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ORIGINAL RESEARCH

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Upregulation of the *ZWINT* expression correlates with prostate cancer progression and immune infiltration

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Abstract

Prostate cancer (PCa), the most prevalent epithelial malignant neoplasm in the male group globally, is the fifth largest cause of cancer-related death in males. *ZW10* Interactor (*ZWINT*) is involved in the chromosome segregation process, which is linked to the formation of several tumor cells. However, its function in PCa remains unknown. Therefore, our aim was to explore the potential mechanisms of *ZWINT* in PCa progression. We obtained RNA-seq as well as clinical data from The Cancer Genome Atlas Program (TCGA), University of California Santa Cruz (UCSC) database. Assessment of *ZWINT* expression in clinical subgroups, immune infiltration, and prognostic relevance using the R program. Search Tool for Recurring Instances of Neighbouring Genes (STRING) tool was applied to construct a *ZWINT* co-expression network and the potential biological functions involved in differentially expressed genes (DEGs) were investigated by enrichment analysis. *ZWINT* was upregulated in prostate cancer tissues and showed to be significantly associated with T stage, N stages, Gleason score (GS), and prognosis of prostate cancer patients. Functional enrichment analysis revealed that *ZWINT*-related genes were mainly related to cell cycle, meiosis, myogenic fiber synthesis, and muscle contraction. In addition, High-expression of *ZWINT* may have possessed immunosuppressive effects through adverse regulation of several immune cells and factors. *ZWINT* is overexpressed in prostate cancer and correlated with immune infiltration, which is expected to be a potential biomarker for PCa prognosis.

Keywords

ZWINT; Prostate cancer; Bioinformatics; Immunity

1. Introduction

Prostate cancer (PCa) ranks as the most prevalent epithelial malignant neoplasm in the male group all over the world. Despite differences in incidence and mortality rates across countries and regions, it remains the world's fifth largest cause of cancer death in males [1]. As a biologically heterogeneous disease, the malignant advances of PCa is associated with interactions between intrinsic germ cell susceptibility, acquired somatic genetic mutations, the cellular microenvironment, and the external environment [2, 3]. Although majority of PCa victims deteriorate gradually, some do so very quickly and proceed into castration-resistant prostate cancer (CRPC). The latter, however, is currently clinically incurable as the main cause of death in PCa. In clinical practice, Prostate-specific antigen (PSA) testing is an effective method for early screening of PCa. However, the European Randomised Study for Screening of Prostate Cancer (ERSPC) trial found that approximately 75% of patients had negative biopsies with a PSA cut-off concentration of 3 μg/L [4]. In addition to being used for screening, PSA in combination with clinical factors is widely accepted to assess the risk of recurrence in patients with prostate cancer. Related studies have shown that approximately 6% of patients suffering from high-grade prostate cancer were in low PSA levels, which may be associated with poor prognosis [5]. In general, PSA is of limited specificity for screening and prognostic assessment of PCa, which may lead to overdiagnosis and overtreatment. With advances in tumor sequencing, genetic testing technologies are commonly applied in disease research. According to the IMPACT study (ClinicalTrials.gov identifier NCT00261456), the incidence of PCa is higher in Breast cancer susceptibility gene 2 (*BRCA2*) carriers compared to *BRCA2* non-carriers [6]. Also, genetic testing identifies whether DNA damage-repair-related genes are expressed abnormally, which in turn allows patients to benefit from targeted drugs [7]. Therefore, the identification of effective prognostic biomarkers for PCa is an important measure to improve the prognosis of PCa patients.

ZWINT, a component of the centromere complex essential for the spindle checkpoint, is involved in mitosis and regulates cell proliferation [8]. *ZWINT* has been linked to chromosomal instability (CIN), and CIN can cause the amplification or

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deletion of whole chromosomes and/or segmental aneuploidy, thereby accelerating tumor evolution and poor clinical outcome [9]. Studies have shown that the deletion of *ZWINT* affects the localization of the ROD–ZW10–Zwilch (RZZ) checkpoint protein to the pro- and mid-phase centromeres which is essential for mitotic checkpoint arrest [10–12]. In recent years, there has been increasing evidence that *ZWINT* plays a cancer-promoting role in a variety of cancers. Obuse *et al*. [13] discovered that *Zwint-1* interacts with the hMis12 complex to link to external kinetochores so that participates in the regulation of the cellular M phase. In colorectal cancer patients, *ZWINT* was significantly associated with the expression of kinase protein family member C1 (*KIFC1*), and silencing *ZWINT* markedly inhibited tumor cell proliferation [14]. As such, we hypothesized that the expression level of *ZWINT* may be involved in the malignant progression of tumor cells. Related studies have shown that high expression levels of *ZWINT* are in close association with the malignant progression of ovarian [15], liver [16], lung [17], and breast cancers [18, 19], whereas its study in PCa has hardly been reported. As a result, We utilized Bioinformatics to explore the correlation of *ZWINT* with PCa progression and immune infiltration for evaluating the value of *ZWINT* as a carcinogenic factor in the personalized treatment of PCa patients.

2. Materials and methods

2.1 Datasets and source

RNA-seq transcriptome data from the TCGA database (https://portal.gdc.cancer.gov/) was used to assess the expression levels of *ZWINT* among various cancer. The GSE46602 dataset (including 34 prostate cancer samples and 16 benign prostate tissue samples) was obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) as a validation set. We then downloaded data on pathological characteristics, survival status, and follow-up of PCa patients through UCSC Xena (https://xenabrowser.net/datapages/).

2.2 Gene expression analysis

Taking the *ZWINT* expression as a reference, 499 PCa samples were divided into high-expression (250) and low-expression (249) group. And R package "DESeq2" [20] was applied for analyzing differentially expressed genes (DEGs) of the groups, with adjusted p-value < 0.05 and $|\log2\ FC| > 1$ as the thresholds.

2.3 Functional enrichment and co-expression network analysis

To reveal the role played by DEGs in molecular functions and biological processes, we conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. In addition, Cytoscape software (version 3.9.1) was applied to visualize the protein-protein interaction (PPI) network of *ZWINT* genes co-expressed constructed from the STRING database (https://cn.string-db.org/).

2.4 Immune infiltration analysis

Using the single sample Gene Set Enrichment Analysis (ssGSEA) algorithm, we presented immune correlation analysis of 24 immune cells to explore potential associations with *ZWINT* expression levels. The correlation coefficients of 24 immune cells with *ZWINT* were calculated using Spearman correlation analysis. Wilcoxon rank sum test was used to examine the difference between subgroups. The tumor-immune system interactions database (TISIDB) database (http://cis.hku.hk/TISIDB/) was used to investigate the association between the *ZWINT* expression levels and the expression of immune checkpoint-associated factors.

2.5 Survival analysis

The Kaplan-Meier method was adopted for survival analysis of the *ZWINT*-expressing group (low/high). Univariate Cox regression analysis was performed to assess the value of clinical variables and *ZWINT* expression on patient prognosis.

2.6 Statistical analysis

All statistical analysis was performed using R software (version 4.1.3, Ross Ihaka and Robert Gentleman, Auckland, New Zealand). The Wilcoxon rank sum test was used to assess the difference in *ZWINT* expression in PCa versus normal tissues. Statistically significant expression of *ZWINT* in 52 pairs of paired samples was assessed using t-test. In addition, logistic regression analysis was performed to assess the correlation between clinical characteristics of PCa and the expression of *ZWINT*. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 ZWINT was overexpressed in PCa

First, we analyzed the expression of *ZWINT* in pan-cancer and PCa using RNA-seq data from Genotype-Tissue Expression Program (GTEx) and TCGA databases. As the Fig. 1 showed that *ZWINT* was significantly upregulated in both most tumor tissues and PCa than normal tissues Fig. 1A,B). Similarly, Results of paracancer paired samples showed that *ZWINT* expression was higher in tumor samples (Fig. 1C). Then, we applied receiver operating characteristic (ROC) curves to evaluate the discriminatory ability and diagnostic value of *ZWINT* expression (Fig. 1D), which showed an area under the curve (AUC) value of 0.828 for the *ZWINT* with confidence interval (CI): 0.791–0.886. External data (GSE46602) furthermore confirmed the high expression of *ZWINT* in PCa (Fig. 1E). As these results suggest, we believe that *ZWINT* has the potential to be a promising biomarker for differentiating prostate cancer tissue from normal tissue.

3.2 The ZWINT expression level correlates with clinical characteristics of PCa

We acquired clinical information such as Age, Tumor Node Metastasis (TNM), PSA, and GS from UCSC Xena database for 499 samples to evaluate the relationship between *ZWINT* expression levels and clinical features of PCa (Table 1). Based

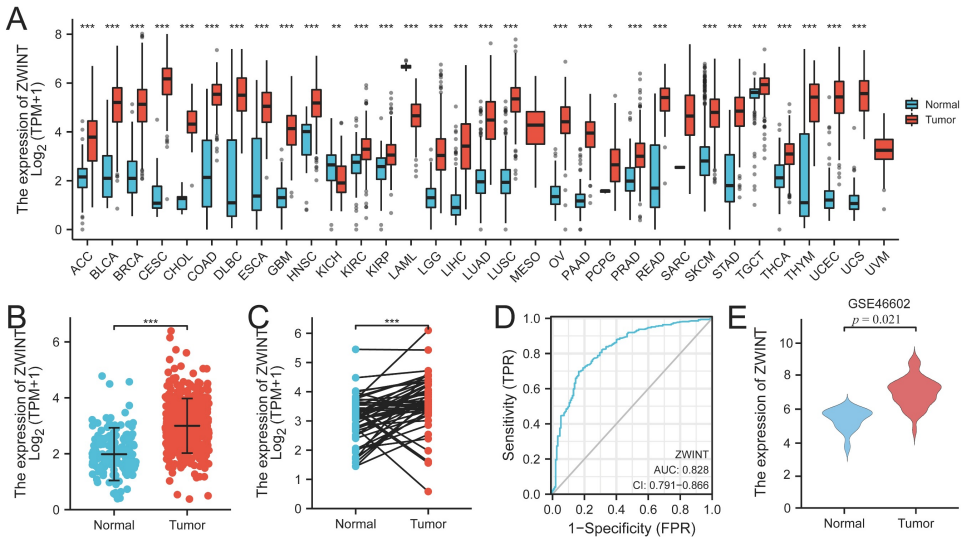

FIGURE 1. Expression analysis of *ZWINT* in cancers. (A,B): Expression of *ZWINT* in 33 cancers and prostate cancer. (C): Expression of *ZWINT* in paired samples of prostate cancer. (D): Sensitivity and specificity of *ZWINT* in diagnosing PCa. (E): Expression of *ZWINT* in GSE46602 validation set. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; *BRCA*: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma; CHOL: Cholangio carcinoma; COAD: Colon adenocarcinoma; DLBC: Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and Neck squamous cell carcinoma; KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute myeloid leukemia; LGG: Brain lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MESO: Mesothelioma; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin cutaneous melanoma; STAD: Stomach adenocarcinoma; TGCT: Testicular germ cell tumors; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine carcinosarcoma; UVN: Uveal melanoma; TPM: Transcripts per million; *ZWINT*: *ZW10* Interactor; TPR: True Positive Rate; FPR: False Positive Rate.

TABLE 1. Clinical information of PCa patients in TCGA database.

Characteristic	n (%)	Median (IQR)
Age	499	61 (56, 66)
>60	275 (55.1%)	
≤60	224 (44.9%)	
T stage	492	-
T2	189 (38.4%)	
T3	292 (59.3%)	
T4	11 (2.2%)	
N stage	426	-
N0	347 (81.5%)	
N1	79 (18.5%)	
M stage	458	-
M0	455 (99.3%)	
M1	3 (0.7%)	
PSA	442	0.1 (0.03, 0.11)
≥4	27 (6.1%)	
<4	415 (93.9%)	
Gleason score	499	-
6	46 (9.2%)	
7	247 (49.5%)	
8	64 (12.8%)	
9	138 (27.7%)	
10	4 (0.8%)	

PSA: Prostate-specific antigen; IQR: InterQuartile Range.

on the median value of *ZWINT* expression levels it was divided into a low expression group (n = 249) and a high expression group (n = 250) (**Supplementary Table 1**), whose results showed statistical differences in T stage ($p = 0.002$), N stage ($p = 0.038$) and Gleason score (GS) ($p < 0.001$) between the two groups. Furthermore, we grouped patients according to TNM stage, age, PSA and GS, and logistic regression analysis showed that high *ZWINT* gene expression was associated with T stage (T3 & T4 *vs.* T2, Odds Ratio (OR) = 1.795, $p < 0.001$), N stage (N1 *vs.* N0, OR = 1.754, $p = 0.029$) and GS (8 & 9 & 10 *vs.* 6 & 7, OR = 2.381, $p < 0.001$) were significantly correlated (Table 2 and Fig. 2A–C).

3.3 Function enrichment analysis

The "DESeq2" package was for analyzing *ZWINT*-related differential genes, as shown in the volcano diagram, 161 up-regulated genes and 128 down-regulated genes were screened (Fig. 3A). According to KEGG analysis, DEGs are mainly involved in the cell cycle, meiosis, luteinizing hormone-mediated oocyte maturation, P53 signaling pathway, and glutathione metabolism (Fig. 3B). The results of GO analysis revealed that the up-regulated genes were enriched in mitosis, nuclear division, chromosome condensation, and microtubule protein binding, while the down-regulated genes were primarily involved in biological processes such as contractile fiber, muscle contraction, myofibril, and structural constituent of the cytoskeleton (Fig. 3C,D).

3.4 Construction of PPI network and verification of hub genes

The top 50 DEGs were explored for potential interactions with each other based on relevance to *ZWINT*. Using the online STRING website, we constructed a PPI network and used Cytoscape software to calculate the six Hub genes with the highest relevance to *ZWINT*: Marker of proliferation Ki-67 (*MKI67*), Anillin (*ANLN*), Ubiquitin Conjugating Enzyme E2 C (*UBE2C*), PDZ binding kinase (*PBK*), Actin alpha cardiac muscle 1 (*ACTC1*), Keratin 14 (*KRT14*) (Fig. 4A). According to correlation analysis, the expression of *ZWINT* was positively correlated with *MKI67*, *ANLN*, *UBE2C* and *PBK* ($p < 0.001$), while it was negatively correlated with *ACTC1* and *KRT1* ($p < 0.001$, Fig. 4B). Interestingly, we found that the expression levels of *MKI67* (Hazard Ratio (HR) = 1.91, $p = 0.003$), *ANLN* (HR = 2.13, $p = 0.001$), *UBE2C* (HR = 2.47, $p < 0.001$), *PBK* (HR = 2.35, $p < 0.001$), *ACTC1* (HR = 0.55, $p = 0.005$), and *KRT14* (HR = 0.65, $p = 0.038$) all indicate worse survival probability of PCa (Fig. 4C–H).

3.5 ZWINT expression correlates with immune infiltration in PCa

By correlation analysis of immune cells, we presented the potential role played by *ZWINT* in the immune function of PCa cells. As shown in Fig. 5A, *ZWINT* upregulation in PCa may inhibit the level of Natural killer cell (NK) ($r = -0.311$, $p < 0.001$) and plasmacytoid dendritic cell (pDC) ($r = -0.230$, $p < 0.001$) infiltration, while positively regulating T helper 2 cell (TH2) ($r = 0.567$, $p < 0.001$). To confirm the above findings, we divided the samples into groups based on the median *ZWINT* expression, and the results showed that NK cells and pDC were significantly enriched in the *ZWINT* low expression group ($p < 0.01$), while TH2 cells were significantly enriched in the high expression group ($p < 0.001$) (Fig. 5B–G). With the aim of further discussing whether *ZWINT* regulates the expression of immune factors, we analyzed the correlation of *ZWINT* with the expression of immunosuppressants and immunostimulants in different cancer species using the TISIDB database (**Supplementary Fig. 1A,B**). The results showed that in PCa, *ZWINT* had no correlation with immunosuppressive factors ($-0.3 < r < 0.3$). Notably, *ZWINT* was negatively correlated with the expression of several immunostimulatory factors, such as Cluster of differentiation 40 (CD40) (*rho* = −0.354, $p = 4.6 \times 10^{-16}$) and Transmembrane protein 173 (TMEM173) (*rho* = −0.32, $p = 3.45 \times 10^{-13}$) (**Supplementary Fig. 1C,D**). The aforementioned results showed that *ZWINT* may have a modulatory role in the immune infiltration of PCa cells.

3.6 Overexpression of ZWINT correlates with a worse prognosis of PCa

Correlation between *ZWINT* expression and PCa prognosis using Kaplan-Meier method. The results showed that Disease free survival (DFS) and Progress free interval (PFI) were significantly worse in the high *ZWINT* expression group than in the low expression group (DFS: HR = 1.66, 95% CI = 1.08–2.55, $p = 0.021$; PFI: HR = 1.75, 95% CI = 1.15–2.66, $p = 0.009$) (Fig. 6A,B). Furthermore, univariate cox regression results showed that T/N stage, PSA level (PSA >4), GS (7 & 8 & 9), and the expression of *ZWINT* were associated with DFS and PFI in PCa (**Supplementary Table 2**).

4. Discussion

An increasing incidence of PCa has been observed worldwide. According to statistics, PCa has become the third most common cancer in the United States in 2022, with the fifth highest mortality rate [21]. Although PSA still plays an important role in the screening and diagnosis of PCa, its limitations in diagnostic specificity (20–25% misdiagnosis rate) have caused unnecessary biopsies and overtreatment [22]. To be sure, The improvements in PSA testing method (fPSA, [−2]proPSA and prostate health index) have been made in the past few years, it could not be widely applied due to experimental conditions and cost constraints [23]. Therefore, searching for new PCa biomarkers is crucial to predict prognosis and enhancing individualized treatment.

Cellular dysregulation caused by the activation or inactivation of related regulators is is a trigger point for of tumor cell proliferation [24]. Related studies have shown that several cell cycle-related genes can serve as potential biomarkers for PCa prognostic and therapeutic targets [25–27]. *ZWINT*, a centromere-associated protein, can directly interact with *ZW10*, co-regulating the mitotic spindle checkpoint process [28]. Overexpression of *ZWINT* in patients with lung cancer has a poor prognosis and may be a potentially therapeutic option [17, 29]. Zhou *et al.*

TABLE 2. Logistic regression analysis of *ZWINT* expression and clinicopathological characteristics.

Characteristic	Total (n)	Odds Ratio (OR)	*p* value
T stage (T3 & T4 *vs.* T2)	492	1.795 (1.244–2.598)	0.002*
N stage (N1 *vs.* N0)	426	1.754 (0.924–2.499)	0.029*
M stage (M1 *vs.* M0)	458	1.957 (0.186–42.276)	0.585
Age (>60 *vs.* ≤60)	499	1.394 (0.979–1.988)	0.263
PSA (≥4 *vs.* <4)	442	1.504 (0.688–3.408)	0.079
Gleason score (>7 *vs.* ≤7)	499	2.381 (1.656–3.443)	<0.001*

*All clinicopathological characteristics with the latter as a reference, * $p < 0.05$. PSA: Prostate-specific antigen.*

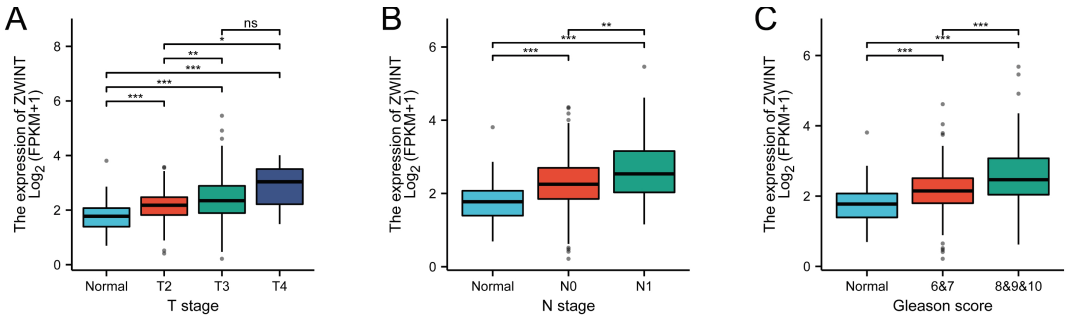

FIGURE 2. The correlation between *ZWINT* expression and clinical characteristics. (A–C): Correlation of *ZWINT* expression with T-stage, N-stage and Gleason score. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, "ns" means no statistical difference). *ZWINT*: *ZW10* Interactor; FPKM: Fragments Per Kilobase Million.

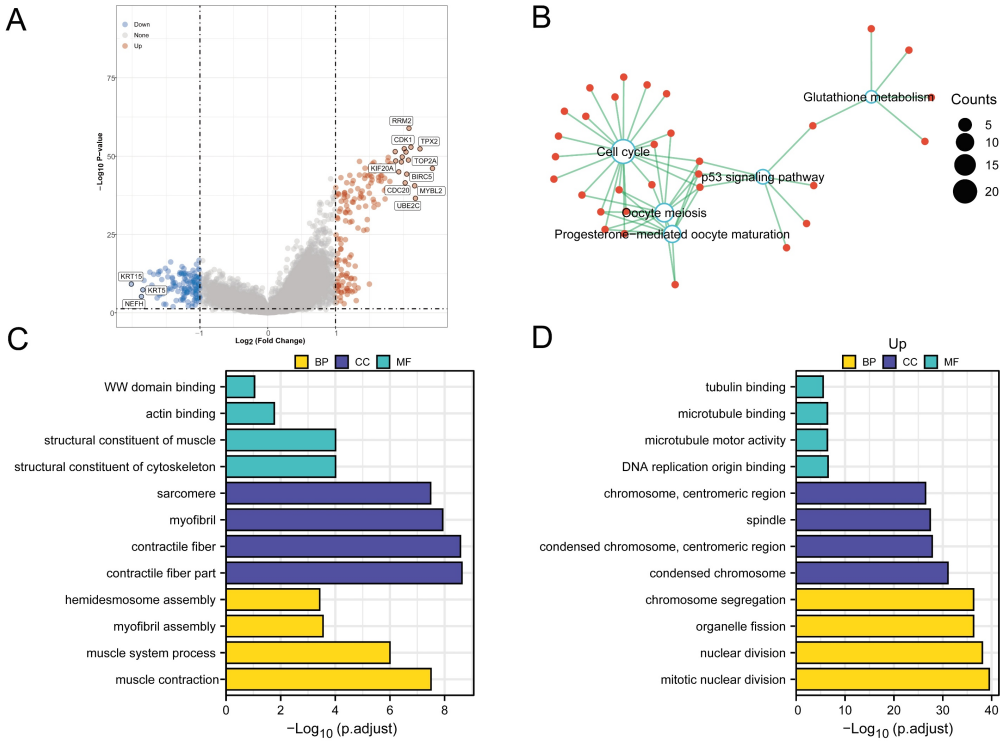

FIGURE 3. Differentially expressed genes and enrichment analysis. (A): Volcano plot of differential genes (up-regulation: 168, down-regulation: 128). (B): KEGG analysis of differential genes. (C,D): GO analysis of differential genes. *KRT15*: Keratin 15; *KRT5*: Keratin 5; *NEFH*: Neurofilament heavy chain; *RRM2*: Ribonucleotide reductase regulatory subunit M2; *CDK1*: Cyclin-dependent kinases 1; *TPX2*: TPX2 microtubule nucleation factor; *KIF20A*: Kinesin family member 20A; *TOP2A*: Topoisomerase (DNA) II Alpha; *BIRC5*: Baculoviral IAP repeat containing 5; *CDC20*: Cell division cycle 20 homologue; *MYBL2*: MYB Proto-Oncogene like 2; *UBE2C*: Ubiquitin conjugating enzyme E2 C; BP: Biological Process; CC: Cell Component; MF: Molecular Function.

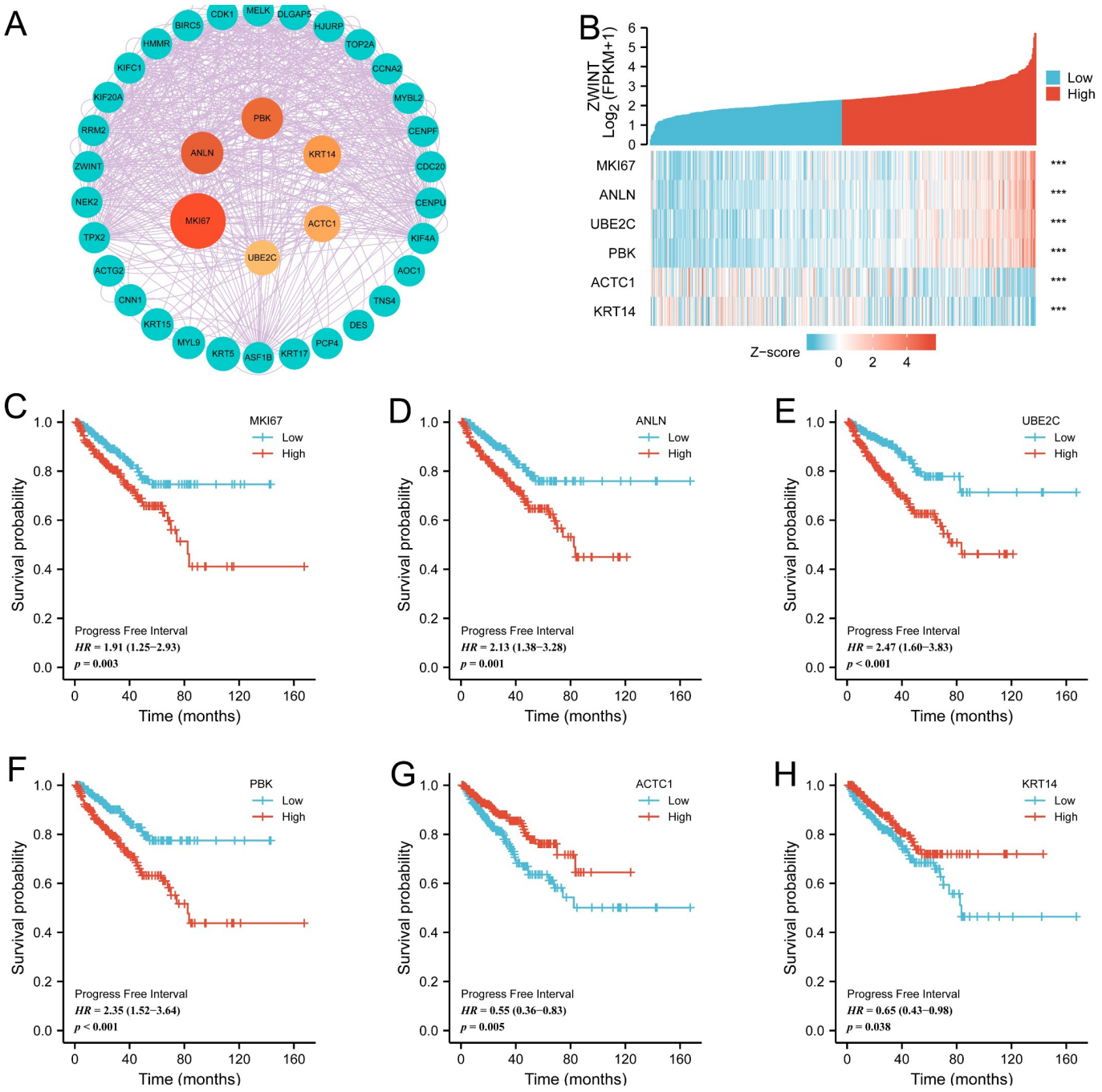

FIGURE 4. Validation of the Hub genes and prognosis analysis. (A): Construction of PPI network. (B): Correlation between Hub genes and *ZWINT* expression (*** $p < 0.001$). (C–H): The Hub genes expression correlates with PCa prognosis. *ZWINT*: *ZW10* Interactor; *RRM2*: Ribonucleotide reductase M2; *KIF20A*: Kinesin family member 20A; *KIFC1*: Kinase protein family member C1; *HMMR*: Hyaluronan mediated motility receptor; *BIRC5*: Baculoviral IAP repeat containing 5; *CDK1*: Cyclin dependent kinase 1; *MELK:* Maternal embryonic leucine zipper kinase; *DLGAP5*: Discs large homolog associated protein 5; *HJURP*: Holliday junction Recognition Protein; *TOP2A*: Topoisomerase (DNA) II Alpha; *CCNA2*: Cyclin A2; *MYBL2*: MYB Proto-Oncogene like 2; *CENPF*: Centromere protein F; *CDC20*: Cell division cycle 20 homologue; *CENPU*: Centromere protein U; *KIF4A*: Kinesin family member 4A; *AOC1*: Amine Oxidase Copper Containing 1; *TNS4*: Tensin 4; *DES*: Desmin; *PCP4*: Purkinje cell protein 4; *KRT17*: Keratin 17; *ASF1B*: Anti-silencing function protein 1 homolog B; *KRT5:* Keratin 5; *MYL9*: Myosin light chain 9; *KRT15*: Keratin 15; *CNN1*: Calponin 1; *ACTG2*: Actin gamma 2; *TPX2:* TPX2 microtubule nucleation factor; *NEK2*: NimA-related protein kinase 2; MKI67: Marker of proliferation Ki-67; ANLN : Anillin; UBE2C : Ubiquitin Conjugating Enzyme E2 C; PBK: PDZ binding kinase; ACTC1: Actin alpha cardiac muscle 1; KRT14: Keratin 14; HR: Hazard Ratio.

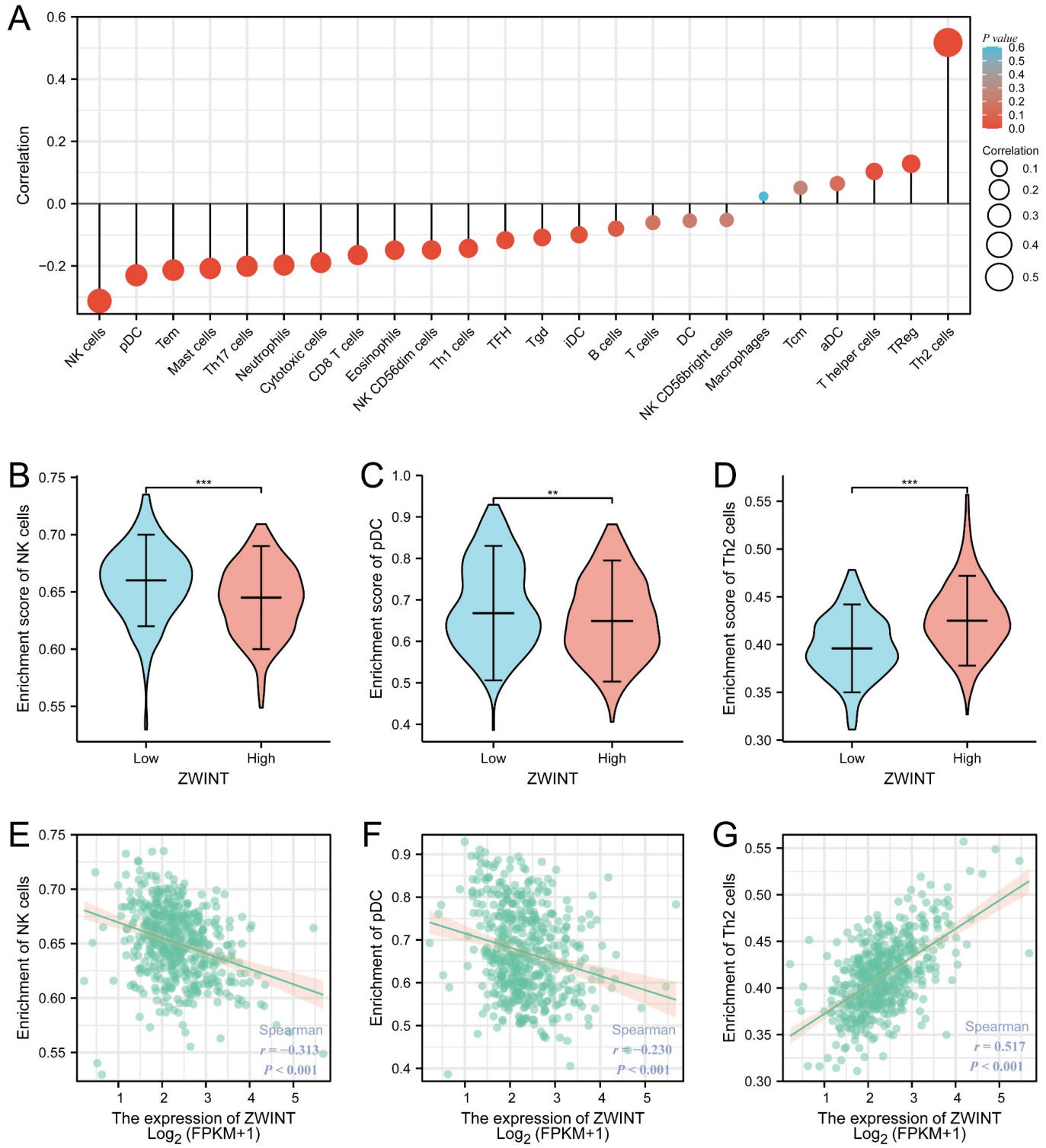

FIGURE 5. The correlation between *ZWINT* expression and immune infiltration. (A): The relevance of *ZWINT* expression to 24 immune cells. (B–G): Enrichment scores of NK, pDC and Th2 cells in *ZWINT* high and low expression groups. (** $p < 0.01$, *** $p < 0.001$). pDC: plasmacytoid dendritic cell; NK: Natural killer cell; Tem: Effective Memory T Cell; Th17: T helper 17 cell; CD8: Cluster of differentiation 8; Th1: T helper 1 cell; TFH: T follicular helper cell; Tgd: T gamma delta cell; iDC: Interdigitating cell; DC: Dendritic cell; Tcm: T central memory cell; aDC: Activated dendritic cells; Th2: T helper 2 cell; *ZWINT*: *ZW10* Interactor; FPKM: Fragments Per Kilobase Million.

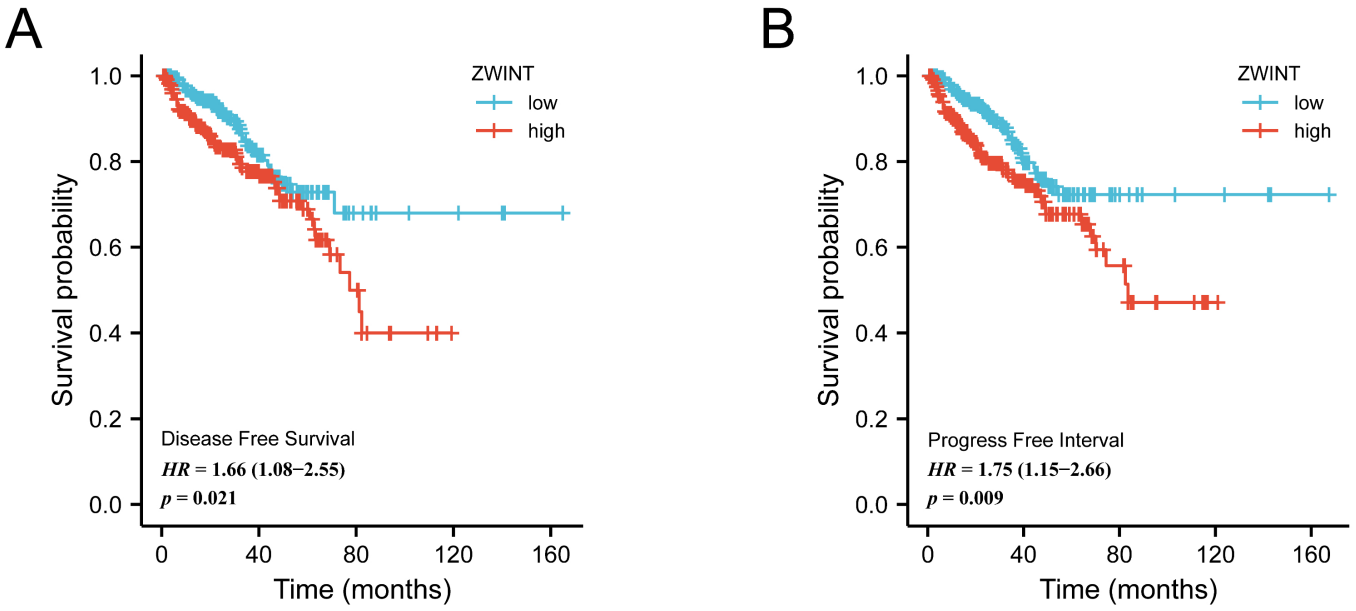

FIGURE 6. *ZWINT* expression correlates with the prognosis of prostate cancer. (A): Disease free survival analysis for PCa samples from the TCGA dataset with high or low *ZWINT* expression. (B): Progress free survival analysis for PCa samples from the TCGA dataset with high or low *ZWINT* expression. *ZWINT*: *ZW10* Interactor; HR: Hazard Ratio.

[19] found that inhibition of *ZWINT* expression inhibited cell proliferation by inducing a G1 phase block in breast cancer cells. Such discoveries indicate that *ZWINT* may have important guidance for cancer progression Furthermore, related studies have demonstrated that *ZWINT* expression is regulated by androgens and is overexpressed in CRPC [30, 31]. However, the correlation between *ZWINT* and PCa remains unevaluated.

In this study, we evaluated the clinical correlation with the upregulation of *ZWINT* in PCa. By bioinformatics analysis, we also investigated the impact of *ZWINT* expression on PCa prognosis and immune cell infiltration. We found that the expression of *ZWINT* was higher in PCa than in normal tissue and was positively correlated with Gleason score and T and N stages, but not with age, PSA or M stage. Furthermore, the *ZWINT* overexpression level was significantly correlated with DFS and PFI in PCa patients. By functional enrichment analysis, it was found that upregulated genes associated with *ZWINT* were significantly enriched in the cell cycle, especially in chromosome segregation. The correct segregation of chromosomes is accomplished by the precise cooperation of centrosomes, Spindle Apparatus, kinetochore, and chromosomes. Errors in this process can lead to abnormal chromosome segregation, resulting in deregulated and abnormal cell proliferation [32]. Lin *et al.* [33] discovered that interactions between Highly expressed in Cancer 1 (*Hec1*), *Zwint-1* and *ZW10* may be necessary to coordinate chromosome segregation accurately. Therefore, we propose that in PCa, *ZWINT* overexpression may affect the chromosome segregation process which promotes tumorigenesis and development. Furthermore, the present study constructed a *ZWINT* co-expression network in which six hub genes significantly associated with PCa prognosis were screened, including *MKI67*, ANLN, *UBE2C*, *PBK*, *ACTC1* and*KRT14*.

Tumor cells live in a microenvironment consisting of immune and inflammatory cells, tumor-associated fibroblasts, mesenchymal tissue, microvasculature, and various cytokines and chemokines [24]. The tumor immune microenvironment (TIME) is a subclass of immune environment composed of immune components and tumor cells, which is mainly divided into tumor-antagonistic immune cells and tumor-promoting immune cells [34, 35]. According to the latest studies, several genes can alter the stability of the tumor microenvironment by regulating the level of immune cell infiltration [36–38]. To evaluate the contribution made by *ZWINT* in TIME, we calculated the relationship between *ZWINT* expression levels and immune characteristics. The results showed that *ZWINT* overexpression was negatively correlated with cellular infiltration of multiple immune cells (NK, pDCs cells, *etc.*) as well as with the expression of immunostimulatory factors (CD40, TMEM173, *etc.*). Previous studies have identified NK cells as an important subpopulation of tumor antagonistic immune cells which exert immune surveillance by exocytosis secretion of cytotoxic granules (*e.g.*, perforin and granzyme) inducing apoptosis in target cells [39]. pDCs can enhance NK cell activity and play a critical role in PCa immune rejection through the release of type I interferon (IFN) [40, 41]. Interestingly, Transmembrane Protein 173 (TMEM173), also known as Stimulation of interferon genes (*STING*), stimulates the release of type I IFN and activates NK, NKT cells and other immune cells [42]. The immunosuppressive microenvironment is considered to be one of the key mechanisms of tumor immune escape. CD40, as an immunostimulant, can initiate CD8+ T cell-mediated cellular immunity by activating DCs [43, 44]. Therefore, we suggest that *ZWINT* overexpression may achieve PCa cellular immune escape by negatively regulating CD40 and TMEM173 expression and inhibiting the infiltration level of NK and pDCs cells, further affecting PCa development and patient prognosis.

5. Conclusions

In summary, we found that *ZWINT* is a cell cycle-associated gene. And *ZWINT* overexpression may be involved in PCa malignant progression by regulating immune cell infiltration. Nevertheless, several limitations are present in our study, such as the fact that most of the data for our study came from public databases, resulting in our inability to obtain complete follow-up data. Furthermore, *ZWINT* involvement in pathways related to the development of PCa needs to be further validated at the molecular level. Overall, our findings suggest that *ZWINT* has the potential to be a novel prognostic biomarker, which provides new insights for personalized treatment of PCa patients.

ABBREVIATIONS

PCa, prostate cancer; *ZWINT*, *ZW10* Interactor; TCGA, The Cancer Genome Atlas; UCSC, University Of California Sisha Cruz; STRING, Search Tool for Recurring Instances of Neighbouring Genes; DEGs, differentially expressed genes; GS, Gleason score; CRPC, Castration-resistant prostate cancer; PSA, prostate-specific antigen; ERSPC, the European Randomised Study for Screening of Prostate Cancer; *BRCA2*, Breast cancer susceptibility gene 2; NCI, National Cancer Institute; NHGRI, the National Human Genome Research Institute; CIN, chromosomal instability; RZZ, ROD–ZW10–Zwilch; *KIFC1*, kinase protein family member C1; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GEO, Gene Expression Omnibus; PPI, protein-protein interaction; ssGSEA, single sample gene set enrichment analysis; ROC, Receiver Operating Characteristic Curve; AUC, area under the ROC curve; GTEx, Genotype-Tissue Expression; OR, Odd Ratio; CI, Confidence interval; ACC, Adrenocortical carcinoma; BLCA, Bladder urothelial carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma; CHOL, Cholangio carcinoma; COAD, Colon adenocarcinoma; DLBC, Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LAML, Acute myeloid leukemia; LGG, Brain lower grade glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin cutaneous melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular germ cell tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine corpus endometrial carcinoma; UCS, Uterine carcinosarcoma; UVN, Uveal melanoma; TPM, Transcripts per million; TPR, True Positive Rate; FPR, False Positive Rate. TNM, Tumor Node Metastasis; IQR, InterQuartile Range; FPKM, Fragments Per Kilobase Million. *KRT15*, Keratin 15; *KRT5*, Keratin 5; *NEFH*, Neurofilament heavy chain; *RRM2*, Ribonucleotide reductase regulatory subunit M2; *CDK1*, Cyclin-dependent kinases 1; *TPX2*, TPX2 microtubule nucleation factor; *KIF20A*, Kinesin family member 20A; *TOP2A*, Topoisomerase (DNA) II Alpha; *BIRC5*, Baculoviral IAP repeat containing 5; *CDC20*, Cell division cycle 20 homologue; *MYBL2*, MYB Proto-Oncogene like 2; *UBE2C*, Ubiquitin conjugating enzyme E2 C; BP, Biological Process; CC, Cell Component; MF, Molecular Function; *KIFC1*, Kinase protein family member C1; *HMMR*, Hyaluronan mediated motility receptor; *BIRC5*, Baculoviral IAP repeat containing 5; *MELK*, Maternal embryonic leucine zipper kinase; *DLGAP5*, Discs large homolog associated protein 5; *HJURP*, Holliday junction Recognition Protein; *CCNA2*, Cyclin A2; *CENPF*, Centromere protein F; *CENPU*, Centromere protein U; *KIF4A*, Kinesin family member 4A; *AOC1*, Amine Oxidase Copper Containing 1; *TNS4*, Tensin 4; *DES*, Desmin; *PCP4*, Purkinje cell protein 4; *KRT17*, Keratin 17; *ASF1B*, Anti-silencing function protein 1 homolog B; *MYL9*, Myosin light chain 9; *CNN1*, Calponin 1; *ACTG2*, Actin gamma 2; *NEK*2, NimA-related protein kinase 2; *MKI67*, Marker of proliferation Ki-67; *ANLN*, Anillin; *PBK*, PDZ binding kinase; *ACTC1*, Actin alpha cardiac muscle 1; *KRT14*, Keratin 14; CD40, Cluster of differentiation 40; TMEM173, Transmembrane protein 173; Tem, Effective Memory T Cell; Th17, T helper 17 cell; CD8, Cluster of differentiation 8; Th1, T helper 1 cell; TFH, T follicular helper cell; Tgd, T gamma delta cell; iDC, Interdigitating cell; DC, Dendritic cell; Tcm, T central memory cell; aDC, Activated dendritic cells; HR, Hazard Ratio; NK, Natural Killer; pDC, plasmacytoid dendritic cell; TH2, helper T cell; PFI, Progress free interval; DFS, Disease-free survival; CRPC, castration-resistant prostate cancer; RRP, radical retropubic prostatectomy; TIME, the tumor immune microenvironment; TME, the tumor microenvironment; IFN, interferon; *STING*, Stimulation of interferon genes; *Hec1*, Highly expressed in Cancer 1.

AVAILABILITY OF DATA AND MATERIALS

The datasets supporting the conclusions of this article are included in this article.

AUTHOR CONTRIBUTIONS

ZLL, XX and JM—Design of the research. ZLL, FXY and YJC—Dataset downloading and analyses. ZLL, XX, FXY and YJC—Manuscript writing. ZLL, XX, FXY, YJC and JM—Manuscript revision. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.jomh.org/files/article/1707275163729313792/attachment/Supplementary%20material.docx.

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