KIF11: A potential prognostic biomarker for predicting bone metastasis-free survival of prostate cancer

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Abstract. Most prostate cancer (PCa) cases remain indolent with a relatively good prognosis. However, bone metastasis of PCa can quickly worsen prognoses and lead to mortality. Metastasis-free survival (MFS), a strong surrogate for overall survival, is widely used in PCa prognosis research. The present study identified molecules that affect bone MFS in PCa, with clinical validation. Three datasets (GSE32269, GSE74367 and GSE77930) were downloaded from the Gene Expression Omnibus database. Hub genes most relevant to clinical traits (bone metastasis-associated morbidity) were identified by weighted gene co-expression network analysis (WGCNA) and subjected to logistic regression analysis. Patient samples were obtained between January 2014 and December 2016, with a clinically annotated follow-up in December 2021. Clinical data and follow-up information for 60 patients with PCa were used in MFS analysis. Tumor samples were retrieved, and immunohistochemistry was performed to detect vascular endothelial growth factor (VEGF). The prognostic potential of the two molecules was assessed using Cox proportional hazards regression analysis. A total of 16 gene modules were obtained via WGCNA, and the tan module, containing 147 genes, was most closely linked to bone metastasis. In total, 877 differentially expressed genes (DEGs) were detected. The DEG-tan module intersection yielded seven hub genes [BUB1, kinesin family member (KIF)2C, RACGAP1, CENPE, KIF11, TTK and KIF20A]. Using univariate and multivariate logistic regression analyses for independent risk factors of bone metastasis, KIF11 and VEGF were found to be significantly associated with a higher T stage, prostate-specific antigen level and Gleason score. In addition, KIF11 and VEGF expression levels were positively correlated (P<0.001). Using univariate Cox analysis, KIF11 and VEGF were found to exhibit a significant association with poor MFS (P<0.05). However, only KIF11 was significantly associated with MFS upon multivariate analysis (P=0.007; hazard ratio, 2.776; 95% confidence interval, 1.315-5.859). Markers of bone metastasis in PCa were identified. Overall, KIF11 is an independent indicator that can predict bone metastasis for patients with PCa, which could be used to guide clinical practice.

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Introduction

Prostate cancer (PCa) is the most frequently diagnosed cancer type in 105 countries (with a total of 36 cancer types identified in 185 countries) and the most common cause of death from malignancy among men (1). Based on data obtained between 2010 and 2014, the number of new cases of PCa was 119.8 per 100,000 men per year, and the number of deaths was 20.1 per 100,000 men per year in the United States of America (2,3). Most PCa cases are indolent, with a low risk of lethality. Death from PCa is mainly caused by metastasis when cancer cells spread to other areas of the body, such as the pelvic and retroperitoneal lymph nodes, the bladder, bones and the brain. Bone metastasis is the most serious type of metastatic PCa (4), and it is a hallmark of progressive and castration-resistant PCa. Bone metastasis develops in multiple stages, including colonization (entrance of circulating cancer cells to the bone marrow compartment), dormancy (adaption of cancer cells to the bone microenvironment and a long dormancy period), reactivation and development, (change of cancer cells from dormant to actively proliferating) and reconstruction (change of the original bone structure and function by the cancer cells) (5). Following bone metastasis, the malignant proliferation rate of cancer cells is appreciably accelerated (6). Patients with bone metastasis have lost their best opportunity for surgery, and subsequently, androgen deprivation therapy (ADT) is applied as the next most common and effective treatment for metastatic PCa (7). However, metastatic PCa eventually develops resistance to castration, with few remaining therapeutic options, and ultimately results in a poor patient prognosis (8,9). Therefore, exploration of the underlying mechanism of bone metastasis in PCa and identification of novel therapeutic targets to address these conditions are urgently needed.

Metastasis-free survival (MFS) is a new index that has been demonstrated as a useful surrogate for overall survival (OS) (10). In recent years, MFS has gradually replaced OS to evaluate the benefits of ADT in patients with primary PCa. OS is the traditional primary efficacy endpoint in clinical studies

of advanced PCa; however, owing to the indolent nature of PCa, adjuvant clinical trials in PCa take more than a decade to reach the irrefutable endpoint of OS (11). In addition, once tumor metastasis occurs, the quality of life and outcome of patients decreases significantly; therefore, it is more reasonable to develop a meaningful treatment plan by assessing metastatic events. Thus, MFS may be a useful indicator of prognoses in PCa research. MFS was defined in clinical trials as the time from enrollment in the study or from treatment initiation until the detection of generalized disease manifesting with the development of distant metastases, or until the patient's death; non-PCa deaths were not counted as an event (12).

Kinesin family member 11 (KIF11), a molecular motor protein essential in mitosis, promotes bipolar spindle formation and chromosome movement during mitosis, and mediates diverse trafficking processes in the cytoplasm during interphase (13). Reports have indicated that KIF11 is overexpressed in various malignancies and is correlated with poor prognoses. Li *et al* (14) showed that migration and invasion abilities decreased after inhibiting KIF11 in breast cancer. The KIF11 inhibitor also significantly reduced the tumor volume. In addition, Daigo *et al* (15) reported that a high level of KIF11 expression is significantly associated with poor prognoses in patients with oral cancer. Piao *et al* (16) showed that the KIF11 expression may be indicative of PCa aggressiveness.

Vascular endothelial growth factor (VEGF) is a cytokine that plays a key role in angiogenesis and is essential in the formation of various solid tumors (17). VEGF is critical for tumor growth. Agents targeting VEGF, including VEGF antibodies, bevacizumab and VEGF receptor tyrosine kinase inhibitors, are gradually being incorporated into clinical cancer therapies, leading to major advances in the treatment of various tumors, such as metastatic breast cancer, non-small cell lung cancer, glioblastoma, renal cell carcinoma, ovarian cancer and cervical cancer (18,19). Angiogenesis is essential in PCa development and metastasis. Moreover, VEGF has already been associated with metastasis and angiogenesis in PCa (20). High VEGF expression levels predict a strong invasive and metastatic capacity of PCa.

A combination of statistical and bioinformatic methods was used in the present study to identify genes that promote bone metastasis in PCa. Bioinformatic analysis represents the application of information technology and computer science in the field of molecular biology, and is widely used in functional analyses of DNA, RNA and proteins; these analysis results are vital for guiding clinical work. KIF11 was identified as an independent risk factor for bone metastasis in PCa in the present study. The study evaluated the correlation between KIF11 and VEGF expression in PCa tissue samples, and investigated the influence of KIF11 expression on MFS in patients with PCa.

Materials and methods

Bioinformatics analysis

Gene profile download and processing. The raw expression profile was downloaded from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) to compare gene expression levels between PCa bone metastasis tissues and primary PCa tissues. The gene expression datasets GSE32269 (21), GSE74367 (22) and GSE77930 (23) were downloaded based on the GPL147, GPL15659 and GPL21289 platforms, respectively. Clinical information on the corresponding samples was available.

A robust multi-array average was used to correct and normalize the raw expression data for each dataset. The three datasets were then merged using the Perl programming language (https://www.perl.org/). The 'SVA' package of R software (http://www.r-project.org/) was performed to eliminate batch effects and other unrelated variables in high-throughput experiments.

Co-expression network construction. The weighted gene co-expression network analysis (WGCNA) R package (https://cran.r-project.org/web/packages/WGCNA/index.html) was used to construct the gene co-expression network in the datasets. The soft-thresholding power β was calculated during the construction of each module using the pickSoftThreshold function of the WGCNA. The power value was screened using a gradient algorithm to test the independence, and the power values of the different modules ranged from 1 to 20. Gene modules were constructed after determining a suitable power value when the index value for the reference dataset exceeded 0.8. A minimum number of 30 was set for each module, and the heatmap tool package was used to analyze and visualize the strength of the correlation between each module. A cut line (0.25) was chosen to generate a dendrogram plot.

Construction of module-clinic trait relationships. Modules from the WGCNA were identified based on gene expression similarities in the samples. The relationship between clinical traits (occurrence or absence of bone metastasis of PCa) and each module was calculated to acquire the module of interest. The gene module most significantly correlated with 'type,' namely the presence or absence of bone metastasis in PCa, was retained for the next step.

Functional enrichment analysis. Metascape (http://metascape.org) is a free, thoroughly maintained and user-friendly gene list analysis tool for gene annotation and analysis. Specifically, it is an automated meta-analysis tool used to understand common and unique pathways within a group of orthogonal target discovery studies. In the present study, Metascape was used to conduct pathway and process enrichment analyses of the gene module most relevant to the clinical traits screened out in WGCNA. Gene Ontology (GO; http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.kegg.jp/) enrichment analysis mainly described the biological processes, cellular components and molecular functions associated with the module genes. Information on the role and function of modular genes was enriched using Metascape. Terms with $P<0.05$, a minimum count of 3, and an enrichment factor of >1.5 were considered significant.

Screening of differentially expressed genes. The differentially expressed genes (DEGs) of bone metastasis in PCa and primary PCa tissues were detected using the 'Limma' package of R software (https://cran.r-project.org/src/contrib/Archive/limma/), with cut-off criteria of $P<0.05$ and absolute $|logFC|>1$. The DEGs and the module of interest from WGCNA were then overlapped to obtain the common DEGs.

Table I. Prostate cancer bone metastasis microarray datasets from different Gene Expression Omnibus datasets.

Series	Platform	Primary samples, n	Bone metastasis samples, n	Total, n
GSE32269	GPL96	22	29	51
GSE74367	GPL15659	11	7	18
GSE77930	GPL21289	13	13	26
Total		46	49	95

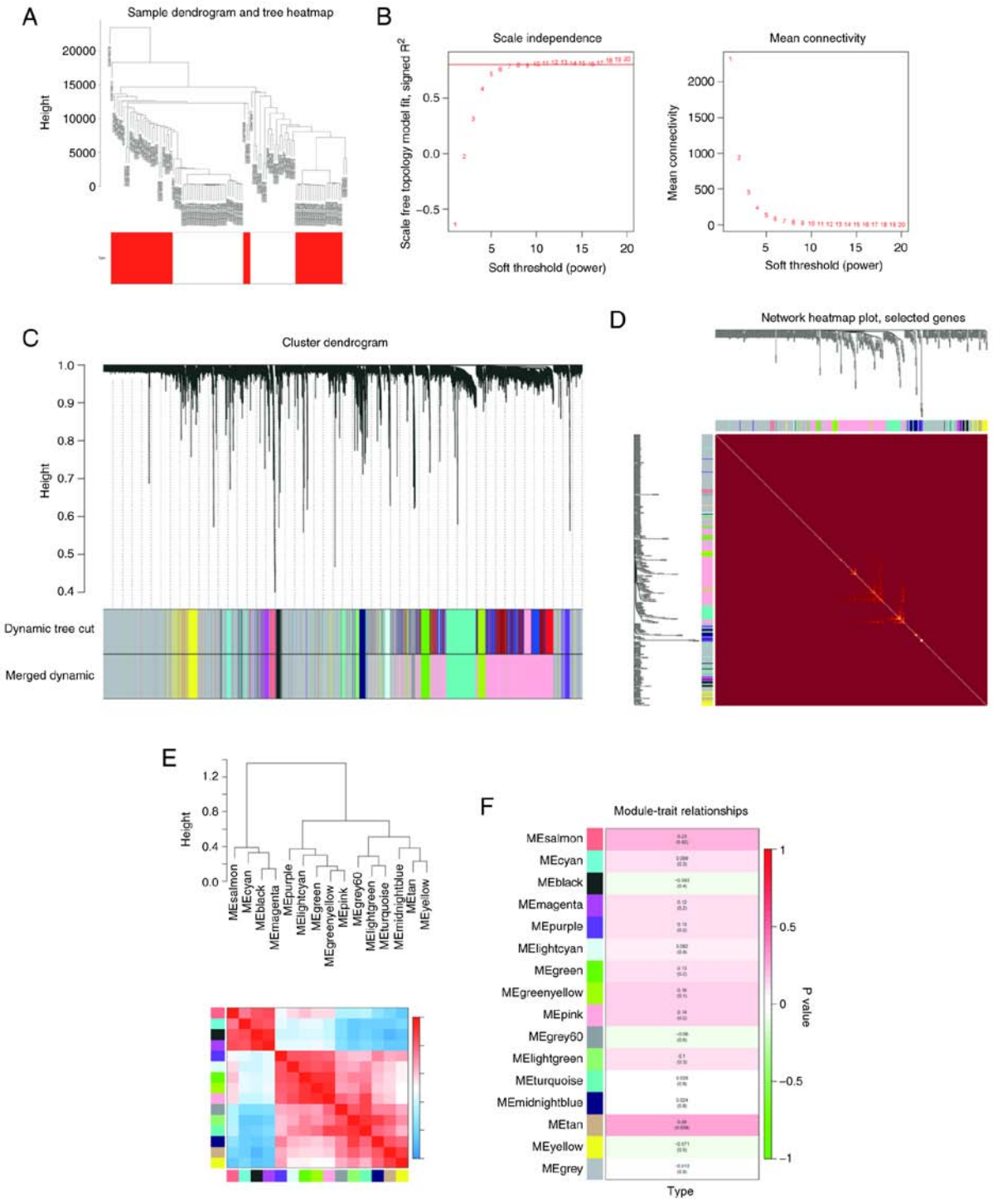

Figure 1. Weighted gene co-expression network analysis of the genes in the merged series. (A) Cluster of patients with clinical information; the red bar represents patients with prostate cancer bone metastases. (B) The lowest power of scale independence. (C) Repeated hierarchical clustering tree of the 23,492 genes. (D) Dendrogram and heatmap of genes. (E) Interactions between these modules. (F) Associations between clinical traits and the modules; the tan module was most relevant to clinical traits.

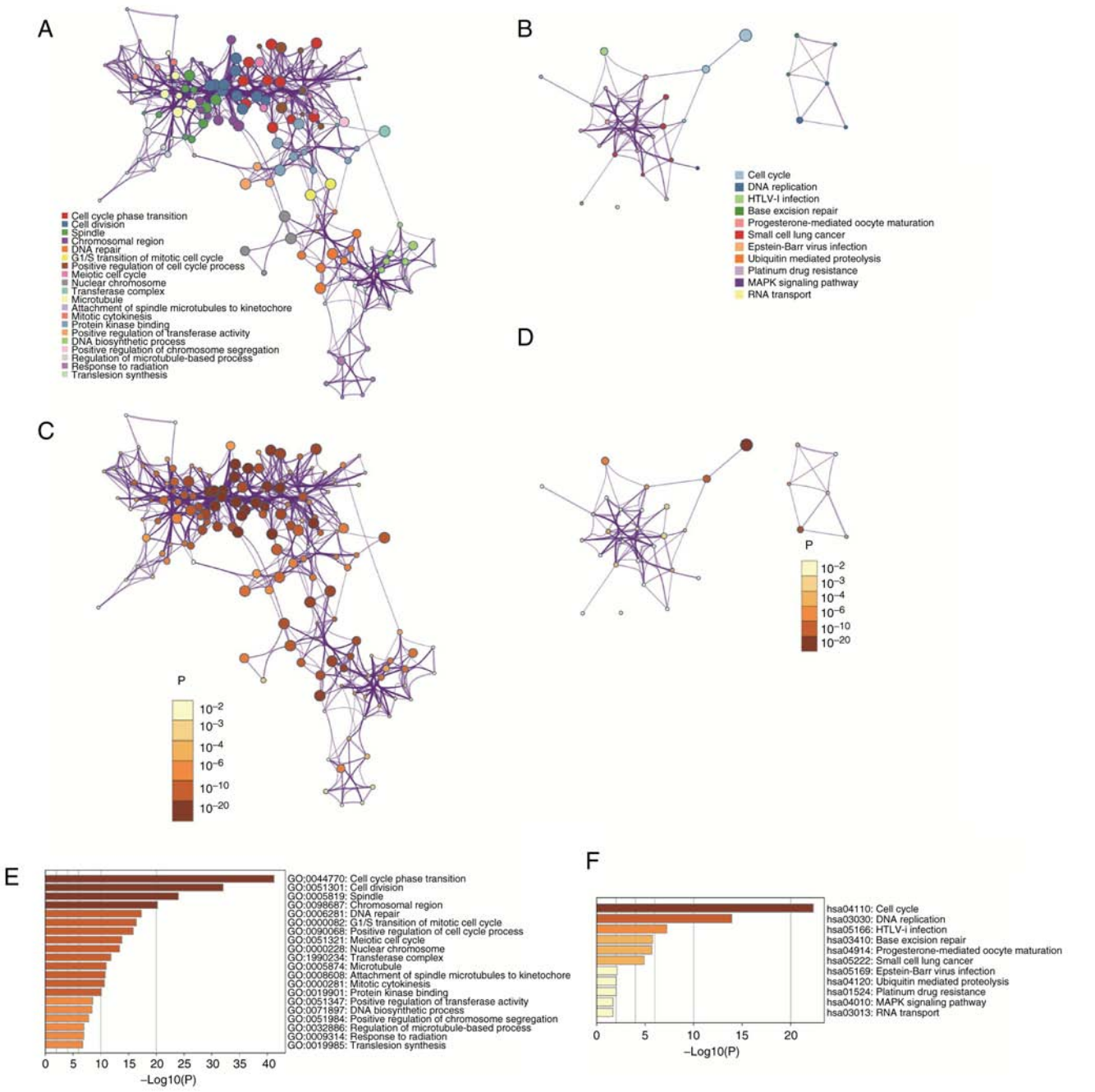

Figure 2. Functional enrichment analysis. (A-F) Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis of the tan module using Metascape.

Construction of protein-protein interaction (PPI) and selection of hub genes. A PPI network was constructed using the STRING online database (http://string-db.org) and imported into the Cytoscape software (https://cytoscape.org/) for visualization and subsequent analysis. Four algorithms were used (betweenness, closeness, Eccentricity and Radiality) in Cytoscape to calculate the top 10 hub genes. A Venn plot was constructed to identify common hub genes. The Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer.pku.cn/) database was used to analyze disease-free survival (DFS) time between samples with high and low expression of the hub genes. The Comparative Toxicogenomics Database (CTD; http://ctdbase.org/) is a web-based database that provides information on the relationship between genes, proteins and diseases; the relationships between gene products and malignancy were analyzed using this database.

Clinical samples and ethics statement. Inclusion criteria: i) Patients diagnosed with PCa; ii) those who refused or were unable to tolerate surgical management; and iii) those who accepted a prostate needle biopsy. Exclusion criteria: i) A history of other malignant tumors; ii) incomplete clinical data; iii) distant metastasis at diagnosis; and iv) a life expectancy of >5 years. A total of 60 patients with primary PCa who visited the Fourth Affiliated Hospital of Hebei Medical University (Shijiazhuang, China) between January 2014 and December 2016 were involved in this study, 45 of whom eventually developed bone metastasis. All patients underwent prostate biopsy

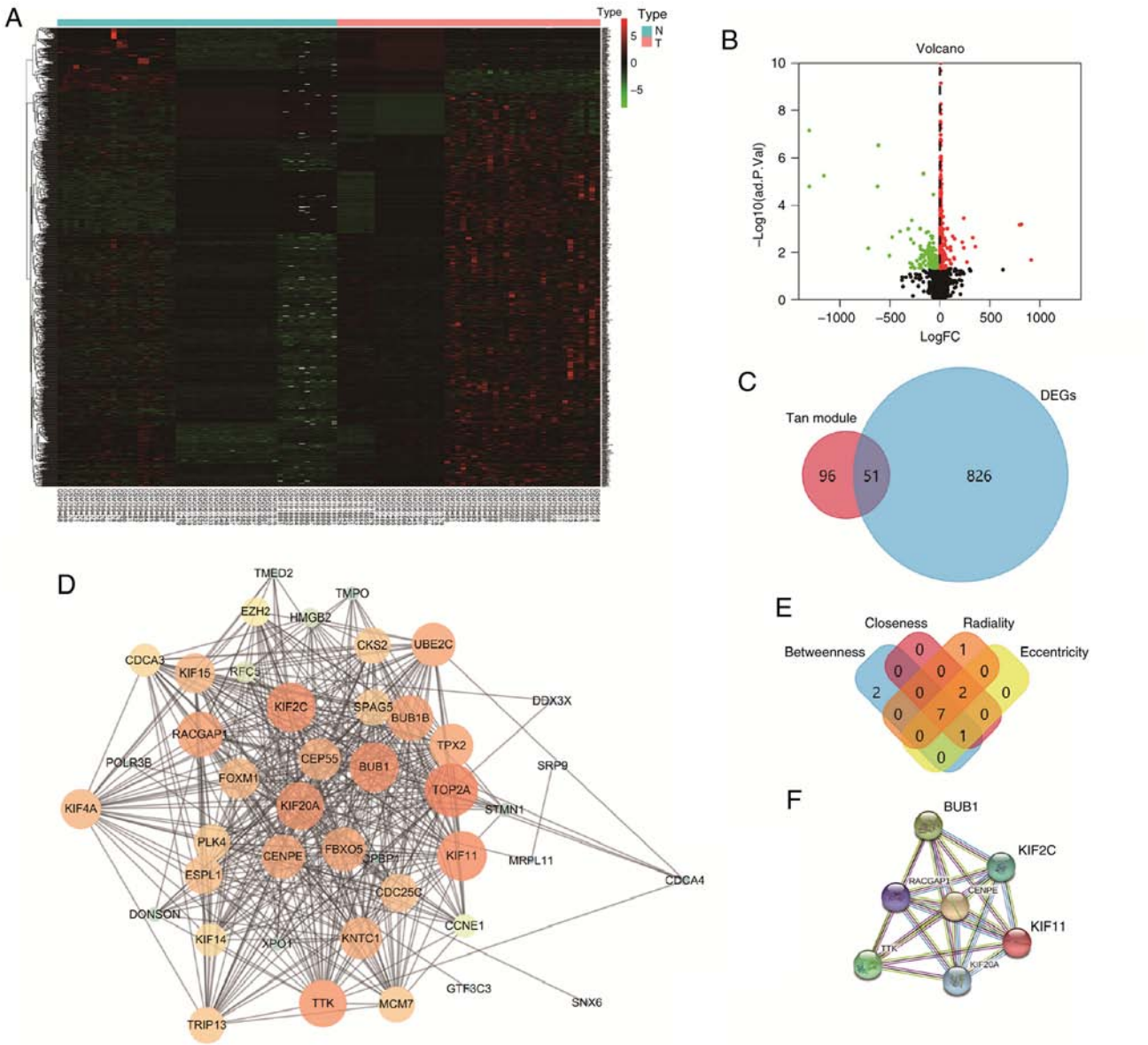

Figure 3. Differential expression analysis of the genes in the merged series. (A) Heatmap of the 877 DEGs in the tan model. (B) Volcano plot of DEGs between the PCa bone metastasis group and primary PCa group. (C) Venn plot of DEGs and tan model genes. (D) PPI network complex for the common DEGs. (E) Common hub genes identified from different algorithms. (F) Common hub genes of the PPI network. DEG, differentially expressed gene; PCA, prostate cancer; PPI, protein-protein interaction; N, primary prostate cancer sample; T, bone metastasis prostate cancer sample.

and ADT. Tissues from PCa biopsies were collected from all patients. Tumor biopsy tissues were fixed in 10% formalin buffer for 24 h at 25˚C, paraffin-embedded and then sectioned to a 5-μm thickness. Tumor biopsy tissues was used to perform H&E and immunohistochemistry. The detailed experimental procedures for H&E staining were as described previously (24). Participants were followed through a time horizon of 5 years. The demographic and clinical characteristics [age, primary tumor size, T stage, prostate-specific antigen (PSA) level and Gleason score (25)], follow-up time and survival information of each patient were collected retrospectively. The staging standard referred to the AJCC Cancer Staging Manual (8th Edition) (26). Furthermore, the patients were not treated with radiochemotherapy.

Ethical approval was obtained from the Ethics Committee of the Fourth Affiliated Hospital of Hebei Medical University (approval no. 2022KY066). Written informed consent was obtained from all participants in this study. All experiments were performed in accordance with the Declaration of Helsinki.

Immunohistochemistry. For immunohistochemistry, biopsy tissues sections were blocked with 3% hydrogen peroxide at room temperature for 10 min. Primary antibody incubation was performed overnight at 4˚C using rabbit KIF11 (1:4,000; catalog no. ab254298; Abcam) and rabbit VEGF (1:100; catalog no. ab52917; Abcam) antibodies. Secondary antibody incubation was performed for 1 h using using goat anti-rabbit IgG antibodies (1:100; catalog no. ab150077; Abcam). Finally, the staining was independently evaluated using a high-resolution optical scanner, Nanozoomer 2.0 HT, by three pathologists blinded to all the data. If differences

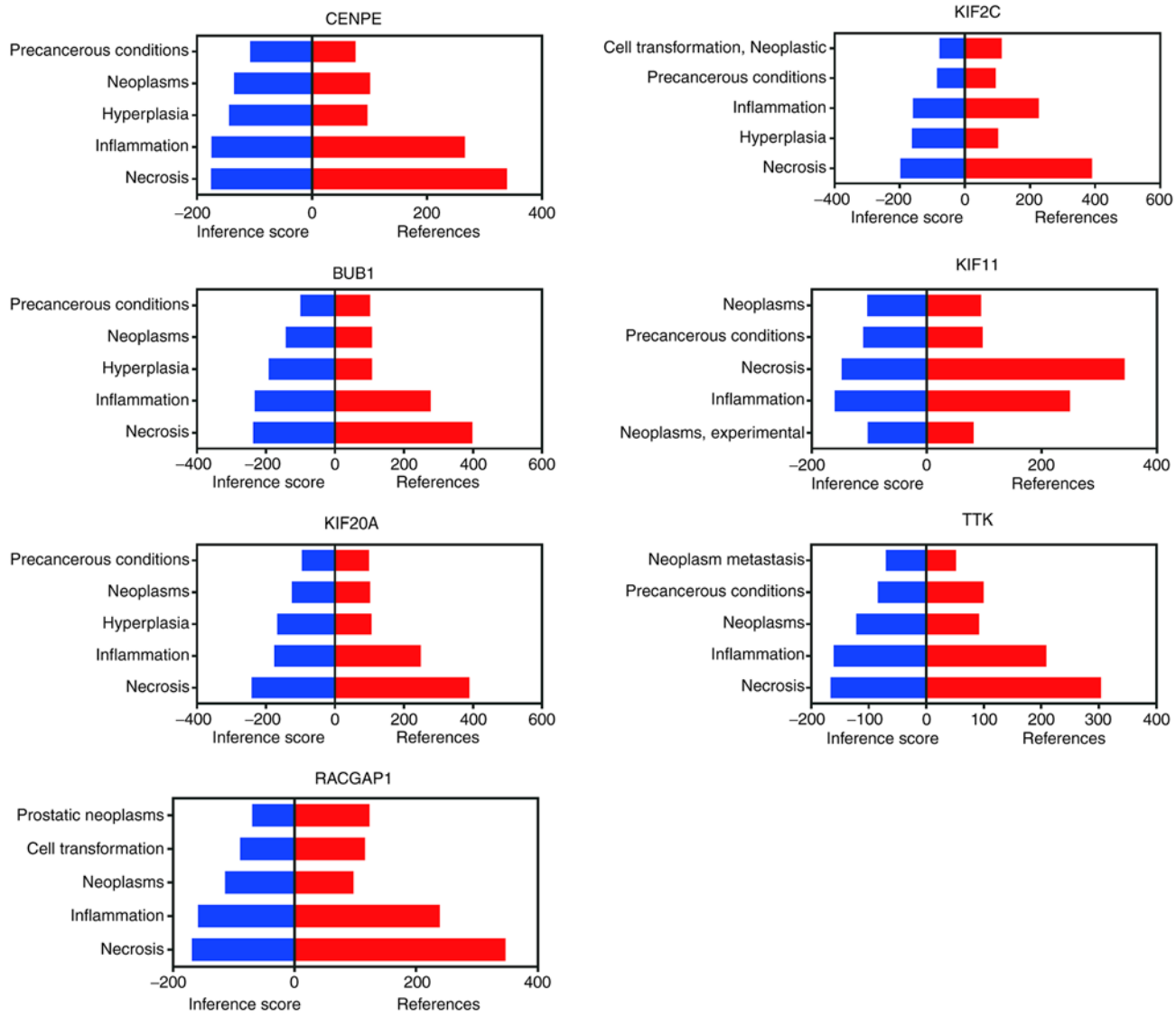

Figure 4. Associations between prostate cancer bone metastasis and common hub genes based on the Comparative Toxicogenomics Database.

were observed, disagreements were settled through discussion. KIF11 and VEGF were scored based on the staining intensity of brown-colored diaminobenzidine (DAB) as follows: Score of 0, <1%; score of 1, 1-25%; score of 2, 25-50%; score of 3, 51-80%; and score of 4, >80%. The intensity of staining was recorded as follows: Grade 0, negative; grade 1, buff; grade 2, brownish-yellow; and grade 3, tan. The product of the staining percentage and intensity grade was used to evaluate the final immunostaining score, and the results were defined as low (score 0-3), moderate (score 4-7) or high (score >7).

Statistical analysis. To determine independent risk factors for PCa bone metastasis, univariate and multivariate logistic proportional hazard regression analyses were conducted for hub genes using bioinformatic analysis. The occurrence of a bone metastasis event or the last follow-up date through to December 2020 was defined as the endpoint. Metastasis-free survival (MFS) was defined as the time from diagnosis to bone metastasis or last follow-up. Correlation analysis was performed using Spearman's ρ test. Cox's proportional hazards regression was used as the univariate and multivariate analysis methodology. The Kaplan-Meier curve method was used to evaluate MFS using the log-rank test. All statistical analyses were performed using SPSS 25.0 (IBM Corp.). $P<0.05$ was used to indicate a statistically significant difference.

Results

Bioinformatic analysis

GEO dataset. The three datasets (GSE32269, GSE74367 and GSE77930) downloaded from the GEO database included 49 PCa samples with bone metastasis and 46 primary PCa samples in total (Table I).

WGCNA construction. All the samples were included in the analysis. The results of the hierarchical clustering analysis showed that there were no obvious outliers, and all 95 samples were included in the co-expression network analysis. A total of 16 corresponding modules were determined (Fig. 1A-E). Subsequently, clinical information (occurrence of PCa bone metastasis) was imported, and the correlation coefficient between each module and PCa bone metastasis was calculated

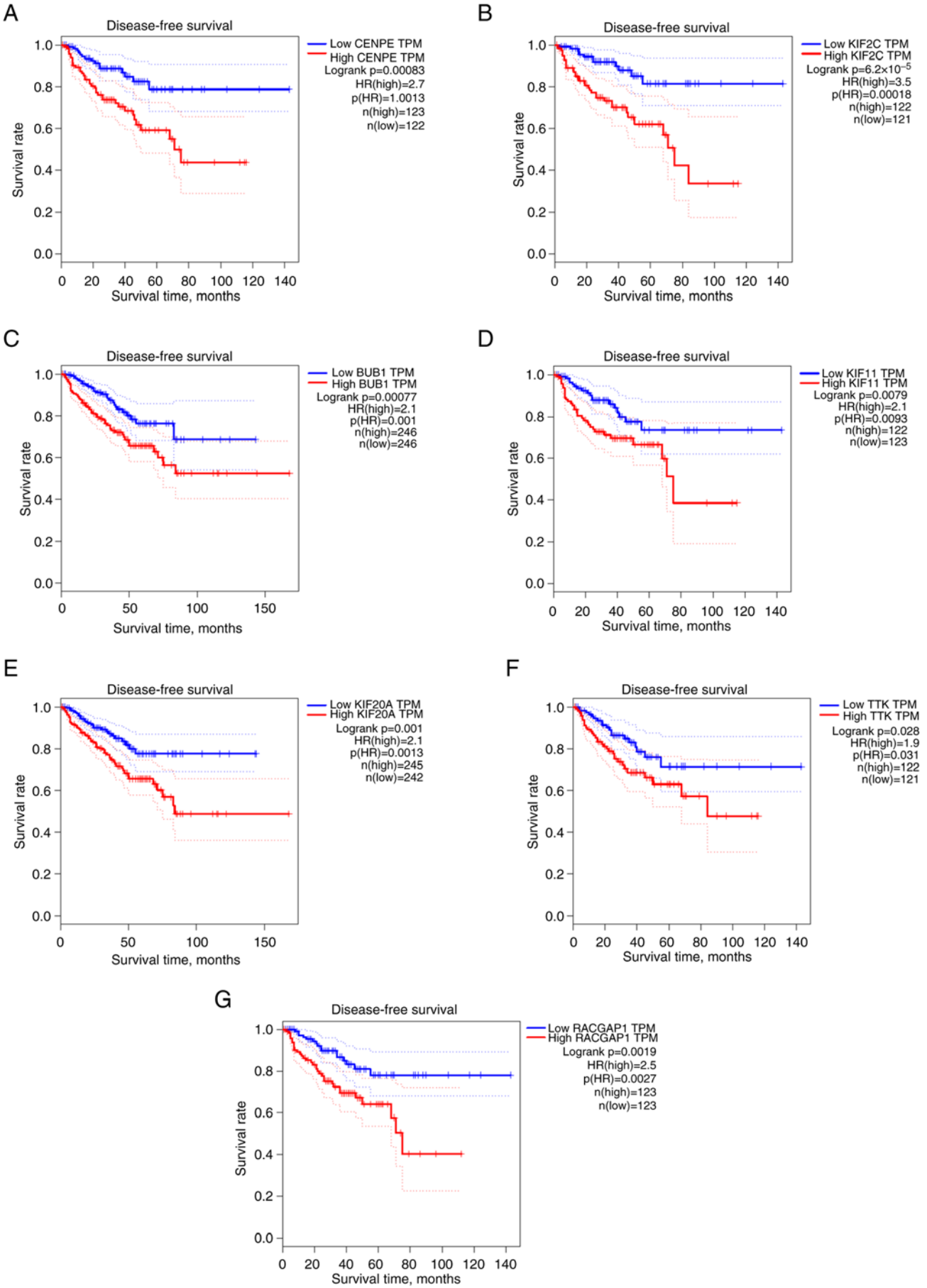

Figure 5. DFS analysis, based on Gene Expression Profiling Interactive Analysis, for the hub genes. (A-G) DFS analysis of seven hub genes (BUB1, KIF2C, RACGAP1, CENPE, KIF11, TTK and KIF20A). KIF11, kinesin family member 11; DFS, disease-free survival; HR, hazard ratio; TPM, transcripts per kilobase million.

Table II. Univariate and multivariate logistic proportional regression analysis to assess the association between hub genes and bone metastasis of prostate cancer.

	Univariate			Multivariate		
Gene	HR	95% CI	P-value	HR	95% CI	P-value
KIF11	2.331	1.049-5.178	0.038	2.331	1.049-5.178	0.038
CENPE	1.681	0.765-3.694	0.196	-	-	-
KIF2C	0.758	0.347-1.658	0.488	-	-	-
BUB1	1.221	0.515-2.456	0.767	-	-	-
KIF20A	0.550	0.250-1.211	0.138	-	-	-
TTK	1.221	0.559-2.667	0.617	-	-	-
RACGAP1	0.646	0.295-1.417	0.276	-	-	-

HR, hazard ratio; CI, confidence interval; KIF11, kinesin family member 11.

Table III. Clinicopathological variables and the expression of KIF11 and VEGF.

	Total patients,	KIF11, n (%)				VEGF, n (%)			
Variable	n	-/+	++	+++	P-value	-/+	++	+++	P-value
Age, years					0.617				0.126
≥65	32	9 (28.1)	11 (34.4)	12 (37.5)		10 (31.2)	6 (18.8)	16 (50.0)	
<65	28	6 (21.4)	8 (28.6)	14 (50.0)		6 (21.4)	12 (42.9)	10 (35.7)	
Primary tumor size, cm					0.633				0.493
<2	33	7 (21.2)	10 (30.3)	16 (48.5)		8 (24.2)	12 (36.4)	13 (39.4)	
≥2	27	8 (29.6)	9 (33.3)	10 (37.0)		8 (29.6)	6 (22.2)	13 (48.1)	
T stage					0.001[a]				0.002[a]
T1/T2	17	9 (52.9)	2 (11.8)	6 (35.3)		10 (58.8)	4 (23.5)	3 (17.6)	
T3	28	5 (17.9)	14 (50.0)	9 (32.1)		6 (21.4)	10 (35.7)	12 (42.9)	
T4	15	1 (6.7)	3 (20.0)	11 (73.3)		0 (0.00)	4 (26.7)	11 (73.3)	
PSA, ng/ml					0.015[a]				0.044[a]
<20	37	13 (35.1)	13 (35.1)	11 (29.7)		14 (37.8)	9 (24.3)	14 (37.8)	
≥20	23	2 (8.7)	6 (26.1)	15 (65.2)		2 (8.7)	9 (39.1)	12 (52.2)	
Gleason score					<0.001[a]				0.001[a]
≤6	9	8 (88.9)	1 (11.1)	0 (00.0)		7 (77.8)	2 (22.2)	0 (00.0)	
7 (3+4)	20	7 (35.0)	10 (50.0)	3 (15.0)		7 (35.0)	7 (35.0)	6 (30.0)	
7 (4+3)	15	0 (00.0)	5 (33.3)	10 (66.6)		1 (6.7)	5 (33.3)	9 (60.0)	
≥8	16	0 (00.0)	3 (18.8)	13 (81.3)		1 (6.3)	4 (25.0)	11 (68.8)	

Pearson's χ^2 test was used. [a]P<0.05. KIF11, kinesin family member 11; VEGF, vascular endothelial growth factor; PSA, prostate-specific antigen.

(Fig. 1F). The results showed that the tan module was most closely associated with PCa bone metastasis (ρ=0.26; P=0.008), including 147 genes. Therefore, the tan module was selected for further analyses.

Functional enrichment analysis. GO and KEGG function enrichment analyses were used to explore the potential functions and pathways of the genes in the tan module of Metascape. The GO analysis results showed that the genes in the tan module mainly regulated (top 5 terms) 'cell cycle phase transition', 'cell division', 'spindle', 'chromosomal regions' and 'DNA repair'. KEGG pathway analysis showed enrichment in the 'cell cycle', 'DNA replication', 'HTLV-I infection', 'base excision repair' and 'progesterone-mediated oocyte maturation' (Fig. 2A-F).

Selection of DEGs. All 49 PCa samples with bone metastasis and 46 primary PCa samples were included in a differential expression analysis. A total of 877 DEGs were screened, and volcano plots and heat maps were subsequently created

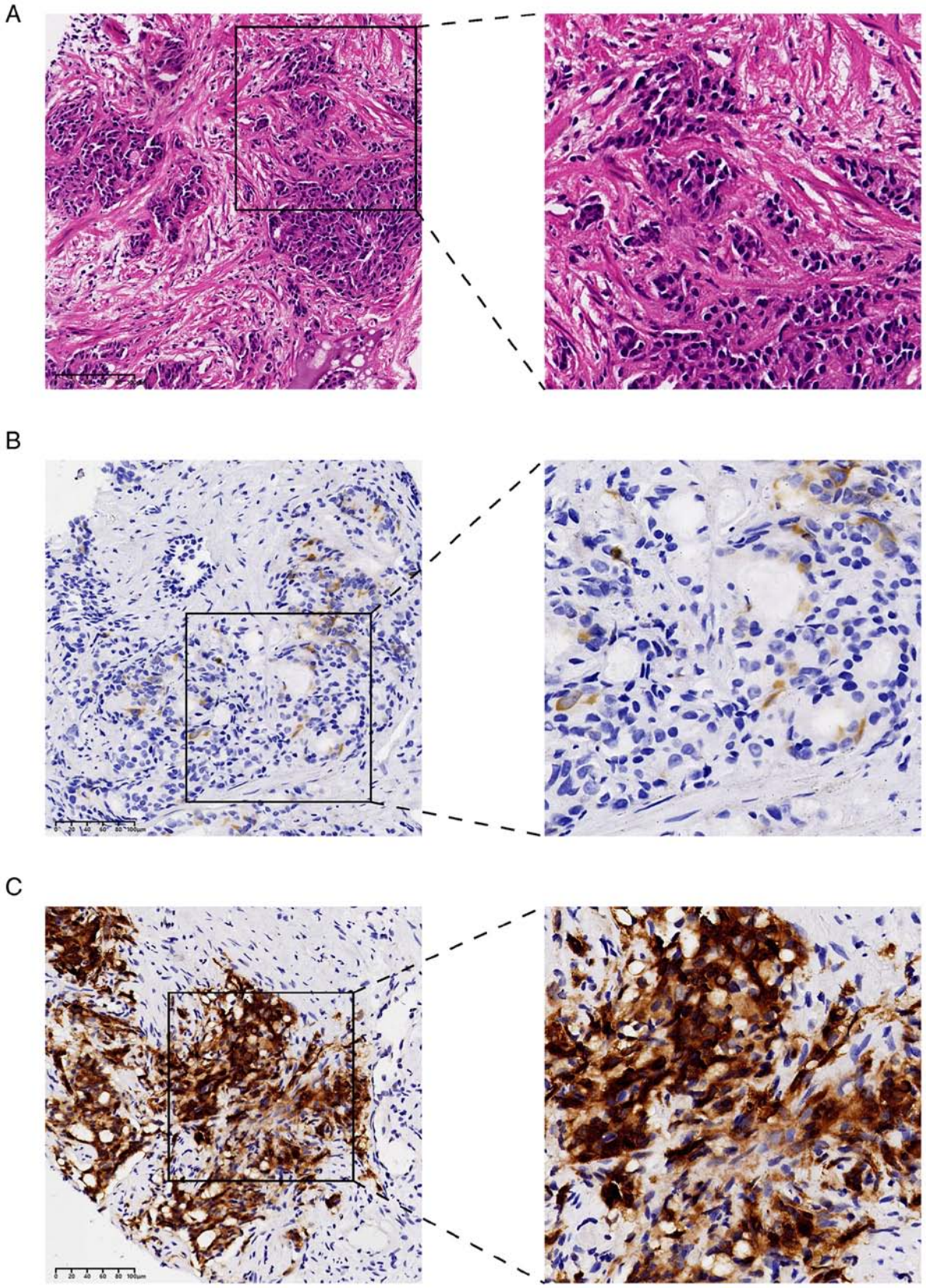

Figure 6. Immunohistochemical staining for KIF11 in PCa bone metastasis and non-metastatic tissues. (A) Hematoxylin and eosin staining of PCa specimens. (B) KIF11 expression in PCa bone metastasis tissue. (C) KIF11 expression in non-metastatic PCa tissue. Magnification, x200 (left) and x400 (right). PCA, prostate cancer; KIF11, kinesin family member 11.

(Fig. 3A and B). A Venn plot was constructed showing the overlap of 51 common genes between the DEGs and tan model genes (Fig. 3C).

Construction of PPIs and selection of hub genes. A PPI network was constructed for the 51 common genes (Fig. 3D). Four different algorithms (betweenness, closeness, eccentricity and

Table IV. Correlation between KIF11 and VEGF expression.

Characteristics	Total patients, n	KIF11			P-value
		-/+, n (%)	++, n (%)	+++, n (%)	
VEGF					<0.001[a]
-/+	16	12 (20.0)	3 (5.0)	1 (1.7)	
++	18	2 (3.3)	8 (13.3)	8 (13.3)	
+++	26	1 (1.7)	8 (13.3)	17 (28.3)	
Total	60	15	19	26	

Spearman's ρ test was used. [a]P<0.05. KIF11, kinesin family member 11; VEGF, vascular endothelial growth factor.

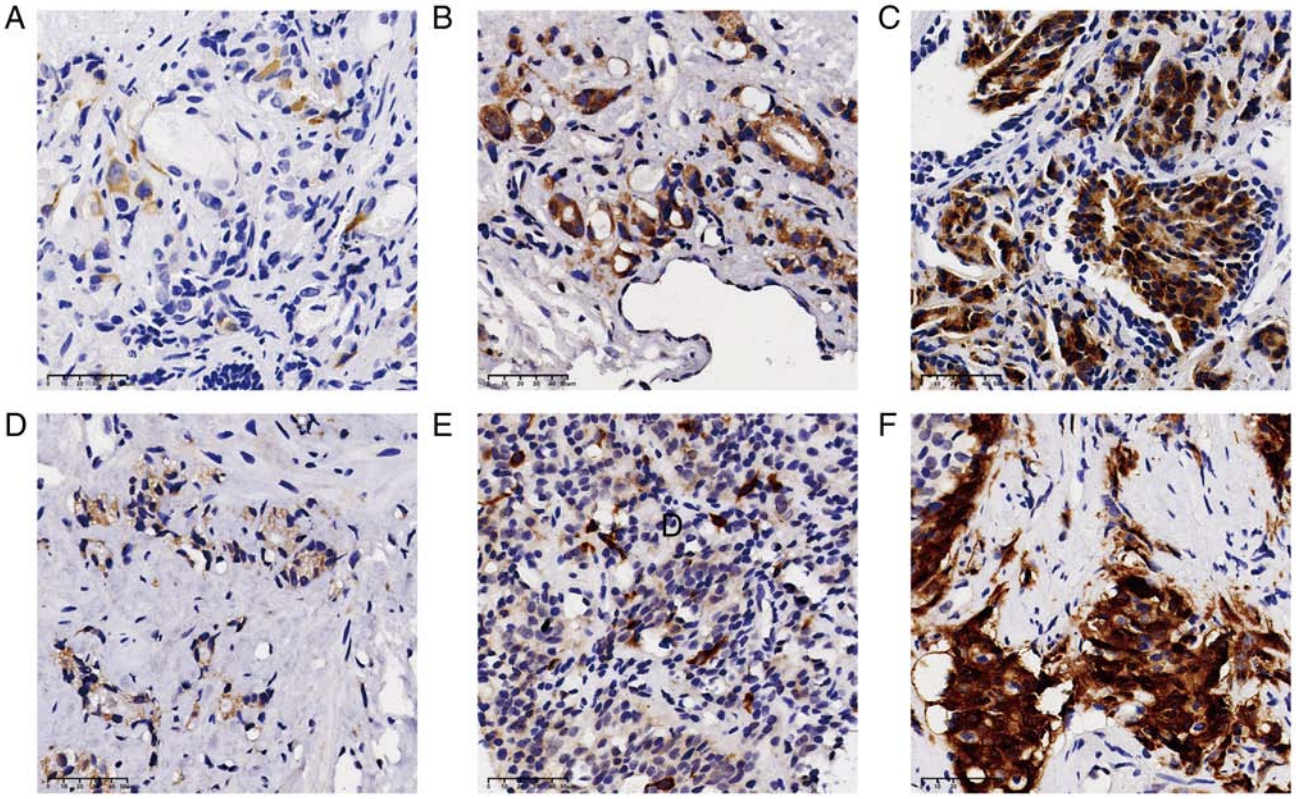

Figure 7. Association between KIF11 and VEGF expression based on immunohistochemical staining. (A-C) Low, moderate and high expression of KIF11. (D-F) Low, moderate and high expression of VEGF. Magnification, x400. VEGF, vascular endothelial growth factor; KIF11, kinesin family member 11.

radiality) were used to calculate hub genes, and the common hub genes of the four different algorithms were obtained (Fig. 3E). Ultimately, seven hub genes (BUB1, KIF2C, RACGAP1, CENPE, KIF11, TTK and KIF20A) were identified (Fig. 3F). The results of the CTD analysis showed that all seven hub genes had a strong correlation with necrosis-related biological process (Fig. 4). The GEPIA website was used to analyze the DFS rates of the seven hub genes. Results from GEPIA showed that all seven genes, including CENPE (log rank P=0.00083; HR, 2.7), KIF2C (log rank P=6.2x10^{-5}; HR, 3.5), BUB1 (log rank P=0.00077; HR, 2.1), KIF11 (log rank P=0.0079; HR, 2.1), KIF20A (log rank P=0.001; HR, 2.1), TTK (log rank P=0.028; HR, 1.9) and RACGAP1 (log rank P=0.0019; HR, 2.5), were significantly associated with DFS (Fig. 5).

Logistics regression analysis. Univariate and multivariate logistic proportional hazard regression analyses were conducted for the seven hub genes (Table II). The results of multivariate regression indicated that only KIF11 (P=0.038; HR, 2.331; 95% CI, 1.049-5.178) represented an independent factor that significantly influenced bone metastasis in PCa.

Expression of KIF11 and VEGF in clinical samples. A summary of the associations between the two proteins (KIF11 and VEGF) and clinicopathological characteristics from the 60 clinical samples is shown in Table III. The expression of KIF11 was low in 15 (25.0%), moderate in 19 (31.7%) and high in 26 (43.3%) patients, whereas the expression of VEGF was low in 16 (26.7%), moderate in 18 (30.0%) and high in

Table V. Univariate Cox proportional regression analysis of clinicopathological factors associated with MFS.

Characteristics	Total patients, n	MFS		
		HR	95% CI	P-value
Age, years				
≥65	32	1.000	-	-
<65	28	1.312	0.725-2.376	0.370
Primary tumor size, cm				
<2	33	1.000	-	-
≥2	27	0.859	0.467-1.581	0.625
T stage				
T1/T2	17	1.000	-	0.001
T3	28	1.613	0.766-3.397	0.208
T4	15	5.217	2.115-12.867	<0.001
PSA, ng/ml[a]				
≤20	37	1.000	-	
≥20	23	2.171	1.174-4.015	0.013
Gleason score[a]				
≤6	9	1.000	-	<0.001
7 (3+4)	20	4.682	1.262-17.378	0.021
7 (4+3)	15	35.443	7.514-167.186	<0.001
≥8	16	30.689	6.940-135.713	<0.001
KIF11[a]				
Low (-/+)	15	1.000	-	<0.001
Moderate (++)	19	3.125	1.175-8.311	0.022
High (+++)	26	16.468	5.618-48.276	<0.001
VEGF[a]				
Low (-/+)	16	1.000	-	0.001
Moderate (++)	18	3.527	1.464-8.496	0.005
High (+++)	26	5.127	2.160-12.167	<0.001

[a]P<0.05. MFS, metastasis-free survival; HR, hazard ratio; CI, confidence interval; KIF11, kinesin family member 11; VEGF, vascular endothelial growth factor; PSA, prostate-specific antigen.

Table VI. Multivariate Cox regression analysis of clinicopathological factors associated with MFS.

Factors	MFS		
	HR	95% CI	P-value
PSA	0.770	0.362-1.637	0.496
VEGF	0.918	0.548-1.536	0.744
T stage	1.665	1.016-2.729	0.043
Gleason score	1.734	1.108-2.714	0.016
KIF11	2.776	1.315-5.859	0.007

MFS, metastasis-free survival; HR, hazard ratio; CI, confidence interval; KIF11, kinesin family member 11; VEGF, vascular endothelial growth factor; PSA, prostate-specific antigen.

26 (43.3%) patients. The expression of KIF11 was significantly higher in bone metastatic PCa tissue than in non-metastatic tissue (Fig. 6). Moreover, both proteins (KIF11 and VEGF) were significantly associated with the T stage (P=0.001 and P=0.002, respectively), PSA (P=0.015 and P=0.044, respectively) and Gleason score (P<0.001 and P=0.001, respectively) (Table III). However, there were no statistically significant differences between KIF11 and VEGF for other clinicopathological features. The results also indicated that the expression of KIF11 was correlated with the expression of VEGF (P<0.001) (Table IV; Fig. 7).

High expression of KIF11 and VEGF leads to poor MFS in patients with PCa. The association between the expression of the two proteins (KIF11 and VEGF) and the MFS of patients with PCa was examined using a univariate Cox analysis. The results showed that the high T stage (P=0.001), high PSA level (P=0.013) and high Gleason score (P<0.001) were significant prognostic factors for poor MFS for patients with PCa. Furthermore, the univariate Cox regression analysis indicated that upregulated KIF11 (moderate P=0.022 and High P<0.001, compared with low expression) and VEGF (moderate P=0.005 and high P<0.001, compared with low expression)

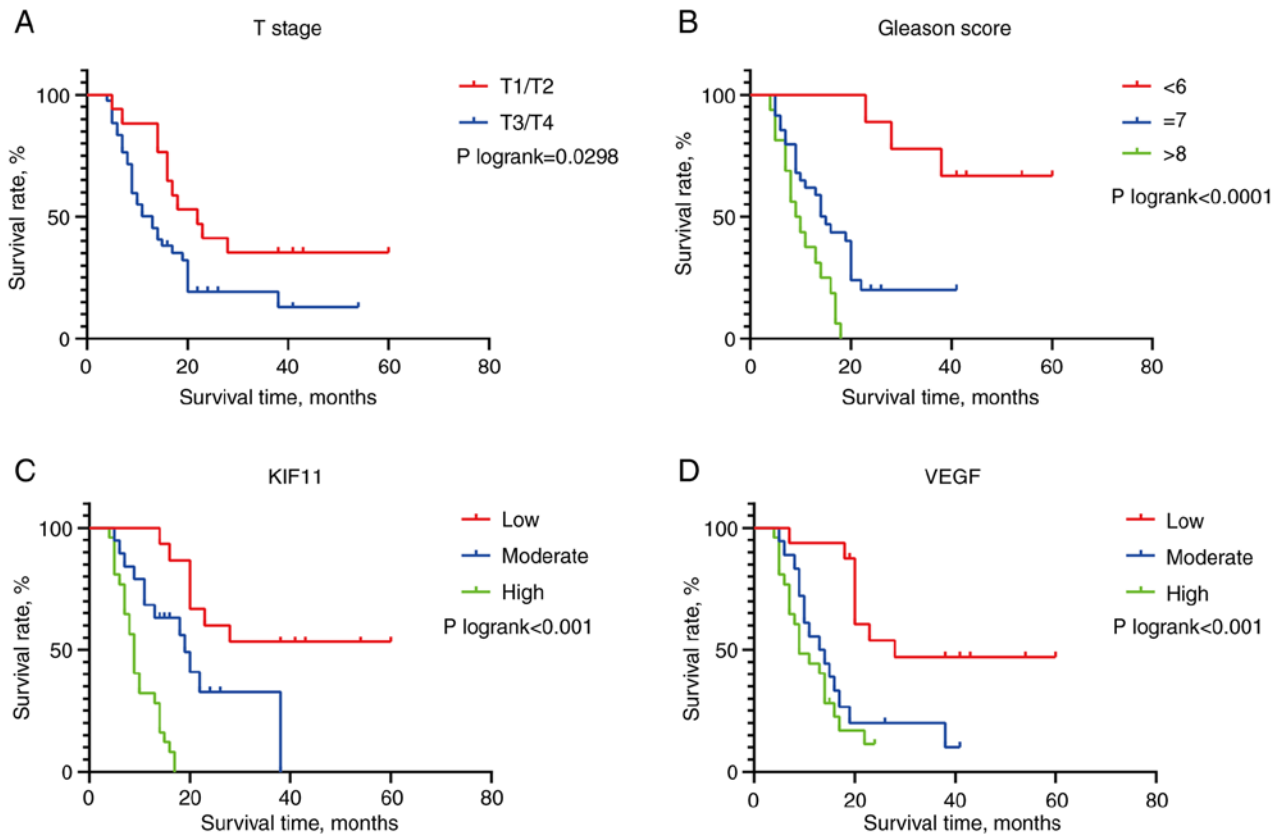

Figure 8. Kaplan-Meier survival plots. Effect of (A) T stage, (B) Gleason score, (C) KIF11 expression and (D) VEGF expression on survival. VEGF, vascular endothelial growth factor; KIF11, kinesin family member 11.

expression was associated the poor prognosis of patients with PCa (Table V).

KIF11: An independent MFS predictor for patients with PCa. The results of the multivariate regression analysis indicated that T stage (P=0.043; HR, 1.665; 95% CI, 1.016-2.729), Gleason score (P=0.016; HR, 1.734; 95% CI, 1.108-2.714) and KIF11 (P=0.007; HR, 2.776; 95% CI, 1.315-5.859) represented independent factors that significantly influenced the bone metastasis of PCa; however, PSA (P=0.496; HR, 0.770; 95% CI, 0.362-1.637) and VEGF (P=0.744; HR, 0.918; 95% CI, 0.548-1.536) showed no significance (Table VI). The Kaplan-Meier survival analysis is shown in Fig. 8, reflecting associations with T stage ($P_{log\text{-}rank}$=0.0298), Gleason score ($P_{log\text{-}rank}$<0.001), KIF11 expression ($P_{log\text{-}rank}$<0.001) and VEGF expression ($P_{log\text{-}rank}$<0.001).

Discussion

Bones represent a frequent site of metastasis in patients with advanced solid tumors, such as breast, lung, thyroid and renal cancer (27). Bone metastasis is the most common type of metastasis in patients with PCa, and it occurs in ~80% of patients with advanced PCa. Skeletal-related events have been correlated with reduced survival rates and quality of life in patients with PCa. The occurrence of bone metastasis leads to a poor prognosis in these patients (28).

KIFs are mainly involved in intracellular transport in various cell types. KIF11, also known as kinesin-5, mediates centrosome separation and the formation of the bipolar mitotic spindle, driving mitosis to support cell proliferation. KIF11 inactivation results in inappropriate cell division and cell cycle arrest during mitosis, which eventually leads to apoptosis (29). KIF11 also appears to have non-mitotic functions. Moreover, it regulates axonal branching and growth cone motility, and has recently been proven to be involved in cell motility (30,31).

WGCNA can determine the correlation between genes and clinical traits as well as quantitatively analyze the strength of the correlations between genes (26). In the present study, evidence from bioinformatic analysis and multivariate logistic regression revealed that, among seven recorded hub genes, KIF11 was an independent factor affecting the bone metastasis of PCa. Thus, we hypothesized that the expression of KIF11 could be used as a prognostic marker of MFS in patients with PCa. Clinical validation revealed that KIF11 was highly expressed in the tissues of patients with PCa and bone metastasis, which suggests that KIF11 may be involved in the bone metastasis process. Furthermore, a significant correlation was found between KIF11 and VEGF expression, suggesting a potential association between KIF11 and tumor angiogenesis. KIF11 may promote the occurrence of bone metastases by influencing angiogenesis. Angiogenesis plays a major role in the development and progression of PCa. However, in the field of PCa-targeted therapy, the performance of anti-angiogenic drugs has been disappointing. Multiple previous clinical trials have often yielded discouraging outcomes (20,32,33). Nevertheless, recent studies have suggested that anti-angiogenic treatment continues to be promising (34,35). Additionally,

combination strategies, such as combination with vaccines, immunotherapy agents and novel poly (ADP-ribose) polymerase inhibitors, have potential applicability as treatment options (36). The present study findings could potentially pave the way for a change in targeted therapy.

In the present study, follow-up data were analyzed by Cox regression analysis. Results from univariate Cox regression indicated that KIF11 and VEGF upregulation were correlated with poor prognoses. PSA is the most common index used in the diagnosis and prediction of prognosis for PCa. However, PSA was not significant in the multivariate analysis results, which indicates PSA may not be an independent MFS prognostic factor of bone metastasis in PCa. This may be related to the fact that PSA can be affected by various clinical situations such as smoking status (37). Meanwhile, KIF11 expression represented an independent risk factor for poor MFS upon multivariate Cox regression analysis. This suggested that KIF11 could be used as a predictor of MFS in patients with PCa. Elucidating the timeline of bone metastasis events is of great significance for patients with PCa, and can contribute to the formulation of clinical medication and markedly improve the quality of life of the patients. KIF11 is a suitable candidate for use in clinical research to assess the risk of bone metastasis in patients with PCa, which may be beneficial to them.

In conclusion, the findings of the present study improve our understanding of the molecular mechanisms underlying bone metastasis in PCa. The results demonstrated that KIF11 may promote bone metastasis and act as a reliable prognostic biomarker for predicting bone metastasis in patients with PCa. This information may be utilized to guide future clinical practices.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Gene Expression Omnibus repository, (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE32269, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74367 and https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE77930).

Authors' contributions

HW and XN were responsible for study conception and design. BL, SL and SW provided administrative support and revised the manuscript. SW, SL, TW, TL and JL were involved in the collection and assembly of data, and performed the experiments. Data analysis and interpretation was performed by HW, BL and SL. HW and XN wrote the manuscript. HW and XN 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

This study complied with the Declaration of Helsinki and was approved by Ethics Committees of The Fourth Hospital of Hebei Medical University (approval no. 2022KY066).

Patient consent for publication

Written informed consent was obtained from all participants in this study.

Competing interests

The authors declare that they have no competing interests.

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