The clinicopathological and prognostic significances of EZH2 expression in urological cancers: A meta-analysis and bioinformatics analysis

YANG-KAI BAI[1], JING SUN[2], YE-SONG WANG[1], NAN ZHENG[1], QING-LE XU[3] and YANG WANG[1]

[1]Department of Urology, Hanzhong Central Hospital; [2]Department of Medical Oncology, Affiliated 3201 Hospital of Xi'an Jiaotong University Health Science Center, Hanzhong, Shaanxi 723000; [3]Department of Urology, Hebei Provincial People's Hospital, Shijiazhuang, Hebei 050000, P.R. China

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Abstract. The Drosophila zeste enhancer homolog 2 gene (enhancer of zeste homolog 2; EZH2) is an important member of the polycomb group (PcG) gene family, which maintains the homologous gene via chromosome modification during embryonic development. EZH2 is overexpressed in various tumors, is closely related to tumor formation and growth, and has a malignant phenotype that promotes tumor cell proliferation, proliferation and metastasis. In the present study, a meta- and bioinformatic analysis was performed using data from multiple online databases until August 30, 2022. EZH2 upregulation was found in kidney, bladder and prostate cancers. EZH2 expression was negatively related to TNM staging and pathological grade in kidney and prostate cancers ($P<0.05$), as well as invasion depth and pathological grade in bladder cancer. According to the KM-plotter database, EZH2 expression was inversely associated with poor overall survival in patients with kidney clear cell renal cell carcinoma (RCC) and papillary RCC and with favorable survival in bladder cancer. EZH2 expression was negatively related to relapse-free survival in kidney papillary RCC and bladder cancer but positively associated with kidney clear cell RCC. According to GEPIA and UALCAN databases, EZH2 expression was higher in tumor tissue than normal tissue. The TIMER database showed that EZH2 was closely associated with the proportion of seven immune cell infiltrates in kidney, bladder, and prostate cancers. High EZH2 expression may be a potential marker of tumorigenesis and metastasis in patients with urological cancers.

Correspondence to: Professor Yang Wang, Department of Urology, Hanzhong Central Hospital, 22 Kangfu Road, Hantai, Hanzhong, Shaanxi 723000, P.R. China
E-mail: 15109160956@139.com

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Introduction

Renal cancer is the most common malignant solid tumor of the kidney. Its incidence accounts for ~3% of systemic malignant tumors, with 85% as clear cell carcinomas (1). Of patients with kidney cancer ~30% have advanced tumors, and ~30% of patients with localized kidney cancer develop metastasis or recurrence after surgery. The main treatment for kidney cancer is surgery, and the treatments for metastatic and advanced cases include radiotherapy, chemotherapy, immunotherapy and molecular targeted therapy (2). Bladder cancer is a common malignant tumor in the urinary tract system, mainly comprising urothelial carcinomas. The pathogenesis of bladder cancer is complex, regulated by various genes and factors, and closely related to the activation of oncogenes (3,4). Prostate cancer is one of the most common malignant tumors in men (5), with a high incidence in European and American countries, and its incidence rate continues to rise in China (6). There is no effective clinical treatment for advanced prostate cancer, leading to a high mortality rate. Therefore, finding suitable molecular targets is very important for the early detection of urinary system tumors and the prognosis of patients.

EZH2 is located on chromosome 7q36.1, has ~40 kb, and contains 26 exons. The EZH2 gene belongs to the PCG (Polycom b group) gene family and has a SET (suvar3-9, enhancer of zeste, trithorax) domain associated with cell proliferation. The EZ gene can regulate cell transduction pathways through the SET domain, inhibiting transcription processes (7,8). As a transcriptional suppressor, EZH2 can significantly inhibit the expression of tumor suppressor or metastasis-related genes. Thus, its high expression or abnormal activation can lead to excessive cell proliferation and malignant transformation and promote tumor invasion and metastasis (9). EZH2 plays a central role in the PG gene family. EZH2 regulates histone methyltransferase and histone deacetylase (HDAC) and is involved in transcriptional suppression (10). As a transcriptional suppressor, EZH2 can reduce the binding of transcription factors to DNA, inhibiting the expression of target genes, and acts on the region where the C-terminus of the pRb2/p130 protein is linked to HDAC1 to restore pRb2/pB0-HDAC1 complex-mediated cyclin A

promoter activity, ultimately promoting cell cycle progression and malignant transformation (11).

Numerous studies have shown that EZH2 is closely related to the occurrence and development of tumors. EZH2 expression increases in breast, prostate, pancreatic, gastric cancers, and other malignant tumors. High EZH2 expression promotes tumor proliferation and metastasis and is closely related to tumor malignancy and poor prognosis (12-15). In the present study, meta- and bioinformatics analyses was conducted to explore the relationship between EZH2 expression and the clinicopathological characteristics and prognosis of urological cancers.

Materials and methods

Literature search. The PubMed (https://pubmed.ncbi.nlm.nih.gov/) and CNKI (https://oversea.cnki.net/index/) databases were utilized on April 22, 2023. The following keywords were used: EZH2 AND (kidney OR bladder OR prostate OR urothelium) AND (cancer OR carcinoma). Searches were not limited by language or year of publication. The inclusion criteria were: i) Articles that detected EZH2 protein levels in kidney, bladder, or prostate cancers by immunohistochemistry; ii) the relationship between EZH2 expression and the pathobiological behavior of renal, bladder, or prostate cancer was assessed. The exclusion criteria included: i) Abstracts, reviews, reviews, and conferences; ii) duplicate published articles; iii) EZH2 levels detected by western blot analysis, reverse transcription PCR, cDNA microarray, or RNA sequencing; and iv) patients did not receive any medical treatment before surgery.

Data extraction and quality assessment. Two reviewers (Y-KB and YW) independently extracted information from all eligible publications according to the inclusion criteria. The following information was extracted from included studies: Name of the first author, year of publication, country, cancer type, antibody company, number of cases and controls, and clinicopathological data of patients. Any disagreements were resolved by discussion until a consensus was reached between the two reviewers. These two reviewers independently assessed the quality of included studies according to the Newcastle-Ottawa Scale (NOS).

Bioinformatics analysis. Using Kaplan-Meier plotter database (https://kmplot.com/analysis), the prognostic significance of EZH2 mRNA expression was analyzed in kidney and bladder cancer. The differences in EZH2 mRNA level were compared between normal tissue and cancer tissue using GEPIA (http://gepia.cancer-pku.cn/) and UALCAN (http://ualcan.path.uab.edu/index.html) databases. In the present study, the TIMER database (https://cistrome.shinyapps.io/timer/) was used to analyze the relationship between EZH2 gene expression and clinical outcomes and immune cell infiltration.

Statistical analysis. Meta-analyses were performed with Revman software 5.3 (https://www.cochrane.org/). The strength of the association between EZH2 expression and tumor risk was assessed by odds ratios (ORs) with 95% confidence intervals. Statistical significance of pooled ORs was determined by Z-test. Heterogeneity effects were quantified by the I^2 test and classified into low, medium, and high degrees of heterogeneity according to cut-off values of 25, 50 and 75%. If there is no significant heterogeneity ($P>0.1$, $I^2<50\%$), a fixed-effects model will be used. Otherwise, a random effects model ($P<0.1$, $I^2\geq50\%$) will be used. Publication bias was assessed by funnel plots and quantified by Begg and Egger tests to assess funnel plot asymmetry (Fig. 3). Two-sided $P<0.05$ was considered to indicate a statistically significant difference.

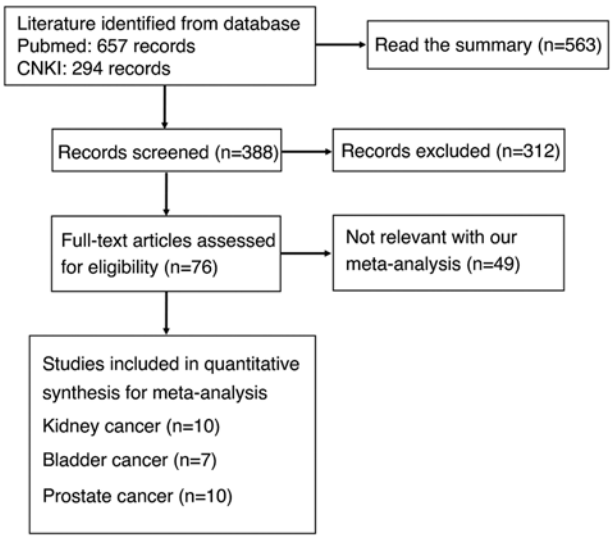

Figure 1. Flow diagram of the selection process in this meta-analysis.

Results

Search results. As shown in Fig. 1, 27 articles on the relationship between EZH2 expression and urological cancers risk, clinicopathological or prognostic parameters were retrieved in PubMed and CNKI by immunohistochemistry for our meta-analysis, including 10 articles on renal cancer (16-25), 7 articles on bladder cancer (26-32) and 10 articles on prostate cancer (33-42). The main characteristics of the included studies are presented in Table I.

Forest plot of OR for the relationship between EZH2 expression and clinicopathological parameters of urological cancer. The present meta-analysis revealed that EZH2 expression was higher in three urological tumor tissues than in normal tissues (Fig. 2A-C, $P<0.01$). A high EZH2 expression was detected in TNM staging III-IV than in I-II (Table II, $P<0.01$). EZH2 overexpression was detected in T3-4 bladder cancer compared with Tis-2 (Table II, $P<0.01$). In three urological tumors, high EZH2 expression was negatively associated with the pathological grade of patients (Table II, $P<0.01$).

Publication bias. As demonstrated in Fig. 3, a heterogeneity test was performed on the included articles. One study at a time was removed from the pooled analysis, the sensitivity of the results was looked at, and this approach was used to coagulate the effect of individual studies on the pooled results. Data that significantly affected heterogeneity were not found in the present study.

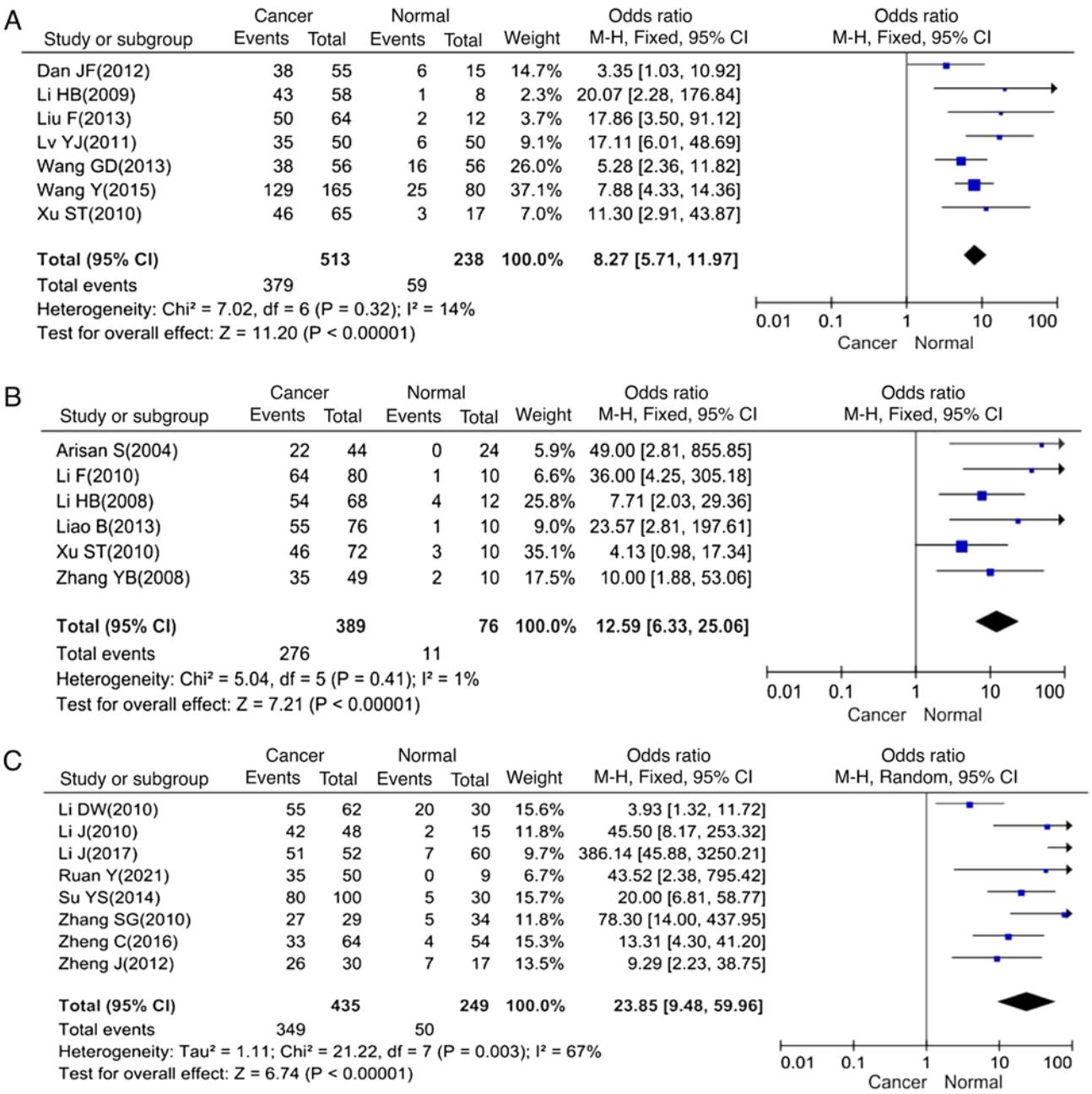

Figure 2. Forest plot of the EZH2 expression in urological cancers. (A) Renal cancer. (B) Bladder cancer. (C) Prostate cancer. EZH2, zeste enhancer homolog 2 gene.

Relationship between EZH2 expression and bioinformatics analysis in kidney cancer patients. According to the Kaplan-Meier plotter, higher EZH2 mRNA levels were negatively associated with the OS rates of kidney clear cell renal and papillary renal cell carcinoma (RCC) (Fig. 4A and B, P<0.01). Higher EZH2 expression was negatively correlated with the relapse-free survival rates in clear cell RCC (Fig. 4C, P<0.01), while it was positively correlated in papillary RCC (Fig. 4D, P<0.01). In GEPIA databases, EZH2 mRNA levels were higher in kidney clear cell renal and papillary RCC than in normal tissues (Fig. 4E and I, respectively, P<0.05). EZH2 mRNA levels were negatively correlated with the TNM stage of kidney clear cell RCC but not in kidney papillary RCC compared with normal tissues (Fig. 4F and J, respectively, P<0.05). Moreover, higher EZH2 mRNA levels were negatively associated with overall survival rates of kidney clear cell RCC and disease-free survival rates of kidney papillary RCC (Fig. 4G and L, P<0.05) and not associated with disease-free survival rates of kidney clear cell RCC (Fig. 4H, P>0.05) or overall survival rates of kidney papillary RCC (Fig. 4K, P>0.05) The TIMER database demonstrated that EZH2 expression was higher in kidney clear cell renal and papillary RCC than in normal tissues (Fig. 5A, P<0.05). Additionally, EZH2 expression was closely associated with the infiltration of seven immune cells (B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils and dendritic cells) in kidney clear cell RCC and kidney papillary RCC (Fig. 5B and D). EZH2 expression was also negatively correlated with patient prognosis (Fig. 5C and E). According to the UALCAN database, EZH2 expression was higher in kidney clear cell RCC, and

Table I. Main characteristics of eligible studies.

First author, year	Country	Antibody supplier	Cases	Ctr	Risk to cancer	Quality	(Refs.)
Lv YJ, 2011	China	Cell Signaling Technology, Inc.	50	50	up	8	(16)
Xu ST, 2010	China	OriGene Technologies, Inc.	65	71	up	8	(17)
Liu F, 2013	China	Invitrogen; Thermo Fisher Scientific, Inc.	64	12	up	8	(18)
Li HB, 2009	China	Signalway Antibody LLC	58	8	up	8	(19)
Dan JF, 2012	China	Cell Signaling Technology, Inc.	55	15	up	8	(20)
Wang GD, 2013	China	Signal	56	56	up	8	(21)
Wagener N, 2010	Germany	Agilent Technologies, Inc.	768			7	(22)
Lee HW, 2012	Korea	Zymed; Thermo Fisher Scientific, Inc.	171			7	(23)
Eichenauer T, 2021	Germany	Abnova	1,809			7	(24)
Wang Y, 2015	China	Signal	165	80	up	8	(25)
Liao B, 2013	China	OriGene Technologies, Inc.	76	10	up	8	(26)
Xu ST, 2010	China	OriGene Technologies, Inc.	72	10	up	8	(27)
Li F, 2010	China	Wuhan Boster Biological Technology, Ltd.	80	10	up	8	(28)
Cai YJ, 2019	China		60			7	(29)
Li HB, 2008	China	Wuhan Boster Biological Technology, Ltd.	68	12	up	8	(30)
Zhang YB, 2008	China		49	10	up	8	(31)
Arisan S, 2005	Istanbul	Abcam	44	24	up	8	(32)
Li DW, 2010	China	OriGene Technologies, Inc.	62	30	up	8	(33)
Zheng J, 2012	China	OriGene Technologies, Inc.	30	17	up	8	(34)
Song L, 2006	China	Abcam	60			7	(35)
Su YS, 2014	China	Santa Cruz Biotechnology, Inc.	100	30	up	8	(36)
Li J, 2010	China	Zymed; Thermo Fisher Scientific, Inc.	48	15	up	8	(37)
Zhang SG, 2010	China	OriGene Technologies, Inc.	29	34	up	8	(38)
Li J, 2017	China	Zymed; Thermo Fisher Scientific, Inc.	52	60	up	8	(39)
Zheng C, 2016	China		64	54	up	8	(40)
Ruan Y, 2021	China	Abcam	50	9	up	8	(41)
Melling N, 2015	Germany	Abnova	10,168			7	(42)

EZH2 expression was elevated in patients with lymph node metastases (Fig. 6A and B, P<0.05). Higher EZH2 expression was negatively associated with OS rates of all cancer patients considering tumor grade (Fig. 6C and D, P<0.05). EZH2 expression was also higher in kidney papillary RCC (Fig. 6E, P<0.05). A higher EZH2 expression was negatively correlated with OS rates of all cancer patients regardless of race and sex (Fig. 6F-H, P<0.05).

Relationship between EZH2 expression and bioinformatics analysis in patients with bladder cancer. The TIMER database demonstrated that EZH2 expression was higher in bladder cancer than in normal tissues (Fig. 7A, P<0.05). EZH2 expression was closely associated with the proportion of immune cell infiltrates in bladder cancer (Fig. 7B). The Kaplan-Meier plotter results are shown in Fig. 7C-E. According to the UALCAN and GEPIA databases, EZH2 expression was higher in bladder cancer than in normal tissues (Fig. 7F and G, P<0.05). Higher EZH2 expression was negatively correlated with disease-free survival rates of bladder cancer (Fig. 7H, P<0.05).

Relationship between EZH2 expression and bioinformatics analysis in patients with prostate cancer. Furthermore, the TIMER results indicated that EZH2 expression was higher in prostate cancer than in normal tissues (Fig. 8A, P<0.05).

Table II. The relationship between EZH2 expression and clinicopathological parameters of urological cancer.

Clinicopathological features	Heterogeneity		Test for overall effect	
	I^2(%)	P-value	Odds ratio (95% confidence interval)	P-value
Renal cancer				
Subtypes (Clear cell RCC/Papillary RCC)	35	0.21	1.02 (0.72-1.44)	0.91
TNM staging (I-II/III-IV)	68	<0.01	0.38 (0.23-0.61)	<0.01
Lymph node metastasis (LN^+/LN^-)	71	0.02	1.57 (0.42-5.85)	0.50
Distant metastasis (M^+/M^-)	73	<0.01	1.35 (0.60-3.03)	0.47
Pathological grade (G1-2/G3-4)	32	0.15	0.51 (0.41-0.64)	<0.01
Bladder cancer				
Depth of invasion (T1-2/T3-4)	0	0.92	0.23 (0.14-0.40)	<0.01
Pathological grade (I-II/III)	0	0.81	0.13 (0.06-0.26)	<0.01
Prostate cancer				
TNM staging (I-II/III-IV)	48	0.09	0.63 (0.58-0.68)	<0.01
Pathological grade (High/Low)	65	<0.01	0.23 (0.10-0.51)	<0.01

TNM, tumor-node-metastasis; LN, Lymph node metastasis.

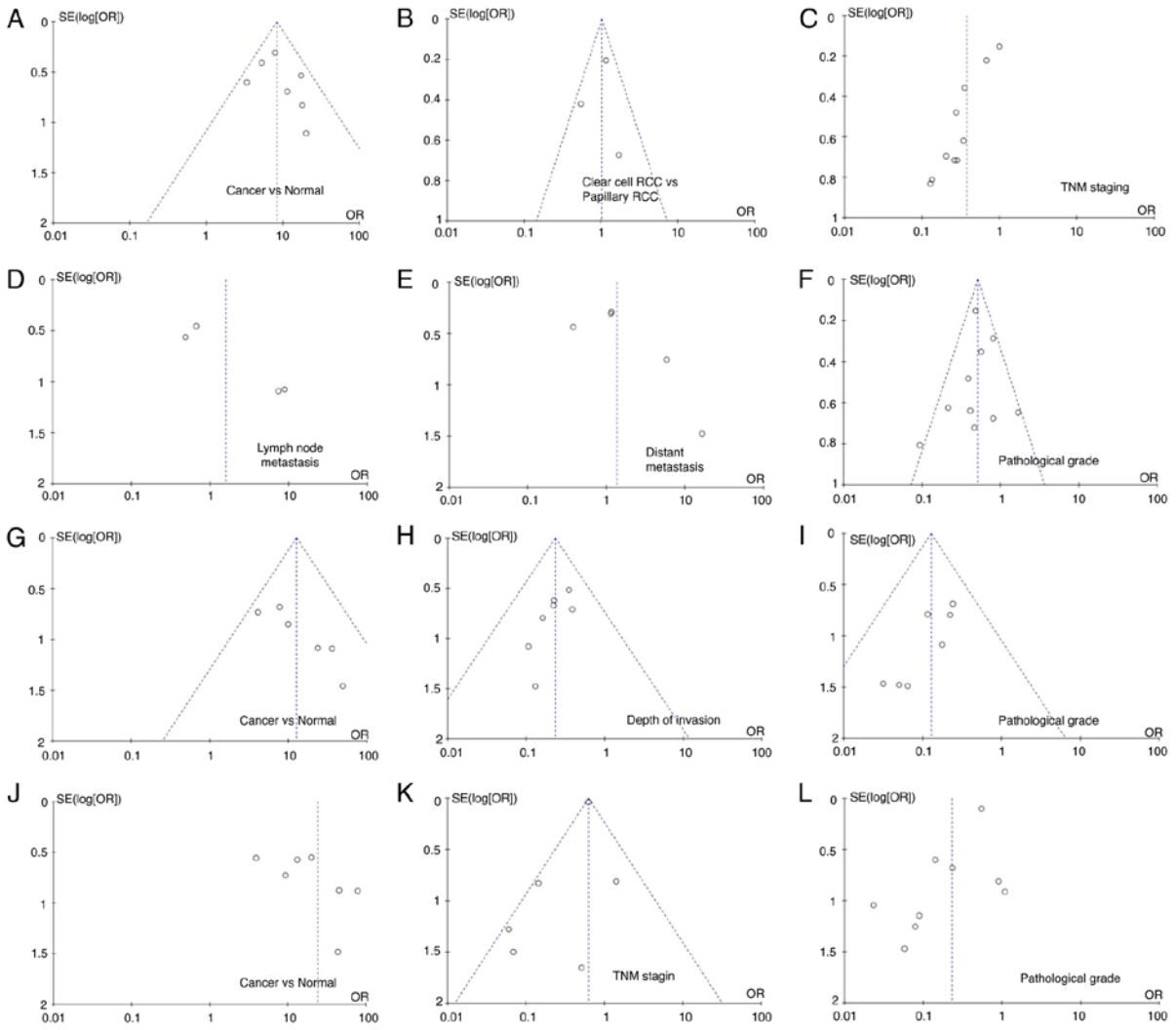

Figure 3. Funnel plot for publication bias test between EZH2 expression and urological cancers or progression. (A-F) Renal cancer. (G-I) Bladder cancer. (J-L) Prostate cancer. EZH2, zeste enhancer homolog 2 gene.

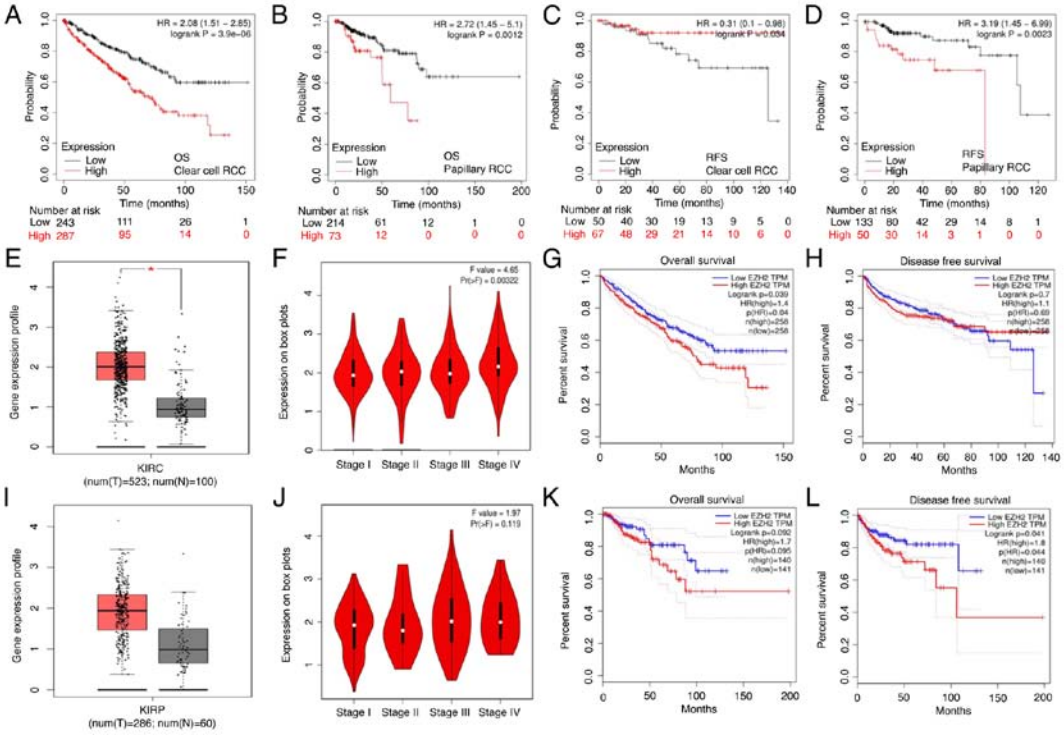

Figure 4. EZH2 mRNA expression in renal cancer. According to the data from Kaplan-Meier plotter, EZH2 mRNA expression was negatively related to both OS rates of the patients with (A) clear cell RCC and (B) papillary RCC, but positively associated with RFS rates (C) in clear cell RCC. EZH2 mRNA expression was negatively related to both (B) overall and (D) RFS rates of the patients with papillary RCC. (E and I) According to GEPIA database, the expression of EZH2 was higher in (E) clear cell RCC and (I) papillary RCC than in normal tissues. (F and J) The expression of EZH2 was negatively correlated with TNM staging of (F) kidney clear cell RCC, but not in (J) kidney papillary RCC compared with normal tissues. (G and L) A higher EZH2 mRNA expression was negatively associated with (G) OS rates of kidney clear cell RCC and (L) disease-free survival rates of kidney papillary RCC. (H and K) The disease-free survival rates of (H) kidney clear cell RCC and (K) OS rates of kidney papillary RCC. *P<0.05. EZH2, zeste enhancer homolog 2 gene; RCC, renal cell carcinoma. OS, overall survival; RFS, relapse-free survival.

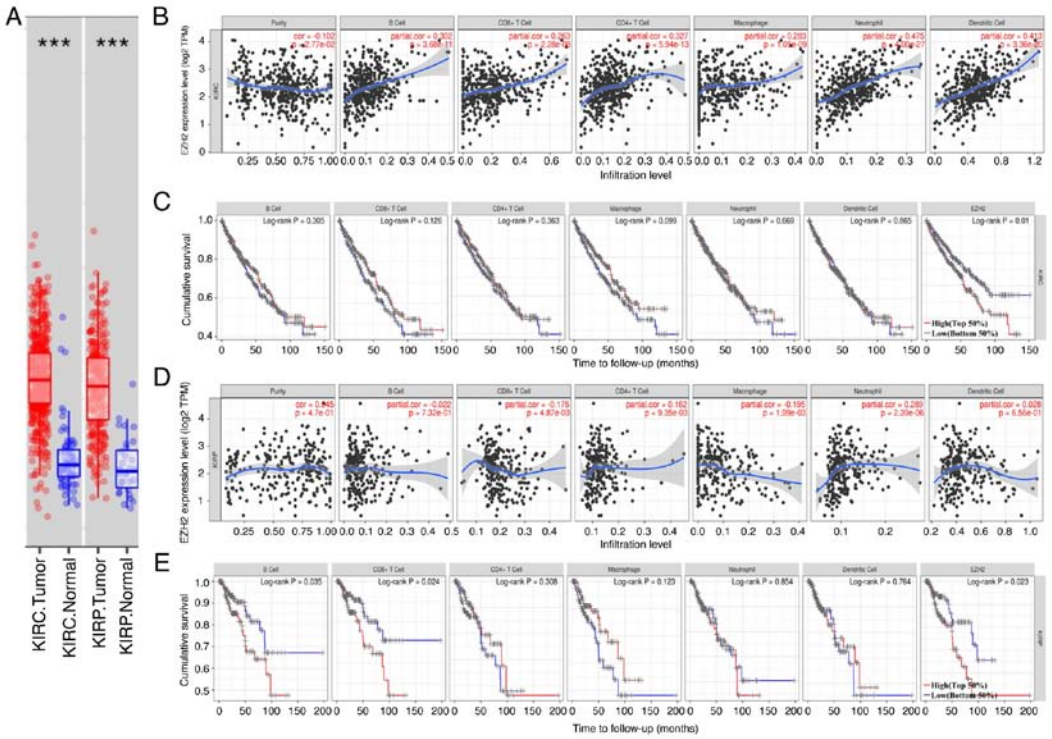

Figure 5. Partial correlation of EZH2 expression levels with immune cells (CD4+ T cells, macrophages, B cells, CD8+ T cells, neutrophils and dendritic cells) in RCC. (A) According to TIMER database, EZH2 expression was higher in clear cell RCC and papillary RCC than normal tissues. (B and D) EZH2 expression closely associated with the proportion of 7 immune cell types (B cells, $CD8^+$ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells) infiltrates in clear cell RCC and papillary RCC. (C and E) EZH2 expression was negatively correlated with patient prognosis. ***P<0.05. EZH2, zeste enhancer homolog 2 gene; RCC, renal cell carcinoma.

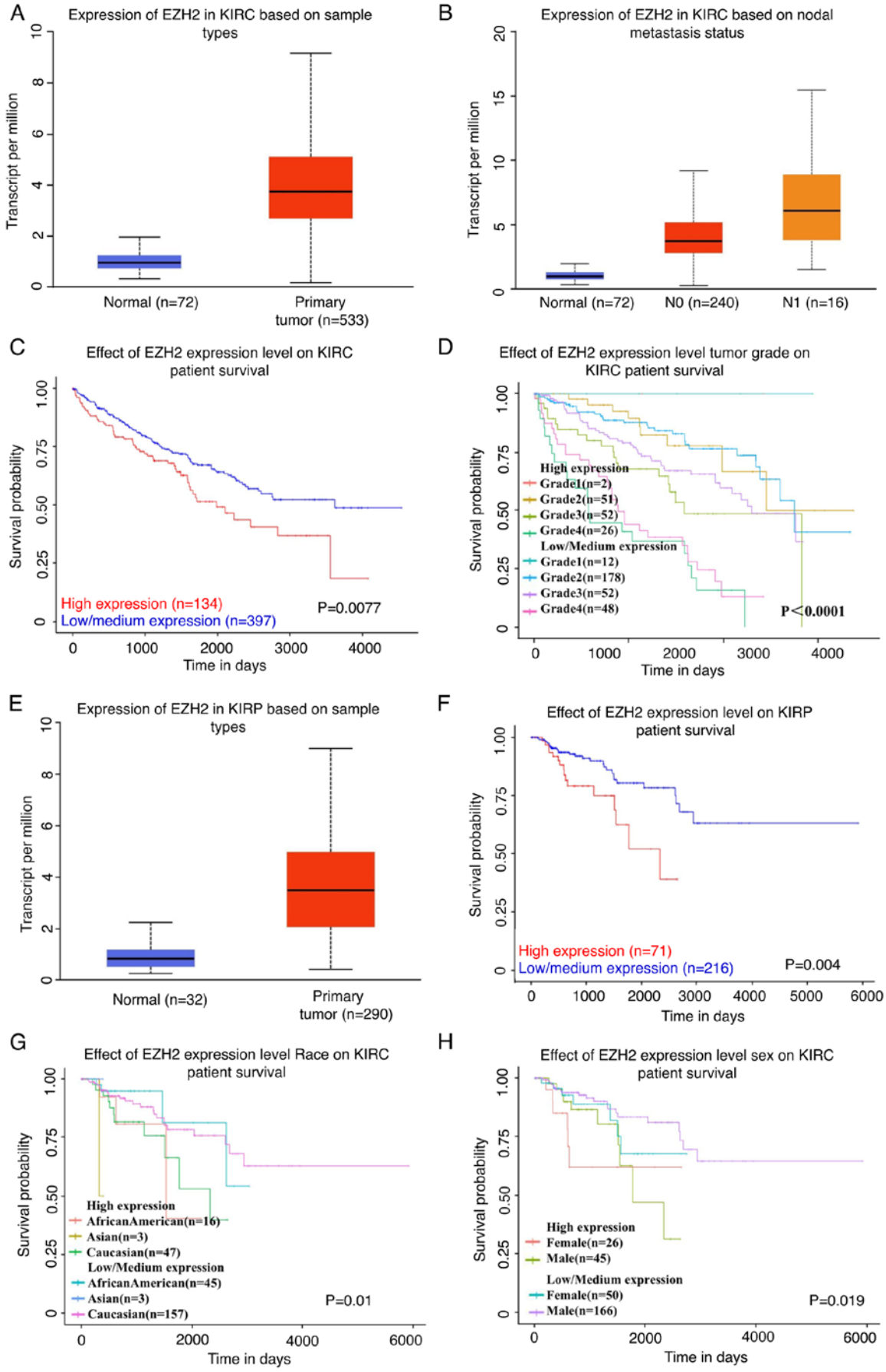

Figure 6. EZH2 mRNA expression in renal cancer. (A and B) According to UALCAN database, EZH2 expression was higher in clear cell RCC, and EZH2 expression was elevated in patients with lymph node metastases. (C and D) A higher EZH2 expression was negatively associated with overall survival rates of all cancer patients, in every tumor grade (1-4). (E) EZH2 expression was also higher in papillary RCC. (F-H) A higher EZH2 expression was negatively correlated with overall survival rates of all cancer patients, independently of race and sex. EZH2, zeste enhancer homolog 2 gene; RCC, renal cell carcinoma.

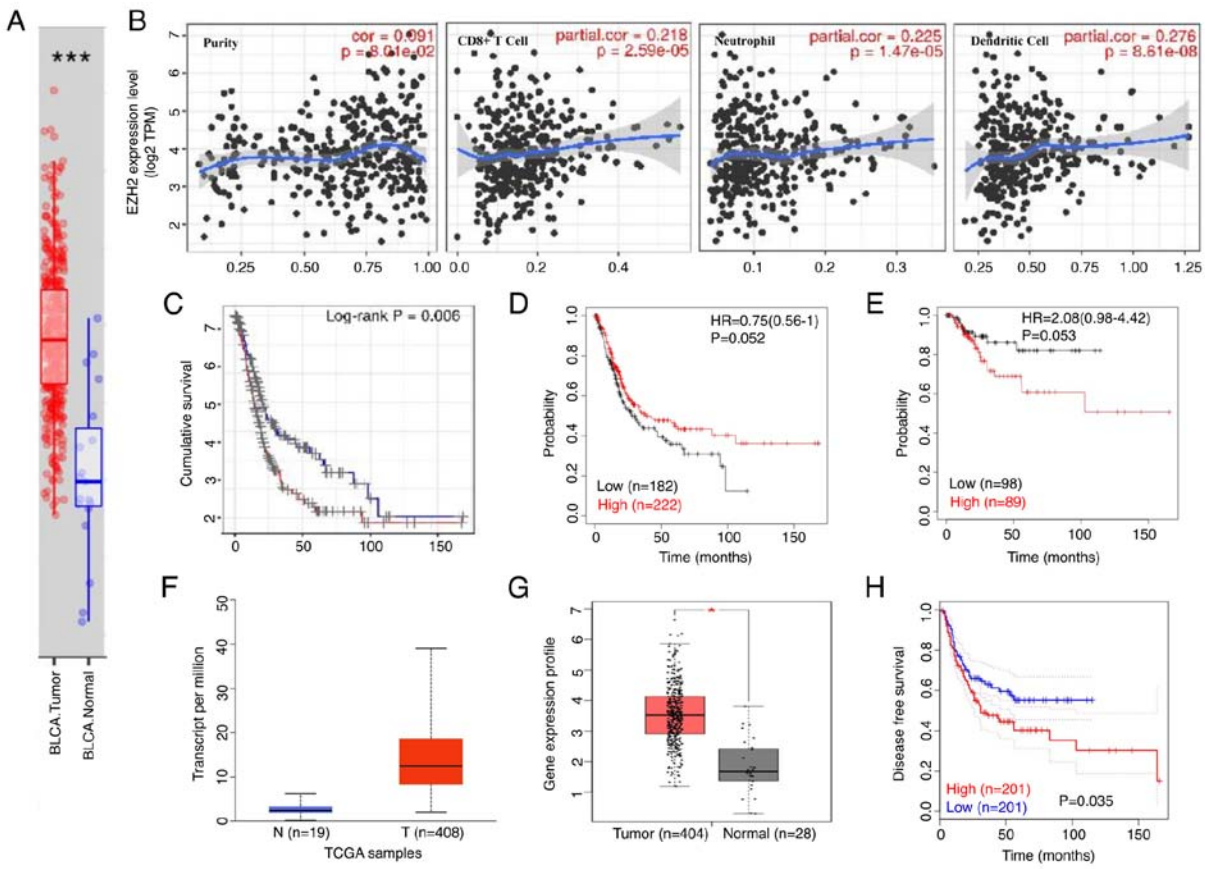

Figure 7. Relationship between EZH2 expression and bioinformatics analysis in patients with bladder cancer. (A) TIMER database demonstrated that EZH2 expression was higher in bladder cancer than in normal tissues. (B) EZH2 expression was closely associated with the proportion of immune cell infiltrates in bladder cancer. (C-E) Kaplan-Meier plotter database results. (F and G) According to UALCAN and GEPIA databases, EZH2 expression was higher in bladder cancer than in normal tissues. (H) A higher EZH2 expression was negatively correlated with disease-free survival rates of bladder cancer. *P<0.05; ***P<0.001. EZH2, zeste enhancer homolog 2 gene; BLCA, bladder cancer.

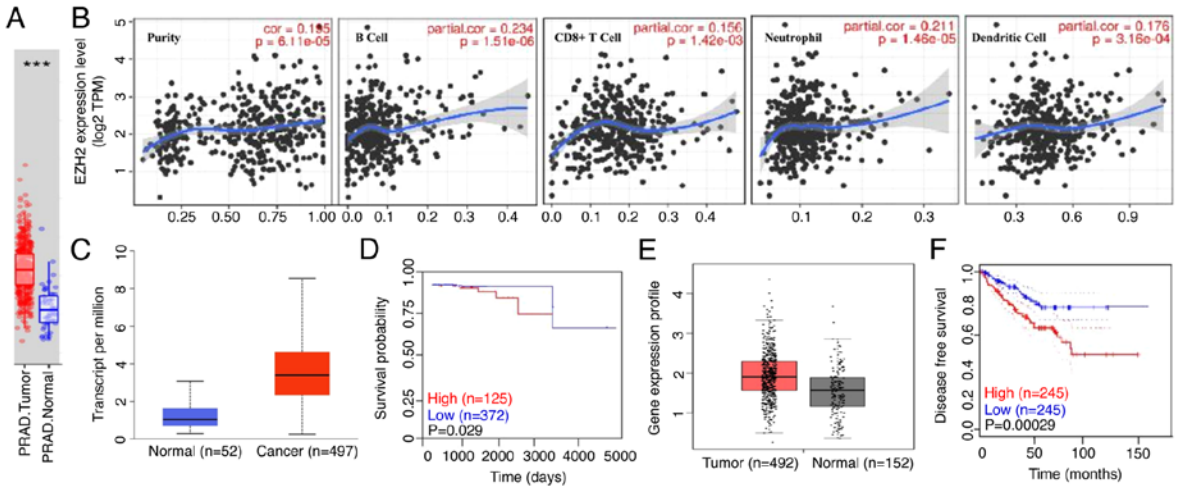

Figure 8. Relationship between EZH2 expression and bioinformatics analysis in patients with prostate cancer. (A) TIMER database demonstrated that EZH2 expression was higher in prostate cancer than in normal tissues. (B) EZH2 expression closely associated with the proportion of immune cell infiltrates in prostate cancer. (C and E) According to UALCAN and GEPIA databases, EZH2 expression was higher in prostate cancer than in normal tissues. (D) According to UALCAN database, a higher EZH2 expression was negatively correlated with overall survival rates of prostate cancer. (F) According to GEPIA database, A higher EZH2 expression was negatively correlated with disease-free survival rates of prostate cancer. ***P<0.001. EZH2, zeste enhancer homolog 2 gene; PRAD, prostate adenocarcinoma.

EZH2 expression was closely associated with the proportion of immune cell infiltrates in prostate cancer (Fig. 8B). The UALCAN and GEPIA results revealed that EZH2 expression was higher in prostate cancer than in normal tissues (Fig. 8C and E, P<0.05). Furthermore, higher EZH2 expression was negatively correlated with the OS rates of prostate

cancer (Fig. 8D, P<0.05) and also negatively correlated with disease-free survival rates of prostate cancer (Fig. 8F, P<0.05).

Discussion

EZH2 is the core catalytic subunit of polycomb repressive complex 2 (PRC2). EZH2 is a tumor-related protein that exhibits histone methyltransferase activity and is commonly present in early embryonic development. EZH2, SUZ12, and EED form the PRC2 complex, which mediates gene silencing by catalyzing histone 3 trimethylation at position 27 (H3K-27me3) and is involved in X chromosome inactivation, cell differentiation, and regulation of embryonic development (43). As a catalytically active subunit, EZH2 is fully involved in the H3K27 process, and EED and SUZ12 jointly maintain PRC2 homeostasis (44). The establishment and maintenance of epigenetic silencing is the basis of cell function. The basic epigenetic systems involved in gene activity suppression are the PcG protein and DNA methylation system. EZH2 interacts with DNA methyltransferase (DNMT) in PRC2 and PRC3, and DNMTs interact and correlate with DNMT activity *in vivo* (45).

Previous studies have indicated that EZH2 expression increases in tumor tissues compared with normal tissues (46). Higher EZH2 expression in tumor tissue leads to inevitably higher severe tumor malignancy and worse prognosis. EZH2 is highly expressed in tumor cells. After interfering with EZH2 inhibition, NK4a/ARF inhibition by PeG will be relieved, resulting in cell senescence. If PeG function is restored, senescent cells will show a naive phenomenon again (18). EZH2 expression is also increased in breast cancer tissues, participating in lymph node metastasis, in the invasion of breast cancer and is closely related to the occurrence of ER-negative breast cancer (47). In breast epithelial cells, abnormally high EZH2 expression can promote cell growth and enhance invasive ability. EZH2 induces G1-S phase cells to enter the division phase, promoting cancer cell proliferation (48). The bioinformatics analysis identified that EZH2 is abnormally highly expressed in hepatocellular carcinoma (HCC) stages 1 to 3. EZH2 can mediate the increase in the modification of histone H3K27me3 near the start site of gene transcription, exerting an epigenetic regulation effect. The EZH2 inhibitor GSK343 impairs HepG2 cell viability and cell proliferation *in vitro* (49).

EZH2 is highly expressed in gastrointestinal malignancies including esophageal, gastric, colorectal and pancreatic cancer (15,50-52). High EZH2 expression can promote the proliferation and invasion of esophageal cancer cells (53). EZH2 induces the epithelial-mesenchymal transition of gastric cancer cells by binding to the PTEN promoter, enhancing migration and invasion ability of cancer cells (54). Particularly, EZH2 enhances the spheroidization ability of gastric cancer cells, indicating its enrichment in cancer stem cells. Additionally, EZH2 inhibits microRNA (miR)-22 through epigenetic modification and regulates H3K27 on the miR-22 promoter, downregulating the galactoprotein-9 and promoting the proliferation and progression of HCC cells. Therefore, inhibiting EZH2 expression can delay the metastatic spread of HCC cells (55).

EZH2 expression is elevated in colorectal cancer tissues, and EZH2 protein is positive in patients with distant metastases. Positive expression rate of EZH2 in tissues of colorectal cancer patients with distant metastasis is higher than in patients without distant metastasis. The multivariate analysis showed that EZH2 protein levels were an independent influencing factor of poor prognosis in patients with colorectal cancer. According to the KM-plotter database, an inverse association between EZH2 expression and poor OS was found in patients with kidney clear cell renal and papillary RCC. In addition, EZH2 overexpression in colorectal cancer tissue is related to the proliferation activity of tumor cells (51). In pancreatic cancer, ANLN-induced EZH2 can upregulate enhancers and regulate cancer cell progression, partially reversing the tumor-suppressive effect of actin-binding protein (ANLN) downregulation (52). By interfering with bladder cancer-related transcription factor 1 (bladder cancer-associated transcript 1, BLACAT1) to block EZH2 recruitment, promote cyclin-dependent kinase inhibitor 1C (cyclin-dependent kinase inhibitor 1C, CDKN1C) expression, and inhibit cyclin expression, thereby inhibiting pancreatic cancer cell proliferation, migration, and aerobic glycolysis (56). The present findings also demonstrated that EZH2 upregulation was negatively associated with TNM staging and pathological grade in kidney and prostate cancers. EZH2 expression was also negatively related to the invasion depth and pathological grade in bladder cancer.

The TIMER database only contains the data of kidney cancer, while the relationship between prostate cancer, bladder cancer and immune cells needs further research in the future, the lack of the use of TISIDB database or other databases as a limitation on this specific aspect, which is also the limitation of the present study.

In conclusion, EZH2 is upregulated in urological cancers. Its high expression was negatively correlated with the invasion depth, TNM staging, and pathological grade of urological cancers at both mRNA and protein levels. The expression of EZH2 was identified to be closely related to four types of immune cells in kidney cancer, which helps with future research. However, the specific mechanism is not yet clear and whether immunotherapy can be considered requires further verification in the future. Moreover, EZH2 expression may be a favorable potential marker for the worse prognosis of urological cancer patients.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Kaplan-Meier plotter (https://kmplot.com/analysis/), TIMER database (https://cistrome.shinyapps.io/timer/), GEPIA (Gene Expression Profiling Interactive Analysis) database(http://gepia.cancer-pku.cn/) and UALCAN (http://ualcan.path.uab.edu/index.).

Authors' contributions

YKB and YW designed the study. YKB and JS prepared figures and tables, interpreted the data and wrote the main manuscript. QLX, YSW and NZ participated in the research of the study and performed the statistical analysis. YKB and YW confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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