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ORIGINAL ARTICLE

MAGI-2 downregulation: a potential predictor of tumor progression and early recurrence in Han Chinese patients with prostate cancer

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Membrane-associated guanylate kinase (MAGUK) family protein MAGUK invert 2 (MAGI-2) has been demonstrated to be involved in the tumorigenic mechanism of prostate cancer. The objective of this study was to investigate the expression of MAGI-2 at mRNA and protein levels. The prognostic value of MAGI-2 in Han Chinese patients with prostate cancer was also investigated. The expression data of MAGI-2 were assessed through database retrieval, analysis of sequencing data from our group, and tissue immunohistochemistry using digital scoring system (H-score). The clinical, pathological, and follow-up data were collected. The expression of MAGI-2 in prostate tumor tissues and prostate normal tissues was evaluated and compared. MAGI-2 expression was associated with clinical parameters including tumor stage, lymph node status, Gleason score, PSA level, and biochemical recurrence of prostate cancer. The relative expression of *MAGI-2* mRNA was lower in the tumor tissue in The Cancer Genome Atlas (TCGA) database and sequencing data ($P < 0.001$). There was no difference in MAGI-2 protein expression between tumor and normal tissues in tissue microarray (TMA) results. MAGI-2 expression was associated with pathological tumor stage ($P = 0.02$), Gleason score ($P = 0.05$), and preoperation prostate-specific antigen (PSA; $P = 0.04$). A positive correlation was identified between MAGI-2 and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) expressions through the analysis of TCGA and TMA data ($P < 0.0001$). Patients with higher MAGI-2 expression had longer biochemical recurrence-free survival in the univariate analysis ($P = 0.005$), which indicates an optimal prognostic value of MAGI-2 in Han Chinese patients with prostate cancer. In conclusion, MAGI-2 expression gradually decreases with tumor progression, and can be used as a predictor of tumor recurrence in Chinese patients.

INTRODUCTION

Prostate cancer is a major health burden globally, with the highest incidence among all malignancies and with a mortality rate ranking second among males in the United States.[1] The incidence of prostate cancer is also growing rapidly in China.[2] Due to the pathohistological heterogeneity of tumors, the prognosis of patients with prostate cancer differed greatly among different races and individuals.[3] Therefore, a prognostic marker with good predictability is essential to estimate patient survival based on specific individual characteristics. Thus, the genetic signature pattern and molecular subtyping for prostate cancer are of great importance in both laboratory and clinical settings.

Membrane-associated guanylate kinase (MAGUK) family protein MAGUK invert 2 (MAGI-2), also known as synaptic scaffolding molecule (S-SCAM), can bind to many receptors including cell adhesion molecules and signaling molecules.[4] MAGI-2 was originally discovered in the brain and is considered as an isoform of MAGI family located in neurons. MAGI-2 interacts with multiple physiologically essential proteins and its deletion could lead to the development of many neurological diseases.[5–7] Further researches indicate that MAGI-2 contains one guanylate kinase (GUK) domain, two tryptophan-tryptophan (WW) domains, and six PDZ (postsynaptic density protein [PSD95], Drosophila disc large tumor suppressor [Dlg1], and zonula occludens-1 protein [zo-1]) domains.[8] These domains are involved in various protein interactions so that MAGI-2 can interact with other scaffold proteins and receptors to support cell junctions. As a tumor suppressor which plays a critical role in maintaining the integrity of cell structures in tissues,[4] MAGI-2 is critical to cell integrity and survival.[9] MAGI-2 is a phosphatase and tensin homolog deleted on chromosome 10 (PTEN)-interacting protein;[10] it can stabilize PTEN expression and enhance its tumor suppressor activity by suppressing AKT activation,[10] thereby reducing tumor metastasis.[11]

Rearrangement of *MAGI-2* gene may be involved in the tumorigenic mechanism of prostate cancer.[12,13] Goldstein *et al.*[14] first reported that MAGI-2 expression in prostate cancer and high-grade prostatic intraepithelial neoplasia (HGPIN) is relatively higher than

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that in benign prostate hyperplasia (BPH) and normal tissues, which indicated that MAGI-2 may conduce to prostate carcinogenesis. Relevant researches conducted in Caucasians have indicated that MAGI-2 can be used for the diagnosis and prognostic prognosis of prostate cancer.[15,16]

In the present study, we aim to investigate the expression profile of MAGI-2 in both Caucasian and Han Chinese populations through database retrieval, analysis of sequencing data from our group, and tissue immunohistochemistry.[17] In addition, we investigated the prognostic value of MAGI-2 in Han Chinese patients with prostate cancer.

PATIENTS AND METHODS

This study was approved by the medical ethics committee of Changhai Hospital (Shanghai, China) and conducted in full accordance with the Declaration of Helsinki. All patients signed consent paper.

Gene expression data acquisition

The Cancer Genome Atlas (TCGA) prostate cancer gene expression data were obtained from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/). We retrieved 495 prostate cancer cases and 52 normal controls, and their clinicopathological characteristics were collected and documented. Besides, the prostate cancer sequencing data of our center were also enrolled. The sequencing data acquisition of 65 treatment-naïve and adjacent normal prostate tissue was done according to a previously reported approach.[17] For further validation, two independent Gene Expression Omnibus (GEO) datasets (GSE6752 and GSE21034) were downloaded to demonstrate the expression of *MAGI-2*. The analysis of GSE25136 data was conducted by NovelBio Inc. Shanghai, China. Data on mRNA expression of *MAGI-2* from these datasets were extracted and analyzed.

Tissue microarray construction

A total of 264 patients who underwent prostatectomy at the Department of Urology, Changhai Hospital, from April 2011 to December 2015, were included in this study. Tissue specimens were obtained from the Department of Pathology. Patients who had either preoperative therapy (endocrine therapy or radiation therapy) or secondary tumor were excluded from the study. The clinical and pathological data were collected from electronic medical history system. The stage of cancers was determined according to the American Joint Committee on Cancer (AJCC) guideline 8th edition.[18] All postoperative patients were recommended to have regular prostate-specific antigen (PSA) tests postoperatively: once every 3 months during the 1st year and once every 6 months from the 2nd to 5th years. The sources of follow-up data were the medical history record during follow-up visits, the database of the authors' unit named "PC-follow," and telephone calls to the patients or relatives. Biochemical recurrence (BCR) is defined as serum PSA levels of above 0.2 ng ml^{-1} continuously, and the time spot of recurrence is set as the date when PSA level was first detected to be 0.2 ng ml^{-1} or higher. Cases with PSA rising to 0.2 ng ml^{-1} within 1 month after surgery were excluded from the calculation. Formalin-fixed and paraffin-embedded tissue samples of prostate cancer were obtained from the archives of the Department of Pathology and subsequently evaluated by two pathologists independently. In total, 455 cores of 1-mm paraffin-embedded tissue were used to construct three tissue microarrays, including tumor tissue ($n = 223$), adjacent normal tissue ($n = 191$), and BPH tissue ($n = 41$).

### Immunohistochemistry and H-score evaluation

Immunohistochemistry (IHC) on sets of 3-μm tissue microarray (TMA) sections was done with MAGI-2 antibody (CSB-PA080079, 1:200,

CUSABIO, Wuhan, China) and PTEN antibody (22034-1-AP, 1:200, Proteintech, Rosement, IL, USA) separately. Thermal repair of antigens after dewaxing and hydrating was done using a fully automated instrument (Leica AutoStainer XL, Wetzlar, Germany) according to the manufacturer's instructions. Subsequently, the sections were stained using the Maxim ready-to-use IHC kit (UltraSensitive™ SP [Mouse/Rabbit] IHC Kit, Maxim, Fuzhou, China) according to the manufacturer's instructions. Before using tissue microarray staining, according to the information provided by the website PROTEIN ATLAS (https://www.proteinatlas.org/), the antibodies were verified, as a positive control. The stained TMA sections were scanned using a scanner Pannoramic MIDI (3D HISTECH, Budapest, Hungary) and observed using the CaseViewer software (3D HISTECH) to plot the region of interest for each core by two independent pathologists. After that, the software would give a H-score through automatic analyses. The cytoplasmic protein expressions of MAGI-2 and PTEN were evaluated using the H-score.[19,20] The formula of H-score is as follows: H-score = (percentage of cells' weak expression × 1) + (percentage of cells' moderate expression × 2) + (percentage of cells' strong expression × 3). H-score ranges from 0 to 300 points, with 300 points indicating strong positivity for all cells.[20] The H-score pattern of MAGI-2 is shown in **Figure 1**. The average of the H-scores is the cutoff value. Above the average, the expression is high; and below the average, the expression is low. The score is rechecked by a senior pathologist.

Statistical analyses

Statistical analysis was performed with SPSS 21.0 software (IBM, Armonk, NY, USA). Comparisons of the H-score between the tumor tissue and normal tissue (including adjacent tumor tissues and BPH tissues) were performed by Wilcoxon signed-rank test. The association between H-score and pathological tumor stage, Gleason score after the surgery, and preoperation PSA level was evaluated using the Kruskal–Wallis
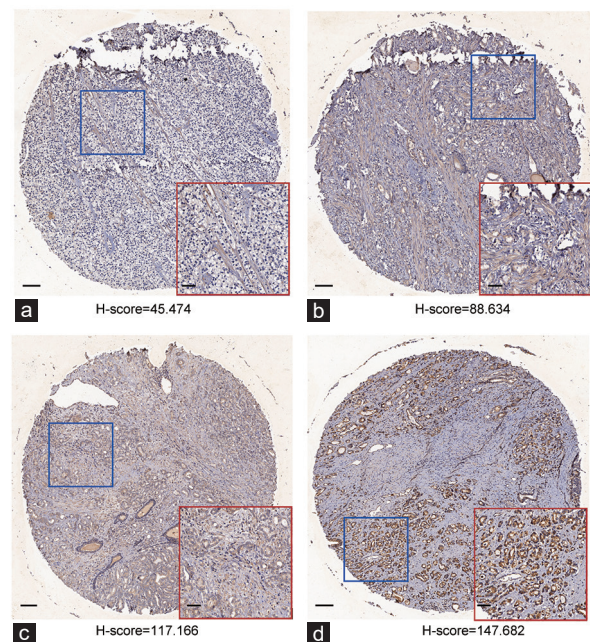
Figure 1: MAGI-2 expression pattern was analyzed by H-score in prostate cancer tissues. The H-scores of (**a**), (**b**), (**c**) and (**d**) are 45.474, 88.634, 117.166 and 147.682, respectively. Scale bars = 100 μm in round slice; scale bars = 50 μm in red square which is a magnified blue box. MAGI-2: membrane-associated guanylate kinase (MAGUK) family protein MAGUK invert 2.

H-test. The Wilcoxon rank test was used to evaluate the associations between H-score and pathological lymph node category, prostate capsule invasion, surgical margin, and seminal vesical invasion. The biochemical recurrence-free analysis was calculated from the surgery date to the last follow-up or the time when recurrence was observed. The association between H-score and biochemical recurrence was analyzed by the log-rank test and Cox proportional hazard regression. Multivariate analysis included factors such as tumor pathology staging, lymphatic category, Gleason score, and PSA level. Recurrence and survival analyses were also conducted in TCGA database using the same approach. Meanwhile, the GEO database was searched to verify the expression level of MAGI-2 in prostate cancer. Correlation analyses between genes or proteins were done using the Spearman's rank correlation test. $P \leq 0.05$ indicates a statistically significant difference (two sided).

RESULTS

MAGI-2 RNA expression

In the 65 pairs of tumor–normal tissue sequencing data, the relative expression of *MAGI-2* mRNA was higher in adjacent normal tissue compared to tumor tissue (**Figure 2a**). *MAGI-2* expression level was not associated with tumor stage (**Figure 2b**) or pathological lymph node status (**Figure 2c**). Similar results were found in the TCGA database: *MAGI-2* mRNA relative expression was lower in the tumor tissue than bengin tissue (**Figure 2d**), while it was associated with tumor stage (**Figure 2e**) and pathological lymph node category (**Figure 2f**). In GSE21034, we found that tumor *MAGI-2* expression with a Gleason score of 6 or less was obviously elevated (**Figure 2g**), and the expression of *MAGI-2* in T2 tumors was ominously higher than that of T3 and T4 tumors (**Figure 2h**). *MAGI-2* was considerably reduced in metastatic tumor tissues in GSE21034 (**Figure 2i**) and GSE6752 (**Figure 2j**).

MAGI-2 expression in TMA

The clinicopathological characteristics of all patients are summarized in **Table 1**. This study included a total of 264 patients, with a mean age of 67.2 (standard deviation [s.d.]: 7.5) years. Data on some patients were not included for analysis because of missing pathological information in the medical record system. In this study, the H-score system was used to measure the expression of MAGI-2 protein in TMA. We calculated the average H-score through further analyses and defined a cutoff value of H-score of 83. An H-score ≥83 indicates a high expression, whereas that <83 indicates a low expression. All results have been visually confirmed by a senior pathologist. The relationship between clinicopathological parameters and MAGI-2 expression is summarized in **Table 2**. There is no difference in MAGI-2 expression between tumor and normal tissues (**Figure 2k**). MAGI-2 expression was associated with pathological tumor stage (**Figure 2l**), with a down-going trend in MAGI-2 expression as the tumor progressed. Moreover, MAGI-2 expression was associated with preoperation PSA level (**Figure 2m**); PSA was higher in patients with high MAGI-2 expression (**Figure 2n**). In addition, patients with high Gleason scores (≥8) had increased MAGI-2 protein expression ($P = 0.05$) than those with low Gleason scores (<8). There was no association between MAGI-2 expression and pathological lymph node category ($P = 0.398$), prostate capsule invasion ($P = 0.711$), seminal vesical invasion ($P = 0.551$), surgical margin ($P = 0.826$), or nerve invasion ($P = 0.579$).

Correlation of MAGI-2 with PTEN expressions

In the TCGA database, we found that the relative expression of *MAGI-2* was positively correlated with *PTEN* expression (**Figure 2o**). Furthermore, a correlation was observed in the TMA data (**Figure 2p**). Representative figures of PTEN are shown in **Supplementary Figure 1**.

Table 1: Baseline characteristics of patients

| Variable	PCa patients	BPH patients
Age (year), mean±s.d.	66.8±7.3	69.1±8.1
BMI (kg m^{-2}), mean±s.d.	24.4±2.7	25.0±3.0
Preoperation PSA (ng ml^{-1}), mean±s.d.	42.28±101.19	7.14±5.55
pT category (AJCC2009), *n* (%)		
pTx	2 (0.9)	–
pT2a/pT2b	38 (17.7)	–
pT2c	83 (38.6)	–
pT3	84 (39.1)	–
pT4	8 (3.7)	–
Gleason score, *n* (%)		
≤6	11 (5.0)	–
3+4	66 (29.7)	–
4+3	37 (16.7)	–
≥8	108 (48.6)	–
Surgical margin, *n* (%)		
Positive	97 (46.0)	–
Negative	114 (54.0)	–
Seminal vesical invasion, *n* (%)		
Positive	54 (25.6)	–
Negative	157 (74.4)	–
Nerve invasion, *n* (%)		
Positive	99 (47.0)	–
Negative	112 (53.0)	–
Lymph node invasion, *n* (%)		
Positive	43 (25.4)	–
Negative	126 (74.6)	–
Prostate capsule invasion, *n* (%)		
Positive	84 (39.8)	–
Negative	127 (60.2)	–

PCa: prostate cancer; BPH: benign prostatic hyperplasia; s.d.: standard deviation; pT category: pathological tumor category; AJCC: the American Joint Committee on Cancer; –: no data; BMI: body mass index

Association between MAGI-2 expression and survival

The follow-up information of 495 cases was retrieved from TCGA database. Of which, 106 (21.4%) experienced BCR after a postoperative follow-up duration of 32.3 (s.d.: 25.4) months. Log-rank test suggested that patients with higher *MAGI-2* expression had longer disease-free survival (**Figure 3a**). Meanwhile, TMA cancer follow-up data were available in 208 of 223 patients; 29 patients were excluded because their PSA levels were rising to 0.2 ng ml^{-1} within 1 month after surgery. One hundred and seventy-nine patients were included in the calculation, with a postoperative follow-up duration of 26.1 (s.d.: 20.2) months. Log-rank test showed that patients with higher MAGI-2 expression had longer BCR-free survival (**Figure 3b**). Eleven of the 208 patients died during the whole follow-up period of 38.8 (s.d.: 16.6) months. The level of MAGI-2 expression did not affect the overall survival of patients (**Figure 3c**). The GEO database (GSE25136) showed that there was no significant correlation between *MAGI-2* expression and recurrence (**Figure 3d**), but an elevated MAGI-2 level was observed in the recurrence-free group.

Univariate Cox analysis showed that high MAGI-2 expression was associated with better BCR-free survival ($P = 0.005$, **Table 3**). After adjusting for other clinicopathological features, multivariate Cox analysis revealed that MAGI-2 expression was not correlated with poor BCR-free survival ($P = 0.135$). After we removed the data of lymph node invasion, tumor stage ($P = 0.005$), PSA ($P = 0.023$),
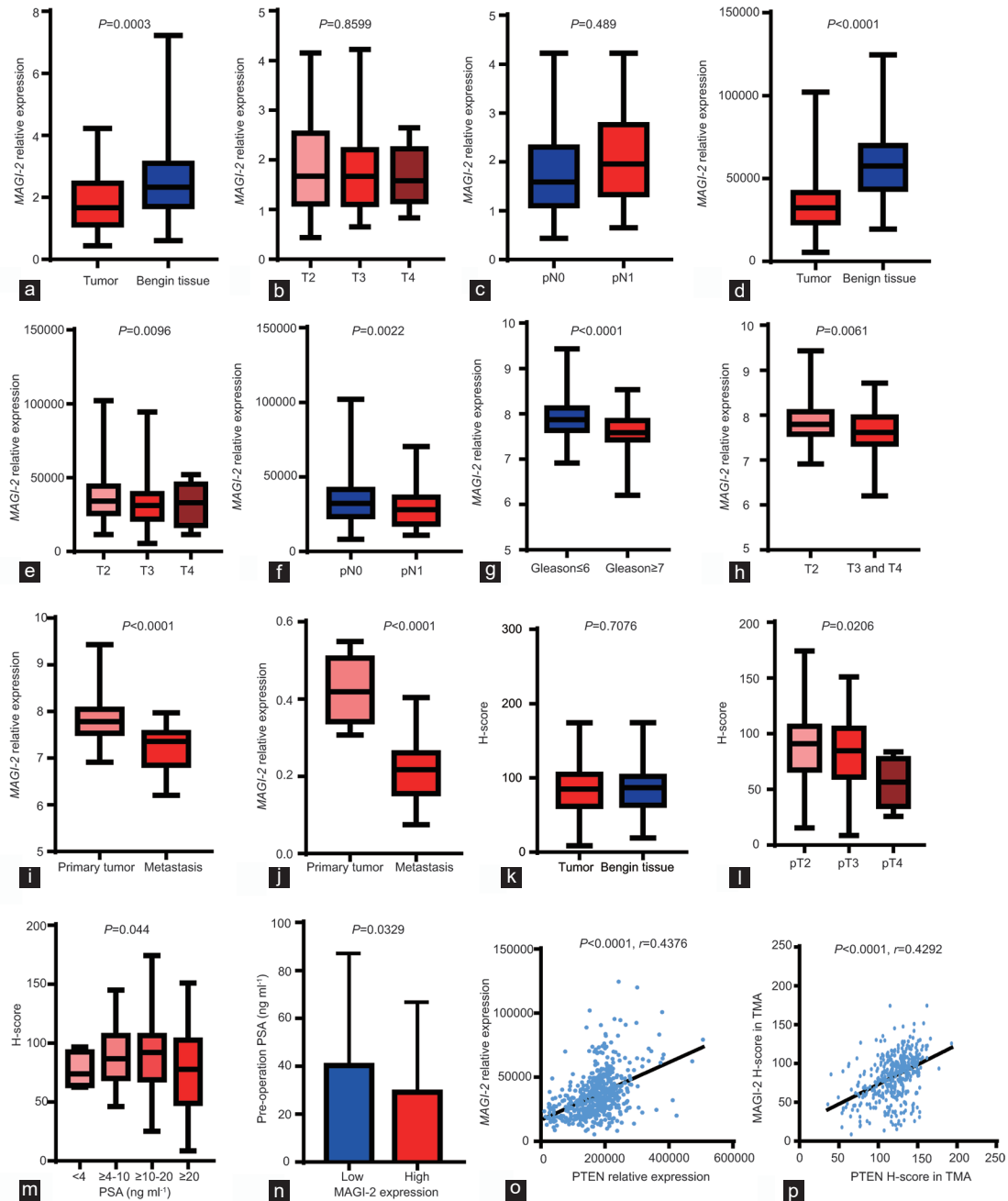
Figure 2: MAGI-2 expression profiles in our 65 pairs of tumor–normal tissue sequencing data, TCGA database, GEO database, and tissue microarray. (**a**) Comparison of *MAGI-2* mRNA relative expression between tumor and benign tissue in our 65 pairs of tumor–normal tissue sequencing data (*P* = 0.0003). *MAGI-2* mRNA relative expression pattern among (**b**) tumor stages (*P* = 0.8599) and (**c**) pathological lymph node status in our 65 pairs of tumor–normal tissue sequencing data (*P* = 0.489). (**d**) Comparison *MAGI-2* mRNA relative expression between tumor and benign tissue in the TCGA database (*P* < 0.0001). *MAGI-2* mRNA relative expression pattern among (**e**) tumor stages (*P* = 0.0096) and (**f**) lymph node categories (*P* = 0.0022) in the TCGA database. Comparison of *MAGI-2* mRNA relative expression (**g**) between Gleason score ≤6 and Gleason score ≥7 (*P* < 0.0001) and (**h**) among T2 and T3 or T4 (*P* = 0.0061) in GSE21034. *MAGI-2* mRNA relative expression in metastatic tumor is statistically significantly elevated than primary tumor in (**i**) GES21034 (*P* < 0.0001) and (**j**) GSE6752 (*P* < 0.0001). Comparison of MAGI-2 protein expression (**k**) between tumor tissue and benign tissue (*P* = 0.7076), among different (**l**) pathological tumor stages (*P* = 0.0206) and (**m**) preoperation PSA levels (*P* = 0.044). (**n**) Comparison of preoperation PSA levels between patients with high or low MAGI-2 protein expression (*P* = 0.0329). A positive correlation was identified between MAGI-2 and PTEN expressions through (**o**) TCGA database (*r* = 0.4376, *P* < 0.0001) and (**p**) tissue microarray data (*r* = 0.4292, *P* < 0.0001). MAGI-2: membrane-associated guanylate kinase (MAGUK) family protein MAGUK invert 2; T2: tumor stage 2; T3: tumor stage 3; T4: tumor stage 4; pN0: pathology lymph node 0; pN1: pathology lymph node 1; PSA: prostate-specific antigen; TCGA: The Cancer Genome Atlas; GEO: Gene Expression Omnibus; PTEN: phosphatase and tensin homolog deleted on chromosome 10.

and Gleason score (*P* = 0.001) were associated with better survival, while MAGI-2 was still not a statistically significant factor (*P* = 0.054).

In TCGA data, univariate Cox analysis demonstrated that the high expression of *MAGI-2* was a protective factor for recurrence (*P* = 0.031,
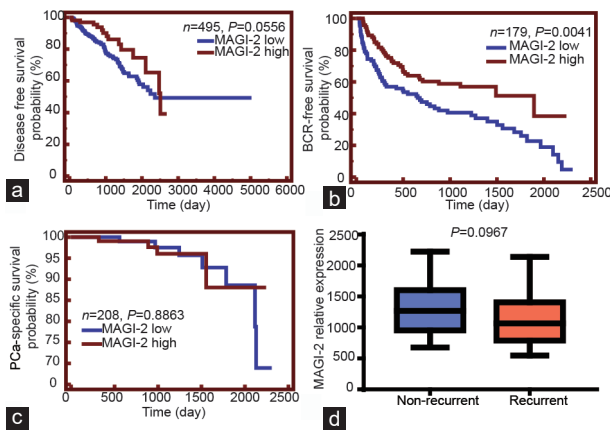
Figure 3: The relationship between MAGI-2 expression and patient prognosis. Kaplan–Meier analysis of (**a**) disease-free survival in patient data retrieved from TCGA database ($n = 495$, $P = 0.0556$), (**b**) BCR-free survival analysis ($n = 179$, $P = 0.0041$), and (**c**) PCa-specific overall survival ($n = 208$, $P = 0.8863$) of patients enrolled in tissue microarray. (**d**) Correlation between MAGI-2 expression and tumor recurrent in GEO dataset. MAGI-2: membrane-associated guanylate kinase (MAGUK) family protein MAGUK invert 2; BCR: biochemical recurrence; PCa: prostate cancer; GEO: Gene Expression Omnibus.

Supplementary Table 1), and multivariate analysis showed that the pT stage was associated with recurrence ($P < 0.0001$).

DISCUSSION

In this study, we investigated the expression of MAGI-2 in prostate cancer and normal prostate tissues, with combined approaches using sequencing data and IHC result of TMA. Higher *MAGI-2* expression in noncancerous prostate tissue was observed than that in prostate cancer tissues at the mRNA level. However, no statistically significant difference was detected in MAGI-2 protein expression between cancerous and normal prostate tissues through TMA. Goldstein *et al.*[14] have demonstrated a higher MAGI-2 protein expression in HGPIN and adenocarcinoma compared to noncancerous prostate tissue ($P < 0.001$) in the US populations. Besides, no difference in MAGI-2 expression between Han Chinese population and Caucasians was reported. Interestingly, mRNA and protein expressions of MAGI-2 were inconsistent in the present study, and MAGI-2 protein expression profile was also different between our study and previous reports. Although many reasons may contribute to the differences, including posttranscriptional regulation, racial difference, sample size, and experimental protocol, it is worthy to note that the precision of evaluating strategies for IHC is relatively suboptimal. The expression calculation method from the study of Goldstein *et al.*[14] is to multiply the percent of the stained region of interest (ROI) by the average absorbance of areas above the threshold. In the present study, a digital assessing method, H-score, was applied in the study in order to do our best to eliminate factitious bias. The explanation of this phenomenon requires further researches.

Furthermore, the expression of MAGI-2 decreased along with increased tumor stage and Gleason score. For further validation, we used two GEO databases (GSE6752 and GSE21034), indicating that *MAGI-2* expression is reduced as tumor progresses, including higher Gleason scores or higher tumor stage and tumor metastasis. In addition, patients with higher PSA level had lower expression of MAGI-2. As we know, MAGI-2 is a tumor suppressor protein

Table 2: MAGI-2 expression status in tissue microarray

Variable	Successfully analyzed (n)	MAGI-2 H-score results		P
		High (n)	Low (n)	
Tissue type	259			
Prostate cancer	220	120	100	0.707
Benign prostate tissue	229	122	107	
pT stage	214			
pTx	2	0	2	0.006*
pT2a/b	38	20	18	
pT2c	83	54	29	
pT3	84	43	41	
pT4	7	1	6	
Gleason score	215			
<8	110	44	66	0.05*
≥8	105	56	48	
Preoperation PSA level (ng ml⁻¹)	218			
<4	4	1	3	0.044*
≥4–10	41	25	16	
≥10–20	73	47	26	
≥20	100	46	54	
pN category	169			
pN0	126	74	52	0.398
pN1	43	21	22	
Prostate capsule invasion	211			
Positive	84	48	36	0.711
Negative	127	70	57	
Surgical margin	211			
Positive	97	54	46	0.826
Negative	114	67	47	
Seminal vesicle invasive	211			
Positive	54	26	28	0.551
Negative	157	92	65	
Nerve invasion	211			
Positive	99	56	43	0.579
Negative	112	62	50	

*$P \le 0.05$. Comparisons of the H-score between the prostate cancer tissue and the normal tissue were performed using the Wilcoxon signed-rank test. Comparisons of the H-score between tumor stage, Gleason score, PSA level, pN category, prostate capsule invasion, seminal vesicle invasion, and nerve invasion were performed using Kruskal–Wallis H test. PSA: prostate-specific antigen; pN category: pathological lymph node category; pT category: pathological tumor category; MAGI-2: membrane-associated guanylate kinase (MAGUK) family protein MAGUK invert 2

Table 3: Univariate and multivariate analyses of survival according to the expression of MAGI-2 in tissue microarray

Variable	BCR-free time	
	HR (95% CI)	P
Univariate		
Low H-score	1	
High H-score	0.560 (0.374–0.836)	0.005*
Multivariate		
MAGI-2 H-score (low/high)	0.703 (0.442–1.116)	0.135
pT stage (pT2/pT3, pT4)	1.470 (0.866–2.497)	0.154
pN category (pN0/pN1)	1.889 (1.088–3.281)	0.024*
Gleason score (<4+4/≥4+4)	1.987 (1.175–3.361)	0.010*
PSA level (>10/≤10 ng ml⁻¹)	2.365 (1.148–4.872)	0.020*

*$P \le 0.05$. BCR: biochemical recurrence; MAGI-2: membrane-associated guanylate kinase (MAGUK) family protein MAGUK invert 2; TMA: tissue microarray; HR: hazard ratio; pT stage: pathological tumor stage; pN category: pathological lymph node category; PSA: prostate-specific antigen; CI: confidence interval

that maintains cell morphology. As tumor progression and PSA increase, MAGI-2 expression gradually decreases. David *et al.*[16] also reported similar results, which showed that MAGI-2 expression is reduced during prostate cancer progression. The role of MAGI-2 in carcinogenesis is closely correlated with its function. Studies have shown that the rearrangement of the *MAGI-2* gene is one of the drivers of prostate cancer.[12] MAGI-2 is involved in the physiology of Par complex and the apical polarity complex of the cell,[21] which is the functional basis of the association between MAGI-2 and cancer pathogenesis. In addition, we confirmed that MAGI-2 expression is positively correlated with PTEN demonstrated by both TCGA database and TMA result. *PTEN* is a tumor suppressor gene that has been detected in many human malignancies.[22] This finding is consistent with that of a previous report, in which an interaction between MAGI-2 and PTEN was demonstrated, and MAGI-2 can stabilize the expression of PTEN and enhance its tumor suppressor activity.[23] In hepatocellular carcinoma, MAGI-2 inhibits cell migration and proliferation via PTEN pathway.[24] After BCR-free analysis of the data in the TCGA database, we found that patients with a high expression of MAGI-2 tended to have longer disease-free survival, although not statistically significant. Subsequently, follow-up data analysis of TMA patients demonstrated that the high expression of MAGI-2 was associated with longer BCR-free survival in univariate analysis. David *et al.*[16] recently explored the potential predicting role of MAGI-2 in prostate cancer prognosis and found that MAGI-2 expression gradually decreased as the tumor progressed. We also found similar pattern in Han Chinese patients with prostate cancer. We believe that there may be no ethnic differences in the expression of MAGI-2. High expression of MAGI-2 is a protective factor for the prognosis of prostate cancer, which is associated with prolonged recurrence-free period. Therefore, we believe that MAGI-2 can be used as a prognostic indicator for biochemical recurrence of prostate cancer.

In multivariate analysis, the expression of MAIG-2 is not an independent risk factor for longer BCR, along with pathological staging. If we enrolled lymph node invasion status into the survival analysis, statistics found no significant effects of tumor stage on BCR-free survival. In contrast, significant effects of tumor stage on BCR-free survival were calculated when we deleted lymph node invasion status in the survival analysis. We can believe that whether statistical significance was calculated during multivariable survival analysis depends on the different weights of the enrolled factors.

In the multivariate analysis of TCGA data, only pT stage was identified as a prognostic factor. TCGA data confirmed this result, but we did not find pT stage to be an independent factor in TMA data ($P = 0.154$). After careful analysis, we believed that it may be also due to the statistically significant heterogeneity ($P = 0.006$, **Table 2**) in pT stage of TMA data, which affects the statistical operation efficiency and leads to a biased Cox analysis result. In the GEO dataset, although we did not identify the high expression of MAGI-2 to be a protective factor ($P = 0.0967$), we can see that there is a clear trend in the nonrecurrent group, which may be attributed to the fact that time factor was not considered in the dataset. Although the two TMAs differ in the number and ethnicity of the included patients, the same conclusions are drawn to illustrate the important role of MAGI-2 in prostate cancer.

There are several limitations in this study. First, the study was a single-center study with a small sample size. Second, this study found that the expression of MAGI-2 was different at mRNA and protein levels, but no further mechanism was explored. Third, as there were few deaths in the TMA samples, the effect of MAGI-2 on death was not further elaborated.

CONCLUSIONS
Our study showed that expression of MAGI-2 was higher in benign prostate than prostate cancer tissues at the mRNA level, and that MAGI-2 protein expression decreased with tumor progression in Han Chinese patients. The correlation between MAGI-2 and PTEN was confirmed, and we found that patients with a high expression of MAGI-2 have a better BCR-free survival, showing their predictive value in the prognosis of Han Chinese patients with prostate cancer.

AUTHOR CONTRIBUTIONS
ZC wrote the manuscript, performed experiments, and acquired, analyzed, and interpreted the data. JJ wrote the manuscript, performed the experiments, and analyzed the data. CK and HX analyzed the data. FBW analyzed the data and designed and supervised the study. YWY interpreted the data. YLX and XC performed the experiments. YHS designed and supervised the study. All authors contributed toward the conception and design of the study and read and approved the final manuscript.

COMPETING INTERESTS
All authors declare no competing interests.

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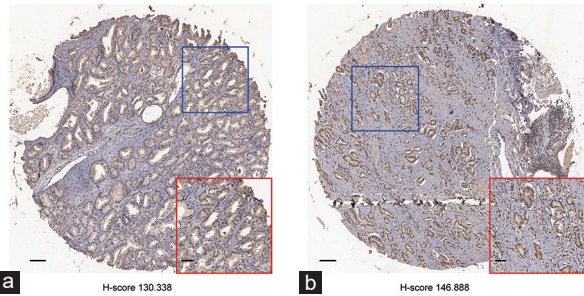
Supplementary Figure 1: PTEN expression in tumor tissues. The H-score of (**a**) and (**b**) are 130.338 and 146.888, respectively. Scale bars = 100 μm in round slice; scale bars = 50 μm in red square which is a magnified blue box. PTEN: phosphatase and tensin homologue deleted on chromosome 10.

Supplementary Table 1: Univariate and multivariate analyses of survival according to the mRNA expression of MAGI-2

Variable	Recurrence-free survival (TCGA)	
	HR (95% CI)	P
Univariate		
MAGI-2 low	1	
MAGI-2 high	1.6 (1.043–2.455)	0.031*
Multivariate		
MAGI2 relative expression (low/high)	1.494 (0.973–2.296)	0.067
pT stage (pT2/pT3/pT4)	3.702 (1.987–6.898)	<0.0001*
pN category (pN0/pN1)	1.397 (0.883–2.21)	0.153

*$P \leq 0.05$. TCGA: The Cancer Genome Atlas; HR: hazard ratio, pT stage: pathological tumor stage; pN category: pathological lymph node category; MAGI-2: membrane-associated guanylate kinase (MAGUK) family protein MAGUK invert 2; CI: confidence interval