 5A2; PCa, prostate cancer; T, tumor; N, non-tumor.

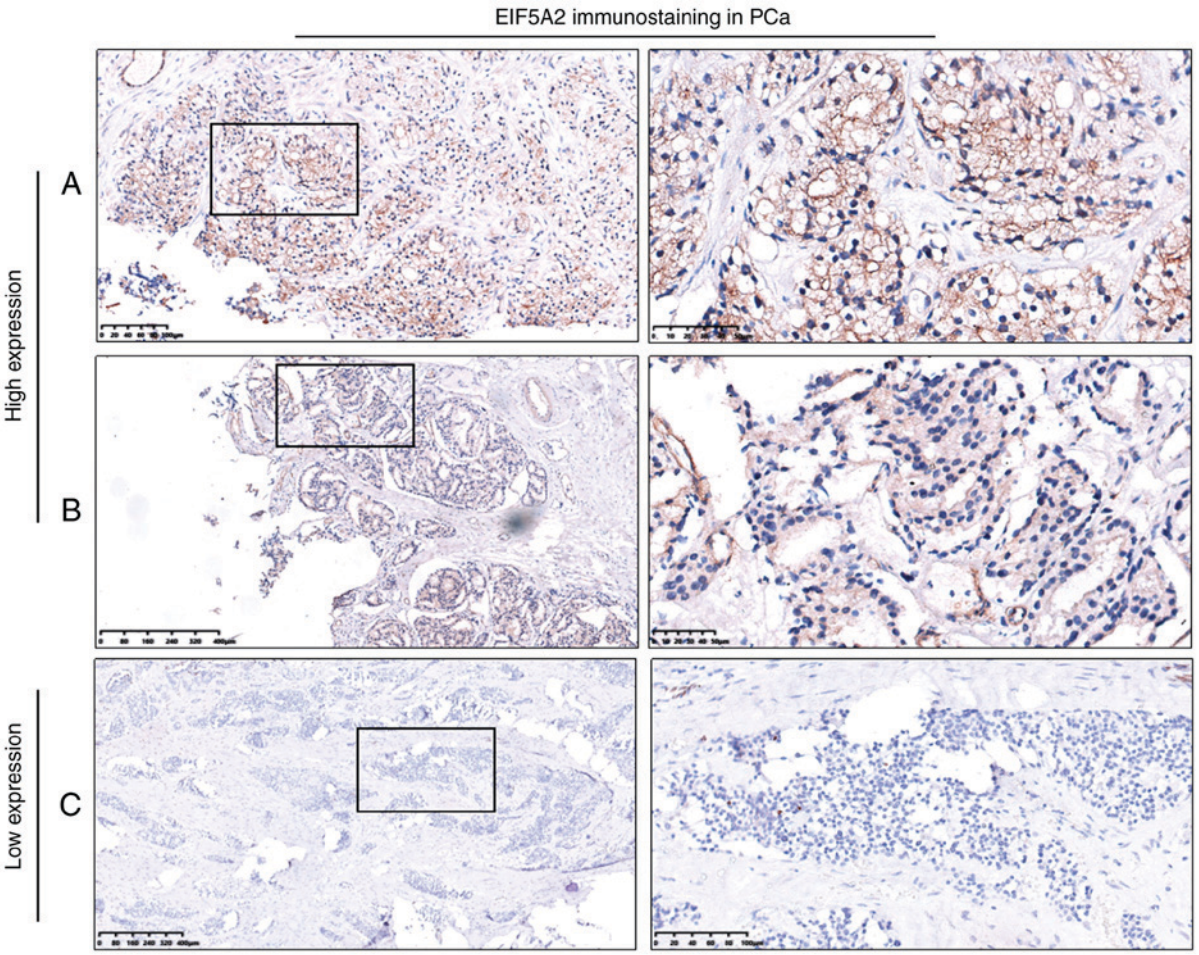

Figure 2. Immunohistochemical staining shows higher expression of EIF5A2 in PCa tissues. (A) High expression of EIF5A2 with a scoring index of 3x4=12. (B) High expression of EIF5A2 with a scoring index of 2x4=8. (C) Low expression of EIF5A2 with a scoring index of 0. The right panels display an enlarged view of the representative EIF5A2 protein expression in the selected zone. EIF5A2, Eukaryotic translation initiation factor 5A2; PCa, prostate cancer.

Table I. Correlation of EIF5A2 expression with clinicopathological parameters of PCa patients.

Variables	N	EIF5A2 expression		P-value[a]
		Low (%)	High (%)	
Age				0.964
<65 y	42	11 (26.2)	31 (73.8)	
≥65 y	30	8 (26.7)	22 (73.3)	
PSA level				0.179
≥10	57	13 (22.8)	44 (77.2)	
<10	15	6 (40.0)	9 (60.0)	
Tumor stage				0.011
T1+T2	43	16 (37.2)	27 (62.8)	
T3	29	3 (10.3)	26 (89.7)	
Biochemical recurrence				0.032
Positive	27	11 (40.7)	16 (59.3)	
Negative	45	8 (17.8)	37 (82.2)	
Gleason score				0.253
<7	56	13 (23.2)	43 (76.8)	
≥7	16	6 (37.5)	10 (62.5)	

[a]χ^2 Test. EIF5A2, Eukaryotic translation initiation factor 5A2; PCa, prostate cancer; PSA, prostate specific antigen.

and protein expression pattern in prostate cancer, RT-PCR and western blot analyses in three sets of freshly resected, paired specimens were conducted first. As shown in Fig. 1A, the SqRT-PCR results indicated that EIF5A2 mRNA levels were significantly higher in PCa tissues than that in adjacent non-tumor tissue in all three specimens. Subsequently, the EIF5A2 protein expression was assessed by Western Blot assay, and consistent with the mRNA expression, the protein level of EIF5A2 was notably up-regulated in PCa tissue compared with that in adjacent non-tumor tissue (Fig. 1B).

EIF5A2 protein levels are elevated in paraffin embedded PCa tissues. To further validate the expression level of EIF5A2 protein in PCa, immunohistochemical staining in a large cohort of PCa tissues including 72 PCa and 20 corresponding non-tumor tissues was conducted. Samples with a staining index ≥6 (median score of EIF5A2 expression in PCa tissues) were defined as high expression and samples with a staining index <6 were defined as low expression. EIF5A2 overexpression was detected in 53 tumor tissue samples (73.6%, 53/72), but in only three adjacent non-tumor tissue samples (15.0%, 3/20; Fig. 2).

EIF5A2 protein expression is associated with aggressive clinicopathological variables. In order to uncover the clinical significance of EIF5A2 in PCa, the associations between the expression of EIF5A2 and clinicopathological parameters were analyzed. High or low expression rates of EIF5A2 protein in PCa with respect to several standard clinicopathological features are shown in Table I. EIF5A2 overexpression was associated with tumor stage (P=0.011) and biochemical recurrence status (P=0.032) However, no significant association was detected between EIF5A2 overexpression and other clinicopathological parameters, including age (P=0.964), serum PSA level (P=0.179) and Gleason Score (P=0.253).

Table II. Multivariate analysis on overall survival (Cox regression model).

Variables	Hazard ratio	95% confidence interval	P-value
Tumor stage[a]	1.427	1.344-2.322	0.013
Biochemical recurrence[b]	1.322	1.658-2.763	0.001
EIF5A2 expression[c]	0.366	0.349-0460	0.021

[a]T1+T2 vs. T3, [b]positive vs negative, [c]high expression vs. low expression. EIF5A2, Eukaryotic translation initiation factor 5A2.

EIF5A2 overexpression predicts inferior prognosis in PCa patients. To associate the EIF5A2 expression levels to clinical outcome, Kaplan-Meier survival analysis was used to analyze the prognostic value of EIF5A2 in PCa patients. As shown in Fig. 3, PCa patients with high EIF5A2 expression had a significantly shorter progression-free survival time than that of those with low EIF5A2 expression (P=0.007). Subsequently, multivariate Cox regression analysis was used to evaluate the potential prognostic significance of EIF5A2 expression. The results showed that high expression of EIF5A2 was an independent prognostic factor for poor overall survival (hazard ratio, 0.366; 95% confidence interval, 0.349-0460; P=0.021; Table II).

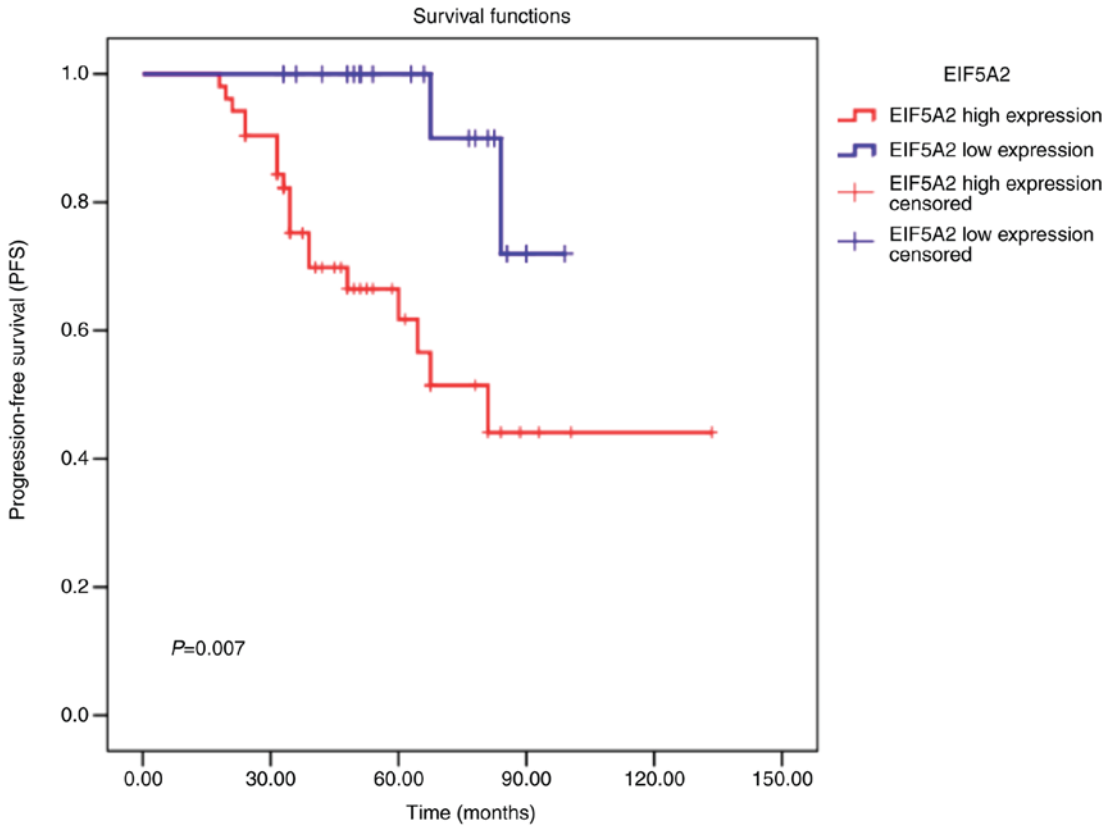

Figure 3. High levels of EIF5A2 predict poor prognosis in PCa patients. Kaplan-Meier analysis of overall survival of PCa patients showed that PCa patients with high EIF5A2 expression had a significantly shorter progression-free survival time than that of patients with low expression of EIF5A2 (P=0.007). EIF5A2, Eukaryotic translation initiation factor 5A2; PCa, prostate cancer.

Discussion

EIF5A2 is thought to be a candidate oncogene that is involved in tumor proliferation, invasion, metastasis, cell aging and drug resistance (9,10). However, the expression pattern and clinical significance of EIF5A2 in PCa are unclear. The present study demonstrated that EIF5A2 was upregulated in PCa tissues at both the mRNA and protein levels; overexpression of EIF5A2 protein was associated with aggressive pathological parameters including tumor stage and biomedical recurrence. Moreover, high expression of EIF5A2 predicted inferior prognosis of PCa patients and may be an independent prognostic marker.

The expression of EIF5A2 is specific to the tissue and cell type. In addition to its expression in the normal testis and brain, EIF5A2 levels are increased in a number of malignancies. It was reported that EIF5A2 was up-regulated in ovary cancer (15), nasopharyngeal carcinoma (16), colorectal carcinoma (17), hepatocellular carcinoma (18), gastric cancer (19) and non-small cell lung cancer (20). A previous study by Li *et al* (21), revealed that EIF5A2 was significantly decreased in prostate cancer when Ephrin type-A receptor 6 was knocked down. However, the expression type of EIF5A2 in prostate cancer cells or tissues, and clinical significance of EIF5A2 have not yet been investigated. Our previous study indicated that EIF5A2 protein levels were elevated in bladder cancer tissues compared with that in adjacent normal bladder tissues by using immunohistrochemical staining (11). Consistent with these results, in the present study, EIF5A2 mRNA and protein levels were shown to be elevated in PCa tissue. These coherent data suggested that EIF5A2 overexpression might be a common event in the tumorigenesis and progression of different cancers. Moreover, the present study demonstrated that upregulation of EIF5A2 was closely associated with aggressive clinicopathological features and might be an independent candidate prognostic marker for PCa patients. These findings are in accordance with previous studies. For instance, in early-stage cervical cancer, EIF5A2 overexpression was correlated with higher International Federation of Gynecology and Obstetrics stage, deep cervical stromal invasion, lymphovascular space involvement and pelvic lymph node metastasis, and might be a prognostic factor for overall survival and disease-free survival (22). In gastric cancer, EIF5A2 overexpression was positively correlated with more advanced pT stage, more advanced pN stage and positive lymphovascular invasion, and predicted poor prognosis (19). However, in nasopharyngeal carcinoma, EIF5A2 expression had no significant association with clinicopathological characteristics, such as tumor stage and world health organization classification (16). These results indicated that, even though different tumor types share similar prognosis predictions in regard to EIF5A2 expression, the biological behavior and anatomical characteristics of EIF5A2 might be distinct among the tumor subtypes.

Accumulating evidence suggests that EIF5A2 serves a vital role in the malignant behavior of diverse cancers. For instance, in hepatocellular carcinoma, EIF5A2 not only promoted cancer cell proliferation, migration and invasion (18), but also

enhanced cell metabolic reprogramming (23). Furthermore, ablation of EIF5A2 could induce tumor vasculature remodeling and improve the tumor response to chemotherapy (24). Recently, Bai *et al* (25) revealed that EIF5A2 contributes to the maintenance of Cluster of differentiation 133+ hepatocellular carcinoma cells, which was the key biomarker to identify and characterize cancer stem cells. A similar influence of EIF5A2 on migration, invasion and chemosensitivity was also observed in esophageal squamous cell carcinoma (26,27) and non-small cell lung cancer (28,29). Although the clinical significance of EIF5A2 in PCa has been demonstrated in the present study, its biological function should be explored in *in vitro* and *in vivo* experiments in future work.

With regard to the mechanisms underlying the role of EIF5A2 in cancer, a variety of downstream events have been reported. EIF5A2 induced cancer cell epithelial-mesenchymal transition contributes to tumor metastasis and/or drug resistance in hepatocellular carcinoma (18), colorectal carcinoma (17,30) and esophageal squamous cell carcinoma (27). In hepatocellular carcinoma, the c-myelocytomatosis/microRNA-29b axis was involved in the process of EIF5A2 maintaining the features of cancer stem cell of hepatocellular carcinoma (25). In addition, EIF5A2 promoted tumor angiogenesis and relied on vascular endothelial growth factor, p38 mitogen-activated protein kinases and the c-Jun N-terminal kinase/c-Jun pathway (24,27). In summary, EIF5A2 has the capacity to play multiple roles in tumorigenesis and tumor progression, which are ascribed to its complicated mechanism. To the best of our knowledge, the present study reported for the first time, the expression type and clinical significance of EIF5A2 in prostate cancer, which will be of great value to link EIF5A2 and progression of prostate cancer. However, more specimens and more in-depth experiments are needed to get a complete picture of EIF5A2 in prostate cancer. In conclusion, the results suggest that EIF5A2 could serve as a novel biomarker for progression-free survival and a potential therapeutic target for the treatment of PCa, but this needs to be investigated further.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YF and JHL designed the current study. JL, HWZ and YC performed the experiments and collected the data. JHW, ZHC and ZHF analyzed the data. YH and WC analyzed the data and prepares the manuscript. JL, HZ and YC contributed equally to this work.

Ethics approval and consent to participate

All the samples were collected with the patient's written informed consent after approval from the Institute Research Medical Ethics Committee of the Hospitals.

Patient consent for publication

Written informed consent for participation and publication was obtained from all patients prior to enrollment in the present study.

Competing interests

The authors declare that they have no competing interests.

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