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High expression of MAGE-A9 correlates with unfavorable survival in hepatocellular carcinoma

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Melanoma-associated antigens (MAGE)-A9 has been reported to play important roles in the development of human cancers. However, the association between MAGE-A9 expression and the clinicopathological characteristics of hepatocellular carcinoma (HCC) is not well understood. The study was to detect the expression of MAGE-A9 in human HCC and investigate the association between its expression and the clinicopathological characteristics of HCC. Reverse transcription-polymerase chain reaction (RT-PCR), one-step quantitative -PCR (qPCR) and immunohistochemistry (IHC) analyses were performed to characterize the expression of MAGE-A9 in HCC cell lines and tissues. Kaplan-Meier survival and Cox regression analyses were employed to evaluate the prognosis of 100 HCC patients. The results showed that the expression of MAGE-A9 in HCC was significantly higher than that in non-cancerous cells and tissues. Moreover, the expression level of the MAGE-A9 protein in HCC was related to the pathological grade ($p = 0.003$), portal vein invasion ($p = 0.001$), distant metastasis ($p = 0.022$) and TNM stage ($p = 0.005$). Cox regression analysis further revealed that MAGE-A9 expression is an independent prognostic factor for disease-free survival ($p = 0.006$) and overall survival ($p = 0.022$). These data are the first to indicate that MAGE-A9 expression is a valuable prognostic biomarker for HCC and that high MAGE-A9 expression suggests unfavorable survival outcomes in HCC patients.

Hepatocellular carcinoma (HCC) is the fifth most common cancer and accounts for more than half a million deaths each year, making HCC the third leading cause of cancer death worldwide[1]. The lethality of HCC and the lack of effective treatments make HCC an enormous challenge. In China, for example, HCC patients account for half of the total global HCC cases, and the township of Qidong in Jiangsu Province of China is one of the highest endemic regions for HCC worldwide[2,3]. Although a growing number of therapy strategies, including liver transplantation, surgical resection, radiofrequency ablation and molecular therapy, have been developed, the overall survival of HCC is still poor and frustrating[4,5]. At present, alpha-fetoprotein (AFP) is the most acknowledged biomarker for early detection and the follow-up of HCC patients during treatment[6]. However, due to the existence of AFP-negative HCC patients, studies that focus on novel biomarkers to distinguish patients with poor prognosis or at high risk of early recurrence of HCC are urgently needed and of great importance.

Melanoma-associated antigens (MAGE) are a group of well-characterized members of the cancer/testis antigen (CTA) family, which represents a unique class of tumor antigens that are expressed in a wide variety of malignant tumors[7,8]. MAGE-A is a subfamily comprising MAGE and MAGE-A antigens that are highly tumor specific; MAGE-A genes are anomalously expressed cancers though rarely expressed in normal tissues[9]. Hence, MAGE-As are rationally identified as ideal targets for cancer immunotherapy, and several MAGE-A-based immunotherapy and targeted therapy strategies have been developed[10-12].

In the case of MAGE-A9, the protein is frequently expressed in various human cancers, including melanoma, head and neck squamous cell carcinoma, non-small cell lung carcinoma (NSCLC) and multiple myeloma[13]. MAGE-A9 was also identified as having prognostic relevance in high-grade bladder cancer, revealing MAGE-A9 expression to be associated with tumor progression[14]. The expression of MAGE-A9 was found to be higher in more advanced stages of renal cancer and was correlated with tumor prognosis[15]. Thus, studies addressing whether the function of MAGE-A9 is analogous in HCC and whether MAGE-A9 can be utilized as a new biomarker for the diagnosis and treatment for HCC are important and of great interest.

This study was designed to detect the expression of MAGE-A9 by reverse transcription-polymerase chain reaction (RT-PCR) in HCC cells, one-step quantitative reverse transcription-PCR (qPCR) in fresh HCC samples and immunohistochemistry (IHC) in HCC tissue microarrays (TMA). Moreover, the relationship of MAGE-A9 expression with the clinicopathological attributes of HCC patients, especially its prognostic significance, was further evaluated.

Results

Summarization of clinical information of 100 HCC patients. The primary clinical information of 100 HCC patients are illustrated in Table 1. A total of 83 men and 17 women, of median age 54.01 years (range 27–73 years) were enrolled in this study. The AFP of 56 patients was high (≥13.2 μg/L), whereas that of the remaining 44 patients was low (<13.2 μg/L). 66 patients encountered Hepatitis B virus (HBV) infection and 42 patients experienced liver cirrhosis. Regarding the pathological grade, 11 patients were at grade 1, 71 were at grade 2, and 18 were at grade 3. Portal vein invasion was detected in 38 patients and 26 patients suffered lymph node metastasis while 6 patients endured distant metastasis. As for TNM stage, 39 patients were in stage I–II while the other 51 patients were in advanced stage III–IV.

Table 1 | Relationship of high MAGE-A9 expression with clinicopathological characteristics in HCC

		MAGE-A9			
Groups	No.	+	%	χ^2	p value
Total	100	55	55.0		
Gender					
Male	83	44	53.0	0.78	0.377
Female	17	11	64.7		
Age (years)					
<60	76	41	53.9	0.14	0.707
≥60	24	14	58.3		
Tumor size (cm)					
>5	58	34	58.6	0.73	0.392
≤5	42	21	50.0		
α-fetoprotein (AFP) status					
High	56	28	50.0	1.29	0.257
Low	44	27	61.4		
Hepatitis B virus infection					
Positive	66	35	53.0	0.30	0.581
Negative	34	20	58.8		
Liver cirrhosis					
Positive	42	21	50.0	0.73	0.392
Negative	58	34	58.6		
Pathological grade					
Grade 1	11	1	9.1	11.74	0.003*
Grade 2	71	41	57.7		
Grade 3	18	13	72.2		
Portal vein invasion					
Positive	38	29	76.3	11.25	0.001*
Negative	62	26	41.9		
Lymph node metastasis					
Positive	26	18	69.2	2.87	0.090
Negative	74	37	50.0		
Distant metastasis					
Positive	6	6	100.0	5.22	0.022*
Negative	94	49	52.1		
TNM stage					
Stage I	13	4	30.8	13.05	0.005*
Stage II	36	15	41.7		
Stage III	45	30	66.7		
Stage IV	6	6	100.0		

*$p < 0.05$.

Detection of MAGE-A9 expression in HCC cell lines by RT-PCR. MAGE-A9 mRNA expression were firstly examined in four HCC cell lines (BEL-7402, SMMC-7721, HepG2 and SK-HEP-1). Two non-cancerous cell lines (LO-2 and HUVEC) were employed as negative control. As shown in Figure 1, MAGE-A9 mRNA expression were detected in four HCC cell lines with high intensities compared with extremely low expression of MAGE-A9 in two non-cancerous cell lines.

Detection of MAGE-A9 expression in HCC tissue samples by qPCR. Total RNA was extracted from 20 fresh HCC tissues and corresponding non-cancerous tissues to evaluate the MAGE-A9 mRNA expression by qPCR test. As is shown in Figure 2, the means of MAGE-A9 mRNA in HCC tissues (4.44 ± 0.342) were significantly higher than that of in corresponding non-cancerous tissues (1.73 ± 0.178) when normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) ($p < 0.05$).

Detection of MAGE-A9 expression in HCC TMA by IHC. IHC was executed to evaluate the MAGE-A9 protein expression. High MAGE-A9 expression was witnessed in 55 of 100 (55%) HCC tissue samples, whereas only 18 cases of 100 non-cancerous normal tissues (18%) exhibited positive MAGE-A9 expression. There was significant difference in high expression rate of MAGE-A9 protein between HCC tissues and non-cancerous tissues ($p < 0.05$). Positive staining was mainly localized in the membrane and cytoplasm of HCC cells and the representative IHC staining for MAGE-A9 protein expression in HCC are shown in Figure 3. The relationship between MAGE-A9 protein expression and clinicopathological parameters was elucidated in Table 1. High MAGE-A9 protein expression was correlated with pathological grade ($p = 0.003$), portal vein invasion ($p = 0.001$), distant metastasis ($p = 0.022$) and TNM stage ($p = 0.005$). In comparison, no significant correlation was discovered between MAGE-A9 expression and other clinical features, including gender, age, tumor diameter, AFP status, HBV infection, liver cirrhosis and lymph node metastasis.

Survival analysis. According to a univariate analysis, several factors were correlated with the disease-free survival (DFS) of HCC patients,

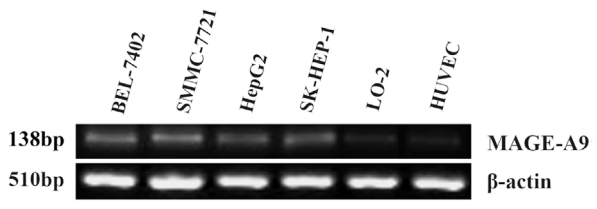

Figure 1 | MAGE-A9 expression in four hepatocellular carcinoma (HCC) cell lines and two non-cancerous cell lines. Reverse transcription-polymerase chain reaction (RT-PCR) analysis showed that MAGE-A9 mRNA expression in HCC cell lines (BEL-7402, SMMC-7721, HepG2 and SK-HEP-1) were detected with high intensities compared with low intensities of MAGE-A9 expression in human liver cell line LO-2 and human umbilical vein-derived endothelial cell line (HUVEC).

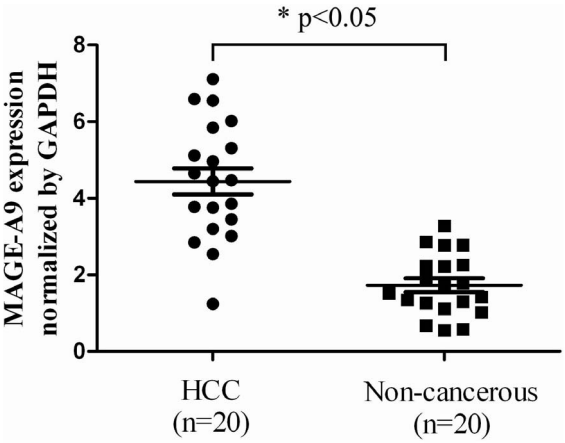

Figure 2 | MAGE-A9 expression in hepatocellular carcinoma (HCC) tissues and tumor adjacent non-cancerous tissues. One-step quantitative real-time polymerase chain reaction (qPCR) demonstrated that the expression of MAGE-A9 in HCC tissues (4.44 ± 0.342) was significantly higher than that of in matched non-cancerous tissues (1.73 ± 0.178), when normalized to the GAPDH internal control. *$p < 0.05$.

including MAGE-A9 expression ($p = 0.001$), tumor size ($p = 0.031$), pathological grade ($p = 0.007$), portal vein invasion ($p = 0.001$), lymph node metastasis ($p = 0.001$), distant metastasis ($p = 0.035$) and TNM stage ($p = 0.001$) (Table 2). All the above items, except for distant metastasis, were also associated with overall survival (OS) in 100 HCC patients (Table 3). By using a multivariate analysis with the Cox regression model, MAGE-A9 expression ($p = 0.006$) and lymph node metastasis ($p = 0.008$) indicated a poor DFS, whereas MAGE-A9 expression ($p = 0.022$) and portal vein invasion ($p = 0.039$) were identified as independent prognostic factors for OS (Table 2 and 3). Kaplan-Meier survival curves subsequently demonstrated that HCC patients with high MAGE-A9 expression presented a significantly unfavorable DFS time and OS time. In addition, HCC patients with lymph node metastasis and positive portal vein invasion experienced poor DFS and OS rates, respectively (Figure 4).

Discussion

MAGE-A antigens have recently been identified as promising immunotherapeutic targets for anticancer therapy, as they are strictly tumor specific and are shared by many types of tumors[7,9,16]. Several basic and clinical trials involving melanoma, esophagus cancer and lung cancer have utilized proteins and peptides derived from some of

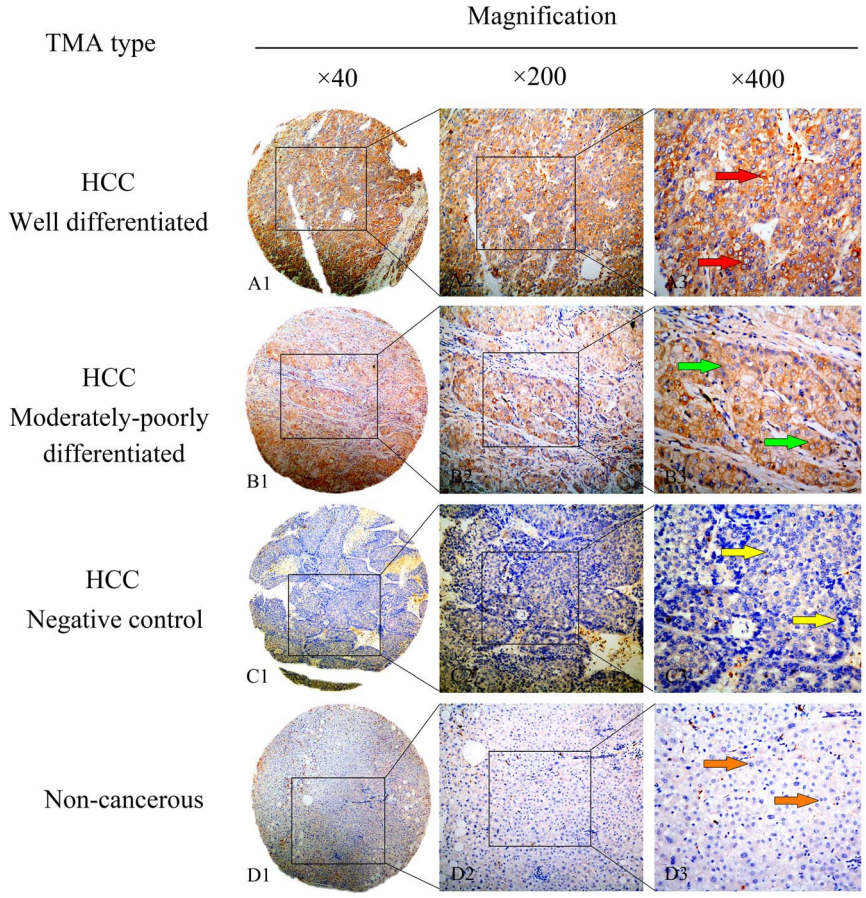

Figure 3 | Representative pattern of MAGE-A9 protein expression in HCC and corresponding non-cancerous tissues with tissue microarray (TMA). A1, A2 and A3 High immunohistochemical (IHC) staining of MAGE-A9 in well differentiated HCC sample. Red arrow shows positive staining in the membrane and cytoplasm of cancer cells. **B1, B2 and B3** High IHC staining of MAGE-A9 in moderately-poorly differentiated HCC sample. Green arrow shows positive staining in the membrane and cytoplasm of cancer cells. **C1, C2 and C3** Negative IHC staining of MAGE-A9 in HCC sample as negative control. Yellow arrow shows negative staining of HCC cells. **D1, D2 and D3** Negative IHC staining of MAGE-A9 in non-cancerous tissue sample. Orange arrow shows negative staining of non-cancerous cells. Original magnification ×40 in A1, B1, C1 and D1; ×200 in A2, B2, C2 and D2; ×400 in A3, B3, C3 and D3.

Table 2 | Univariate and multivariate analysis of prognostic factors in HCC for disease-free survival

	Univariate analysis			Multivariate analysis		
	HR	p > |z|	95% CI	HR	p > |z|	95% CI
MAGE-A9 expression						
High versus Low	3.39	0.001*	1.898–6.046	2.54	0.006*	1.299–4.954
Gender						
Male versus Female	1.08	0.648	0.580–2.403			
Age (years)						
<60 versus ≥60	1.66	0.146	0.838–3.285			
Tumour size (cm)						
>5 versus ≤5	1.83	0.031*	1.057–3.177	1.44	0.255	0.768–2.707
α-fetoprotein status						
High versus Low	1.01	0.971	0.603–1.692			
Hepatitis B virus infection						
Positive versus Negative	1.21	0.486	0.699–2.128			
Liver cirrhosis						
Positive versus Negative	0.89	0.660	0.522–1.509			
Pathological grade						
Grade 1 and 2 versus Grade 3	0.45	0.007*	0.252–0.807	0.74	0.378	0.384–1.438
Portal vein invasion						
Positive versus Negative	3.26	0.001*	1.930–5.520	1.79	0.073	0.948–3.368
Lymph node metastasis						
Positive versus Negative	2.67	0.001*	1.548–4.605	2.29	0.008*	1.238–4.229
Distant metastasis						
Positive versus Negative	2.52	0.035*	1.068–5.966	0.60	0.317	0.216–1.644
TNM stage						
Stage I-II versus Stage III-IV	0.32	0.001*	0.184–0.565	0.77	0.480	0.370–1.598
MAGE-A9 expression+ α-fetoprotein status						
Both high versus Others situations (High + Low or Low + High or Both low)	1.60	0.093	0.925–2.785			

*p < 0.05.

Table 3 | Univariate and multivariate analysis of prognostic factors in HCC for overall survival

	Univariate analysis			Multivariate analysis		
	HR	p > |z|	95% CI	HR	p > |z|	95% CI
MAGE-A9 expression						
High versus Low	3.22	0.001*	1.804–5.737	2.17	0.022*	1.121–4.205
Gender						
Male versus Female	1.13	0.740	0.554–2.297			
Age (years)						
<60 versus ≥60	1.72	0.121	0.867–3.396			
Tumour size (cm)						
>5 versus ≤5	1.84	0.031*	1.058–3.183	1.44	0.244	0.778–2.679
α-fetoprotein status						
High versus Low	1.03	0.896	0.618–1.734			
Hepatitis B virus infection						
Positive versus Negative	1.19	0.539	0.682–2.078			
Liver cirrhosis						
Positive versus Negative	0.84	0.519	0.494–1.428			
Pathological grade						
Grade 1 and 2 versus Grade 3	0.44	0.005*	0.245–0.783	0.79	0.455	0.425–1.467
Portal vein invasion						
Positive versus Negative	3.33	0.001*	1.966–5.645	1.95	0.039*	1.035–3.656
Lymph node metastasis						
Positive versus Negative	2.42	0.001*	1.408–4.159	1.74	0.066	0.964–3.133
Distant metastasis						
Positive versus Negative	2.10	0.088	0.896–4.917			
TNM stage						
Stage I-II versus Stage III-IV	0.35	0.001*	0.200–0.612	0.86	0.690	0.411–1.800
MAGE-A9 expression + α-fetoprotein status						
Both high versus Others situations (High + Low or Low + High or Both low)	1.59	0.100	0.915–2.748			

*p < 0.05.

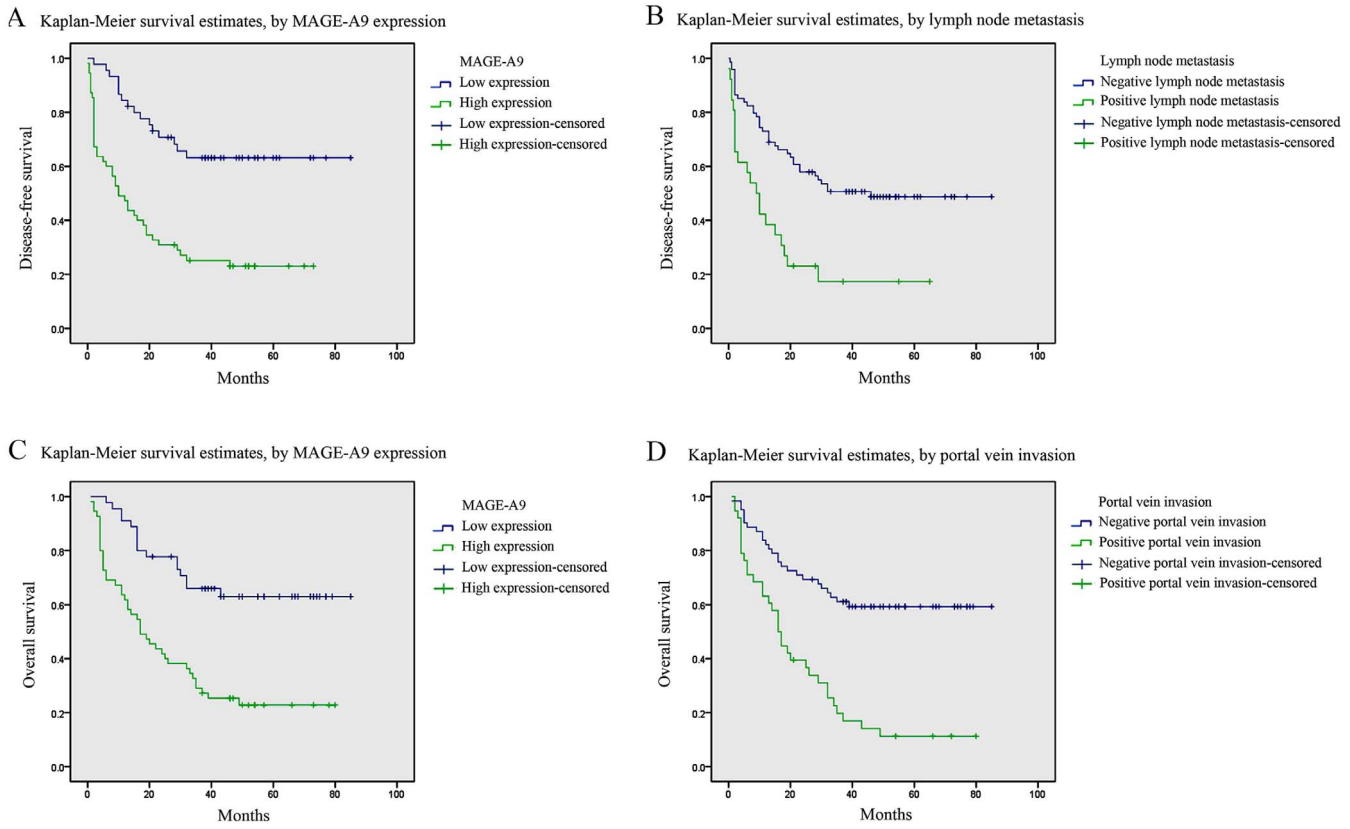

Figure 4 | Survival analysis of 100 HCC patients by Kaplan-Meier method. (A) Disease-free survival rate in patients with high MAGE-A9 expression (green line) was significantly lower than that in patients with low MAGE-A9 expression (blue line). (B) Disease-free survival rate in patients with positive lymph node metastasis (green line) was significantly lower than that in patients with negative lymph node metastasis (blue line). (C) Overall survival rate in patients with high MAGE-A9 expression (green line) was significantly lower than that in patients with low MAGE-A9 expression (blue line). (D) Overall survival rate in patients with positive portal vein invasion (green line) was significantly lower than that in patients with negative portal vein invasion (blue line).

these antigens and have shown encouraging results[10–12,17,18]. Although the normal physiologic role of MAGE-A antigens remains unknown, their contribution to the development of cancers has been investigated recently. It is reported that MAGE expression is directly regulated by microRNAs, such as miR-34a, and that MAGE-A proteins can inhibit p53 function through direct and indirect mechanisms[9,19,20]. With regard to MAGE-A9, high protein expression has been detected in several human cancers[13], MAGE-A9 also shows prognostic relevance in bladder cancer and renal cancer and is correlated with tumor progression[14,15]. Regardless, the detailed understanding of the function of MAGE-A9 in hepatocellular carcinoma, especially regarding prognostic characteristics, is limited.

In this study, we first investigated MAGE-A9 mRNA expression in cell lines by RT-PCR. The results showed elevated MAGE-A9 expression in four HCC cell lines compared to non-cancerous cell lines. Subsequently, the expression of MAGE-A9 mRNA in fresh HCC tissues and matched non-cancerous tissues was evaluated by qPCR. The results suggested a significantly higher level of MAGE-A9 expression in HCC tissue samples than in non-cancerous tissue samples. Moreover, TMA with HCC specimens was constructed, and an IHC analysis was performed to further prove that MAGE-A9 protein expression in HCC is also higher than in non-cancerous tissues, with statistical significance. In a report by Picard V et al, a high MAGE-A9 transcript level was found in superficial and invasive tumors, whereas no transcript was detected in normal urothelium[21]. These data are consistent with our results and support our findings. In addition, certain clinical parameters, including the pathological grade, portal vein invasion, distant metastasis and TNM stage, were correlated with MAGE-A9 protein expression. Similarly, in a panel of 493 primary bladder tumors, Bergeron et al described that MAGE-A9 was associated with a higher grade and tumor recurrence[14].

For survival analysis, Cox proportional hazards regression models, in which the effect of covariates is to multiply the hazard function by a function of the explanatory covariates, have achieved widespread application in the analysis of time-to-event data, with censoring and covariates[22]. In the present study, a univariate analysis was firstly chosen to detect important factors that may influence the prognosis of HCC patients; a multivariate analysis was then performed to identify the authenticity and validity of the prognostic factors detected. Finally, we screened the valid prognostic factors (MAGE-A9 expression and lymph node metastasis status for DFS and MAGE-A9 expression and portal vein invasion status for OS), and the data demonstrated that high MAGE-A9 expression was associated with a poor prognosis in patients with HCC. Patients expressing high levels of MAGE-A9 exhibited unfavorable outcomes for both DFS and OS. These results were in line with previous studies in renal cell carcinoma[15] and bladder cancer[21]. A Kaplan-Meier analysis also verified that HCC patients with high MAGE-A9 expression showed a significantly unfavorable life span, including DFS and OS.

The research to date concerning the role of MAGE-A9 in cancers has been unthorough and inadequate. However, a growing number of studies focusing on the MAGE-A family have indicated the critical role of different members of the MAGE-A family in cancer development, and MAGE-As have been identified as appealing targets for cancer immunotherapy or chemo-immunotherapy[7,9,20,23,24]. Accordingly, several MAGE-A-based immunotherapy therapeutics have been developed and showed positive results[10,12]. Our research team also constructed an anti-MAGE-A1 immunotoxin and verified

its anti-tumor effectiveness[11]. Considering the oncogenic role of MAGE-A9 in cancer, the application of treatment targeting MAGE-A9 is anticipated, and a fully human anti-MAGE-A9 antibody is currently being generated by our research group.

However, there were some limitations to the present study. For example, we failed to collect the clinical information of HCV infection and alcohol consumption of the HCC patients, factors that are considered to be important elements of HCC etiology. We will consummate our studies in the future by improving the clinical data collection.

In summary, we conclude for the first time that MAGE-A9 can be recognized as a prognostic factor in HCC and that targeting MAGE-A9 may provide a promising therapeutic strategy for HCC treatment. Further studies that include more clinical samples of HCC are necessary to confirm our findings and to elucidate the possible mechanisms of MAGE-A9 characteristics in HCC.

Methods

Cell lines and cell culture. Four HCC cell lines (BEL-7402, SMMC-7721, HepG2 and SK-HEP-1), human liver cell line LO-2, and human umbilical vein-derived endothelial cell line (HUVEC) were obtained from the cell bank of the Chinese Academy of Science (Shanghai, China). All the cells were cultured routinely by our laboratory in DMEM medium (Gibco, Invitrogen, Carlsbad, California, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), penicillin (100 U/mL) and streptomycin (100 lg/mL).

Patients and tissue samples. A total of 100 formalin-fixed, paraffin-embedded HCC tissues and 100 matched tumor-adjacent normal tissues were obtained from the Affiliated Hospital of Nantong University from 2003 and 2010. Before surgical therapy, none of the patients had received neoadjuvant chemotherapy, radiation therapy or immunotherapy. Related original clinical data, including gender, age, tumor size, AFP status, HBV infection, liver cirrhosis, pathological grade, portal vein invasion, lymph node metastasis, distant metastasis and TNM stage, were also collected simultaneously. Clinical staging was performed according to the 2002 American Joint Committee on Cancer/International Union Against Cancer TNM staging system[25]. A panel of 20 fresh HCC tissues and corresponding adjacent non-cancerous tissues, obtained from the Department of Pathology, the Affiliated Hospital of Nantong University were also enrolled in this study. Written informed consent was accomplished from the patients for publication of this study. Study protocol was approved by the Ethics Committee of Nanjing Medical University and all experimental methods were carried out in accordance with approved guidelines of Nanjing Medical University.

RT-PCR and qPCR analysis. Total RNA was extracted from cell lines and 20 fresh HCC tissues and corresponding adjacent non-cancerous tissues using the Trizol reagent (Life Technologies, Inc., Grand Island, NY) according to the manufacturer's guidelines for performing RT-PCR and qPCR analysis, respectively. The prepared RNA (5 μg) was mixed with oligo-dT primers and reverse-transcribed with MMLV reverse transcriptase (Promega, United States). The primers for MAGE-A9 were as follows: forward primer 5′- CAC TGT ATG TCA TCT CTG -3′; reverse primer 5′- ACT ACT GTC ATT CAT TAA CT -3′. For RT-PCR, the transcription levels of β-actin served as a loading control and the primers for β-actin were as follows: forward 5′- CTC CAT CCT GGC CTC GCT GT-3′, reverse 5′- GCT GCT ACC TTC ACC GTT CC-3′. For qPCR, the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA level was used to standardize the measurements of the target gene and the primers for GAPDH were as follows: forward primer 5′-TGC ACC ACC AAC TGC TTA GC-3′ and reverse primer 3′-GGC ATG GAC TGT GGT CAT GAG-5′. PCR amplification was executed in 20 μL using a thermocycler (Biometra, Germany). A SensiMixTM One-Step Kit (Quantace, Berlin, Germany) was employed to carry out qPCR analysis with a Real Time PCR system (Bio-Rad Laboratories, Hercules, CA) according to the standard protocol. Total RNA extraction, amplification conditions, RT-PCR and one-step qPCR procedure were described in our previous publication[26,27].

TMA construction and IHC analysis. 100 HCC tissues and matched non-cancerous tissues were prepared and TMA was produced by Alenabio Biotech Co., Ltd (Xi'an, China). Core tissue biopsies (2 mm in diameter) were taken from individual paraffin-embedded sections and arranged in the new recipient paraffin blocks. The TMA was cut into 4 μm sections and placed on super frost charged glass microscope slides. IHC analysis was performed as described previously[1,26]. TMA sections were incubated with a primary monoclonal mouse anti-MAGE-A9 antibody (1 : 250, Abcam, England) in phosphate-buffered saline (PBS) and then incubated with horseradish peroxidase-conjugated antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) after washing. Negative controls were included by replacement of the primary antibody with PBS.

MAGE-A9 immunostaining was scored by two independent pathologists according to intensity and percentage of MAGE-A9-positive cells. Staining intensity was scored as follows: 0 (negative), 1 (weakly positive), 2 (moderately positive), and 3 (strongly positive). The percentage of MAGE-A9-positive cells was also scored according to 4 categories, in which 1 was given for 0–10%, 2 for 11–50%, 3 for 51–80%, and 4 for 81–100%. The product of the intensity and percentage scores gave rise to the final staining score. The degree of MAGE-A9 staining was quantified using a two-level grading system as follows: <3 indicates low or no expression while 3–9 indicates high expression. The cutoff point for the MAGE-A9 expression score that was statistically significant in terms of survival was set using the X-tile software program (The Rimm Lab at Yale University; http://www.tissuearray.org/rimmlab) as described previously[28].

Statistical analysis. The MAGE-A9 mRNA expression in fresh HCC tissues compared with matched non-cancerous tissues was analyzed with the Wilcoxon signed rank nonparametric test. The significance of MAGE-A9 protein expression on clinical parameters of HCC was detected by chi-square test. Univariate and multivariate analyses were performed using Cox proportional hazards regression models to identify important factors that statistically associated with disease-free survival and overall survival status. The Kaplan-Meier method was employed to explore the associations between MAGE-A9 expression and the outcome of HCC patients. For all tests, the significance level for statistical analysis was set at $p < 0.05$. All the statistical analyses were conducted by using STATA Version 12.0 (Stata Corporation, College Station, TX, USA) and SPSS 16.0 (SPSS Inc, Chicago, IL).

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Author contributions

Y.M. and J.Z. designed the study; X.F.G., M.Y.F., Z.J.G. and F.Z. collected the tissue samples; Y.M., F.Z., X.J.T., X.F.G. and M.Y.F. executed the RT-PCR and qPCR test; L.X., J.L. and L.Q. performed and evaluated the IHC analysis; Z.J.G., Y.Q.D., H.H.N., W.Z. and Y.F.Z. collected clinical data and participated in the evaluation of the IHC data; Y.M. drafted the manuscript; Y.M. and J.Z. supervised the study. All authors read and approved the final manuscript.

Additional information

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