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ORIGINAL ARTICLE

MAGE-A gene expression in peripheral blood serves as a poor prognostic marker for patients with lung cancer

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Keywords
Biological marker; lung cancer; *MAGE*; metastasis; prognosis.

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Abstract

Background: *MAGE-A* genes belong to the cancer/testis antigens family. The prognostic significance of *MAGE-A* expression in the peripheral blood of patients with lung cancer is unknown. Therefore, this study evaluated the expression and possible prognostic significance of *MAGE-A* in the peripheral blood of patients with lung cancer.

Methods: In this study, we detected *MAGE-A* gene expression in the peripheral blood of 150 patients with lung cancer and 30 healthy donors using multiplex semi-nested PCR and analyzed their correlation with clinicopathological risk factors.

Results: *MAGE-A* expression was associated with factors indicating poor prognosis. The expression of *MAGE-A* and each individual *MAGE-A* gene were also associated with low overall survival in patients with lung cancer.

Conclusion: The expression of *MAGE-A* genes in peripheral blood may act as a poor prognostic marker in patients with lung cancer.

Introduction

Lung cancer is one of the most common cancers in the world. The American Cancer Society estimated that in 2015, lung cancer would account for 221 200 estimated new cases and 158 040 deaths, representing the highest rates of all cancers.[1] The latest statistics indicate that lung cancer still has the highest incidence and is the leading cause of death from malignant tumors.[2] Lung cancer mainly includes non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). As there are few obvious symptoms, it is difficult to detect lung cancer in early stages when there are more therapeutic options. In recent years, the combination of radiotherapy and chemotherapy, surgery, and advanced imaging technology for the diagnosis and treatment of lung cancer patients has led to significant progress; however, the average five-year lung cancer survival rate is still < 20%. Therefore, individual drugs, biomarkers, and immunotherapy have attracted increasing attention among experts.

Studies have found that circulating tumor cells (CTCs) exist in the peripheral blood of patients with malignant tumors and that distant metastasis and recurrence are the main causes of death in patients with breast cancer.[3] The main pathway of metastasis is via the separation of tumor cells from the primary tumor mass and subsequent migration toward blood vessels. CTCs have been proven to predict prognosis in patients with malignant tumors.[4–6] Although the CellSearch system was approved by the United States (US) Food and Drug Administration (FDA) in 2004, there is still no standard method to separate or identify CTCs because of the relatively low detection rate. Therefore, it is necessary to develop new molecular markers to detect CTCs in the peripheral blood of cancer patients.

Cancer/testis antigens (CTA) are not expressed in adult tissues but are found in stem cells and a variety of tumor tissues. Melanoma-associated antigens (MAGE) belong to the CTA family, and were first discovered by Van der Bruggen *et al.*[7] The MAGE family contain at least 55 family

members, which have been divided into two sub-families: MAGE-I (MAGE-A, MAGE-B and MAGE-C) and MAGE-II (MAGE-D).[7,8] MAGE-A antigens are strictly tumor-specific, include 12 family members (MAGE-A1–MAGE-A12) and are expressed in various tumor tissues, including NSCLC,[9,10] laryngeal squamous cell carcinoma,[11] breast cancer,[12] bladder carcinoma[13] and glioma.[14] The expression of *MAGE-A* in blood and bone marrow has been detected using real-time reverse transcriptase (RT)-PCR analysis.[9,10] The prognostic significance of *MAGE-A* expression in the CTCs of patients with lung cancer is unknown. Because *MAGE-A1*, *MAGE-A2*, *MAGE-A3*, *MAGE-A4*, and *MAGE-A6* have highly similar sequences, it is difficult to detect these genes using specific primers.

In this study, we detected *MAGE-A* gene expression in the peripheral blood of patients with lung cancer using multiplex semi-nested PCR and analyzed their correlation with clinicopathological risk factors. We observed that peripheral blood in metastatic tumor patients had a higher checkout rate of multiple *MAGE-A* genes. Thus, *MAGE-A* gene expression in peripheral blood may act as a poor prognostic marker in patients with lung cancer.

Methods

Patients and clinical data

Clinicopathological data of 150 patients with lung cancer and 30 healthy volunteer donors were retrospectively obtained from the Department of Thoracic Surgery, the Fourth Hospital of Hebei Medical University, from September 2011 to September 2016. All patients provided written informed consent. The Medical Ethics Committee of the Fourth Hospital of Hebei Medical University approved the study.

The healthy volunteer donors had no history of carcinoma. None of the lung cancer patients had undergone preoperative adjuvant chemotherapy or radiotherapy. Clinicopathological data collected included gender, age, smoking, pathological type, histological grade, Union for International Cancer Control (UICC) stage, lymph node metastasis, distant metastasis, and tumor size.

Blood collection

Ten milliliters of blood was obtained from the volunteer donors and patients before surgery. Blood samples were collected in tubes containing sodium citrate and processed within four hours of collection. The blood samples collected from the 30 healthy volunteer donors were used as a negative control in reverse transcriptase (RT)-PCR assay.

RNA preparation and complementary DNA synthesis

Peripheral blood cells were collected with red blood cell lysis buffer. Total RNA from peripheral blood cells was extracted from using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. All RNA preparation and handling steps took place in a laminar flow hood, under RNAse-free conditions. Prior to RT-PCR analysis, the RNA extracted from peripheral blood was checked for quality using an ultraviolet spectrophotometer (EMCLAB Instruments GmbH & Co., Bad Wildbad, Germany) and agarose gel electrophoresis. The isolated RNA was dissolved in 20 μL of diethylpyrocarbonate water and stored at −80°C until used. The RNA concentration was routinely measured using a spectrophotometer, and its quality was evaluated by visualization after agarose gel electrophoresis and ethidium bromide staining. One microgram of total RNA was used to generate the first-strand complementary (c) DNA using a RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, Waltham, MD, USA) in a total volume of 20 μL. Reverse transcription was carried out at 42°C for 1 hour.

Multiplex semi-nested PCR

Molecular analysis of *MAGE-A* gene expression in lung tissues, including *MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4*, and *MAGE-A6*, was conducted using multiplex semi-nested RT-PCR. cDNA was amplified using Go Taq Green Master Mix (Promega, Madison, WI, USA). The PCR reaction mixture for the external product (first cycle) consisted of: 5 μL of cDNA product of the reverse transcription, 25 μL of PCR Master Mix (2×), 0.5 μL of primers (F1, 10 μM), 0.5 μL of primers (R1, 10 μM), 0.5 μL of primers (F2, 10 μM), 0.5 μL of primers (R2, 10 μM), and 18 μL of H_2O. The PCR reaction mixture for the internal product (second cycle) consisted of: 5 μL of external PCR product, 50 μL of PCR master mix (2×), 0.5 μL of primers (F3, 10 μM), 0.5 μL of primers (R3, 10 μM), 0.5 μL of primers (F4, 10 μM), 0.5 μL of primers (R4, 10 μM), and 43 μL of H_2O. The primers were derived from previous studies.[15] The expression of a housekeeping gene, *GAPDH*, was measured as an internal control. The first set of primers were: MAGE-1F: 5′-ACTGGCCCTGGCTGCAAC-3′, MAGE-1R: 5′-GCCCTGACCAG AGTCATCAT-3′(993bp), MAGE-1-1F: 5′-ACTGGCCTTGGCTGCAAC-3′, MAGE -1-1R: 5′-CCCTGACGAGAGTCATCATG-3′(965bp), The second set were: MAGE-1F:5′-ACTGGCCCTGGCTGCAAC-3′, MAGE-2R:5′-AGGCCCTG GGCCTGGTG-3′(914bp), MAGE1-1F:5′-ACTGGCCTTGGCTGCAAC-3′, MAGE -2-12R:5′-AGGCCCTGGGCTTGGTG-3′(893bp). GAPDH:

Forward: 5′-ACCTGACCTGCCGTCTAGAA-3′ Reverse: 5′-TCCACCACCCTGTTGCTGT A-3′.

To evaluate external and internal PCR reactions, the same PCR conditions were used: (i) initial denaturation at 95°C for 5 minutes; (ii) 32 cycles of denaturation at 95°C for 45 seconds, annealing at 65°C for 45 seconds, extension at 72°C for 1.5 minutes; and (iii) final extension at 72°C for 6 minutes. For *GAPDH*: (i) initial denaturation at 95°C for 5 minutes; (ii) 22 cycles of denaturation at 95°C for 15 seconds, annealing at 58°C for 15 seconds, extension at 72°C for 20 seconds; and (iii) final extension at 72°C for 6 minutes. PCR product was analyzed using 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

Restriction endonuclease treatment

In multiple *MAGE-A* positive blood specimens, the multiple *MAGE-A* products were treated with different restriction endonucleases (5 units of Bcl I, 5 units of EcoR I, 5 units of Eco47 III, 5 units of Sph I and 5 units of Afl III). The specific length of the restriction fragments is shown in Table 1. Restriction fragments were analyzed using 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistical analysis

All statistical analyses were conducted using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). The potential association between *MAGE-A* gene expression and clinicopathological risk factors was analyzed using chi-square or Fisher's exact tests, as appropriate. Overall survival was estimated using the Kaplan–Meier method. Univariate and multivariate analysis of overall survival for prognostic factors was analyzed by Cox proportional hazards regression model. $P < 0.05$ was considered statistically significant.

Results

Expression of MAGE-A genes in peripheral blood

We examined the expression of *MAGE-A* genes, including *MAGE-A1*, *MAGE-A2*, *MAGE-A3*, *MAGE-A4*, and *MAGE-A6*, in the blood samples of 150 lung cancer patients and 30 healthy donors using multiplex semi-nested RT-PCR.

Table 1 Restriction endonuclease for multiple *MAGE-A* gene products, and the restriction fragments for each *MAGE-A* gene

Restriction endonuclease	*MAGE* gene	PCR product length (bp)	Fragment length (bp)
Bcl I	*MAGE-A1*	893	106,787
Sph I	*MAGE-A2*	914	21,22,151,720
EcoR I	*MAGE-A3*	914	167,747
Eco47 III	*MAGE-A4*	917	375,542
Afl III	*MAGE-A6*	914	22,172,282,438

MAGE-A was not detected in the peripheral blood samples of the 30 healthy donors but was found in 26 of the 150 (17.3%) lung cancer samples (Fig 1a). Figure 1b shows the representative lung cancer blood samples with positive *MAGE-A* expression after internal PCR (second PCR cycle).

After restriction endonuclease treatment of multiple *MAGE-A* products (second PCR cycle), the expression pattern of each *MAGE-A* gene was identified. *MAGE-A* was expressed in the peripheral blood of lung cancer patients in the following order: *A2 > A6 > A4 > A3 > A1.* As shown in Table 2, *MAGE-A1* was positive in 2.7% (4/150) of patients, *MAGE-A2* in 15.3% (23/150), *MAGE-A3* in 9.3% (14/150), *MAGE-A4* in 12% (18/150), and *MAGE-A6* in 14% (21/150). Five patients were positive for only one *MAGE-A* gene, one for two genes, nine for three genes, 10 for four genes, and one patient for all five genes.

Examples of restriction endonuclease treatment of the multiple *MAGE-A* products from two patients are shown in Figure 2. Multiple *MAGE-A* products were found in the peripheral blood of patients 35 and 42. The multiple *MAGE-A* products (second PCR cycle) were digested with Bcl I, Sph I, EcoR I, Eco47 III, and Afl III, respectively. The digestion products were then separated by agarose gel electrophoresis. We observed fragment patterns to identify the individual *MAGE-A* genes. In patient 35, no *MAGE-A1* or *MAGE-A4* fragments were observed, but a 720 bp fragment was observed after Sph I digestion, 747 bp after EcoR I digestion, and 438 bp after Afl III digestion, indicating that *MAGE-A2, MAGE-A*3, and *MAGE-A6* existed in the PCR product (Fig 2a). The *MAGE-A* expression pattern in

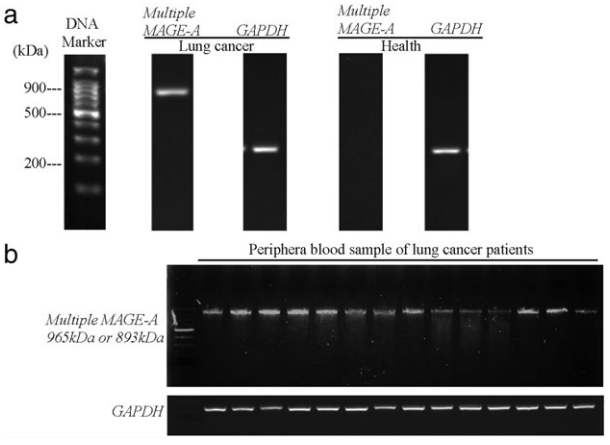

Figure 1 (**a**) Representative blood samples of multiple *MAGE-A* and *GAPDH* control products of the internal PCR (second PCR cycle) of lung cancer patients and healthy volunteers. (**b**) Representative blood samples with positive *MAGE-A* products of the internal PCR (second PCR cycle) in patients with lung cancer.

Table 2 Expression rate of *MAGE-A* genes in peripheral blood of lung cancer patients

Group	*MAGE-As*	*MAGE-A1*	*MAGE-A2*	*MAGE-A3*	*MAGE-A4*	*MAGE-A6*
Positive case	26	4	23	14	18	21
Positive rate	17.3%	2.7%	15.3%	9.3%	12%	14%

patient 42 is shown in Figure 2b. No *MAGE-A1, MAGE-A3* or *MAGE-A6* expression was observed, but a 720 bp fragment was observed after Sph I digestion, and 542 bp was after Eco47 III digestion, indicating that *MAGE-A2* and *MAGE-A4* existed in the PCR product.

Correlation between MAGE-A expression in peripheral blood and clinicopathological factors in lung cancer patients

The correlation between *MAGE-A* gene expression in peripheral blood and the clinicopathological factors of patients with lung cancer was statistically evaluated (Table 3). Multiple *MAGE-A* expression in the peripheral blood of lung cancer patients was positively associated with age ($P = 0.029$), UICC stage ($P < 0.001$), tumor size ($P < 0.001$), lymph node metastasis ($P < 0.001$), and distant metastasis ($P < 0.001$). No correlation was observed for multiple *MAGE-A* gene expression and gender, smoking, pathological type, or histological grade.

MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, and *MAGE-A6* were expressed more frequently in patients with distant metastasis compared to patients without distant metastasis (all $P < 0.001$). *MAGE-A2* expression was positively correlated with gender ($P < 0.001$) and age ($P = 0.004$) in lung cancer patients. *MAGE-A2* ($P < 0.001$), *MAGE-A3* ($P = 0.007$), *MAGE-A4* ($P = 0.001$), and *MAGE-A6* ($P < 0.001$) expression was positively correlated with UICC stage in lung cancer patients. *MAGE-A2* ($P < 0.001$), *MAGE-A4* ($P = 0.001$), and *MAGE-A6* ($P = 0.001$) expression was positively correlated with tumor size in patients with lung cancer. *MAGE-A2* ($P < 0.001$), *MAGE-A3* ($P = 0.004$), *MAGE-A4* ($P < 0.001$), and *MAGE-A6* ($P < 0.001$) expression was positively correlated with lymph node metastasis in lung cancer patients. No other correlations were observed between individual *MAGE-A* gene expression and clinicopathological factors.

Correlation between MAGE-A gene expression in peripheral blood and overall survival in lung cancer patients

The 150 lung cancer patients were followed-up for three to 60 months; only 10 patients were lost to follow-up. Figure 3 shows the Kaplan–Meier plots of multiple and individual *MAGE-A* gene expression levels in relation to overall survival. Overall survival of patients with multiple *MAGE-A* expression in peripheral blood was significantly lower than in patients with single *MAGE-A* gene expression ($P = 0.001$). Moreover, low overall survival was also associated with individual *MAGE-A* gene expression: *MAGE-A1*, $P = 0.143$; *MAGE-A2*, $P = 0.001$; *MAGE-A3*, $P < 0.001$; *MAGE-A4*, $P < 0.001$; and *MAGE-A6*, $P = 0.032$.

To further evaluate the prognostic significance of *MAGE-A* expression we performed univariate analysis of other clinicopathological factors in relation to overall survival. *MAGE-A* expression ($P = 0.047$), clinical stage ($P = 0.021$), lymph node metastasis ($P < 0.001$), and distant metastasis ($P < 0.001$) were prognostic factors for

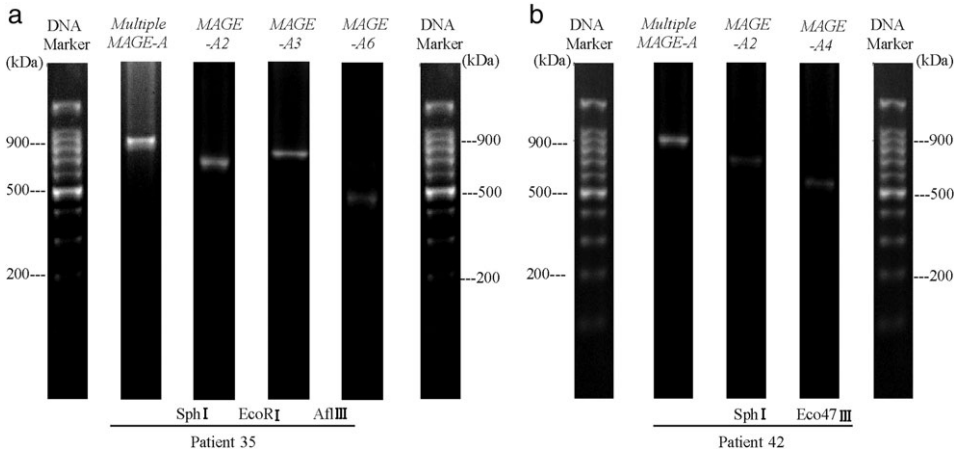

Figure 2 *MAGE-A* expression pattern by restriction endonuclease treatment in: (**a**) patient 35 and (**b**) patient 42.

Table 3 Correlation between *MAGE-A* expression in peripheral blood and clinicopathological factors in patients with lung cancer

Clinicopathological factors	N	Multiple *MAGE-A* *n* (%)	*MAGE-A1* *n* (%)	*MAGE-A2* *n* (%)	*MAGE-A3* *n* (%)	*MAGE-A4* *n* (%)	*MAGE-A6* *n* (%)
Gender							
Men	107	17 (10.3)	2 (1.9)	6 (5.6)	8 (7.8)	13 (12)	15 (14)
Women	43	9 (34.9)	2 (4.7)	17* (40.5)	6 (14)	5 (12)	6 (14)
Age/year							
< 60	81	9(11)	2 (2)	6 (7)	7 (7)	7 (7)	11 (14)
≥ 60	69	17* (25)	2 (3)	17* (25)	7 (10)	11 (16)	10 (14)
Smoking							
Yes	102	18 (18)	2 (2)	18 (18)	10 (10)	14 (14)	16 (16)
No	48	8 (17)	2 (4)	5 (10)	4 (8)	4 (8)	5 (10)
Pathological type							
Adenocarcinoma	68	12 (18)	2 (3)	10 (15)	7 (10)	6 (9)	11 (17)
Squamous cell carcinoma	44	5 (11)	1 (2)	5 (11)	4 (10)	4 (10)	5 (11)
Other	38	9 (24)	1 (3)	8 (21)	3 (8)	8 (21)	5 (13)
Histological grade							
G1	52	5 (10)	1 (2)	4 (8)	3 (6)	4 (8)	6 (12)
G2	47	8 (17)	1 (2)	10 (21)	5 (11)	5 (11)	6 (13)
G3	51	13 (25)	2 (4)	9 (18)	6 (12)	9 (18)	9 (18)
UICC stage							
I–II	93	4(4)	1 (1)	4 (4)	4 (4)	5 (5)	5 (5)
III–IV	57	22* (39)	3 (5)	19* (33)	10* (18)	13* (23)	16* (28)
Tumor size (cm)							
≤ 5	80	4(5)	1 (1)	3(4)	4 (5)	3 (4)	4 (5)
> 5	70	22* (31)	3 (4)	20* (29)	10 (14)	15* (21)	17* (24)
Lymph node metastasis							
N_0	112	7 (6)	2 (2)	7 (6)	6 (5)	6 (5)	7 (6)
N_+	38	19* (50)	2 (5)	16* (42)	8* (21)	12* (32)	14* (37)
Distant metastasis							
M_0	132	11 (8)	1 (1)	11 (8)	5 (4)	10 (8)	11 (8)
M_+	18	15* (83)	3* (17)	12* (67)	9* (50)	8* (44)	10* (56)

*Significant difference, $P < 0.05$. UICC, Union for International Cancer Control.

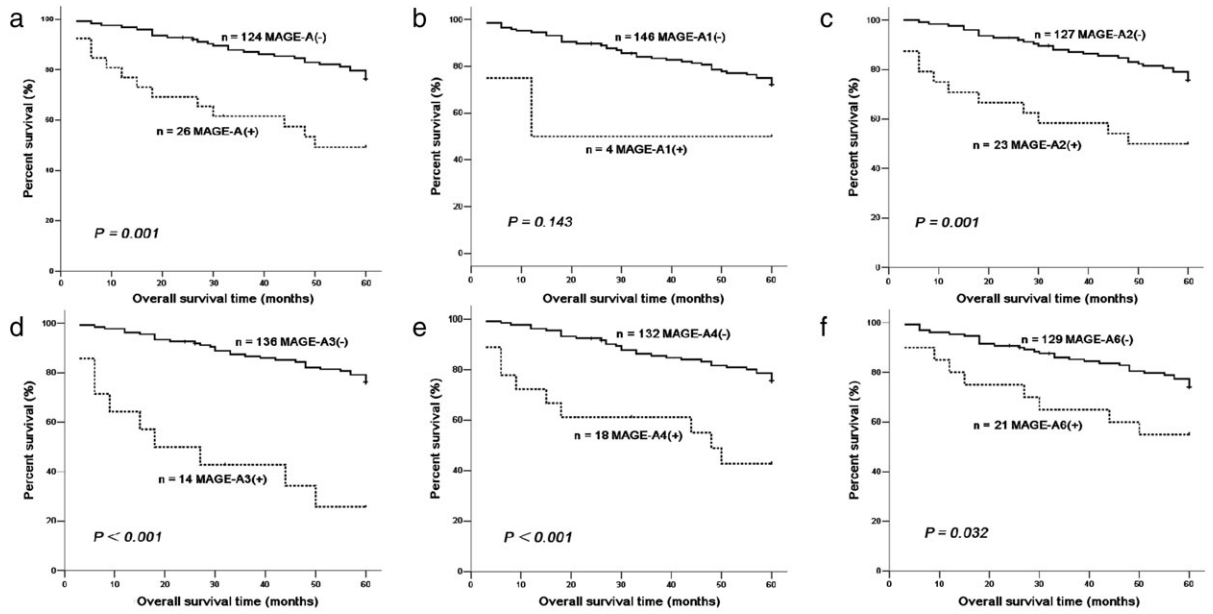

Figure 3 Kaplan–Meier curves showing overall survival in relation to (**a**) multiple *MAGE-A* and (**b**–**f**) each *MAGE-A* member.

overall survival (Table 4). Lymph node and distant metastases were used for logistic regression analysis (tumor size, $P > 0.05$). Multivariate analysis showed significantly longer survival in patients without lymph node or distant metastases, while patients with multiple *MAGE-A* expression achieved longer survival (Table 4).

Discussion

A significant part of the development of tumor metastasis is the spread of tumor cells into blood circulation. CTCs appear in the blood at very low frequencies. It is estimated that every million white blood cells has < 1 CTC. Identifying and isolating CTCs is of primary importance. Many techniques are used to identify and isolate CTCs, but the immunomagnetic CellSearch system (Veridex, Raritan, NJ, USA) is the gold standard for detecting CTCs, and has been approved by the US FDA for predicting metastasis in breast, prostate, and colorectal cancers.[16,17] However, the technical platform based on cell surface markers cannot detect the CTCs that are downregulated or missing epithelial markers. Therefore, it is necessary to find new markers to identify CTCs in the peripheral blood of patients with malignant tumors.

Most studies detect CTCs using immunocytochemistry and RT-PCR. Foreign scholars have examined CTC messenger RNA levels in the peripheral blood of patients with primary breast and colorectal cancers using multiplex PCR.[18,19] There are few primer sites aimed at single genes because of the highly homologous sequences of the *MAGE-A* gene. In this study, we identified multiple *MAGE-A* genes (*MAGE-A1*, *MAGE-A2*, *MAGE-A3*, *MAGE-A4*, and *MAGE-A6*) as potential markers for detecting CTCs in the peripheral blood of patients with lung cancer using multiple semi-nested PCR and restriction endonuclease treatment. Although a variety of tumor tissues express *MAGE-A* genes, there is currently no evidence that *MAGE-A* genes can be ideal markers for CTCs in cancer patients.

In this study, we designed two primer sites covered by two forward and two reverse primers, and used amplified hydrolysis products (MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4 and MAGE-A6) and multiplex semi-nested PCR. *MAGE-A* expression was found in 26 of the 150 (17.3%) blood samples of lung cancer patients. Each *MAGE-A* gene was detected by the corresponding restriction endonuclease treatment. *MAGE-A1* was positively expressed in the peripheral blood of 2.7% (4/150) of patients, *MAGE-A2* in 15.3% (23/150), *MAGE-A3* in 9.3% (14/150), *MAGE-A4* in 12% (18/150), and *MAGE-A6* in 14% (21/150). The relationship between *MAGE-A* gene expression and clinicopathological factors was then analyzed in lung cancer patients. Statistical analysis showed that the rate of *MAGE-A* and *MAGE-A2* gene expression increases with age. This could be related to the methylation state and instability factors of the body that increase with age.[20] Gene demethylation may occur with age and induces silent gene expression.[20] The expression of *MAGE-A* in lung cancer was correlated with clinical stage, tumor size,

Table 4 Univariate and multivariable analyses of prognostic factors for overall survival of lung cancer

	Univariate analysis			Multivariate analysis		
Variable	HR	*P*	95% CI	HR	*P*	95% CI
MAGE-A expression Positive vs. negative	1562	0.047*	1.006–2.426	9.073	< 0.001*	3.405–24.178
Gender Male vs. female	1.363	0.093	0.949–1.958			
Age (years) <60 vs. ≥ 60	1.122	0.497	0.805–1.563			
Smoking Yes vs. no	1.317	0.117	0.933–1.859			
Pathological type Adenocarcinoma vs. squamous cell carcinoma vs. other	1.192	0.101	0.967–1.469			
Tumor size (cm) ≤ 5 vs. > 5	1.179	0.331	0.846–1.642			
Histological grade I vs. II vs. III	1.033	0.744	0.849–1.255			
Clinical stage I and II vs. III and IV	1.495	0.021*	1.061–2.105			
Metastatic state of lymph node Yes vs. no	3.309	< 0.001*	2.203–4.971	11.803	< 0.001*	4.683–29.748
Distant metastasis Yes vs. no	7.372	< 0.001*	4.289–12.672	5.939	< 0.001*	2.756–12.795

*Significant difference, $P < 0.05$. CI, confidence interval; HR, hazard ratio.

lymph node metastasis, and distant metastasis. Previous studies have reported that CTCs are more common in advanced metastatic malignant tumors.[21] Under normal conditions, the larger the neoplasm, the easier the tumor cells detach and enter the blood system. After entering peripheral blood, tumor cells can be recognized and removed by the human immune system. However, when immune function is low, part of the CTCs is able to survive. Lianidou *et al.* showed that CTCs could be used as an indicator of recurrence and metastasis in breast cancer.[22] In our study, *MAGE-A* gene messenger RNA could only be detected when tumor cells entered the peripheral blood of patients with lung cancer. As such, the *MAGE-A* gene may represent a specific marker in the peripheral blood of lung cancer patients. Multiple *MAGE-A* expression in the peripheral blood of patients with lung cancer was positively associated with poorer outcomes, including lymph node and distant metastases.

Regarding the individual *MAGE-A* genes, *MAGE-A1*, *MAGE-A2*, *MAGE-A3*, *MAGE-A4*, and *MAGE-A6* expression was more frequent in patients with distant metastasis compared to patients without. In lung cancer patients, *MAGE-A2*, *MAGE-A3*, *MAGE-A4*, and *MAGE-A6* expression was positively associated with lymph node metastasis; *MAGE-A2*, *MAGE-A4*, and *MAGE-A6* expression was positively associated with tumor size; and *MAGE-A2*, *MAGE-A3*, *MAGE-A4*, and *MAGE-A6* expression was positively associated with UICC stage. These results indicated that *MAGE-A* expression may represent an important biological marker to evaluate prognosis in patients with lung cancer.

Multiple *MAGE-A* expression in the peripheral blood of patients with lung cancer was positively correlated with distant metastasis. Further survival analysis revealed that overall survival of patients with multiple *MAGE-A* or *MAGE-A2*, *MAGE-A3*, *MAGE-A4*, and *MAGE-A6* expression in peripheral blood was significantly lower than in patients without the corresponding expression.

Multivariate regression analysis was performed including factors that were filtered by binary logistic regression. Multiple *MAGE-A* expression and lymph node and distant metastases may be risk factors for five-year survival of lung cancer patients. Our study was conducted using patient data over five years and our follow-up duration was 3–60 months, which may represent a limitation. These limitations may have affected the results of statistical analysis.

Our study results are the first to suggest the significance of *MAGE-A* expression in the peripheral blood of patients with lung cancer. Multiple *MAGE-A* expression may prove to be a novel and reliable prognostic marker for patients with lung cancer.

The detection rate of *MAGE-A* expression was higher in advanced tumors, which may limit the application of this biomarker for early stage cancer. In addition, the *MAGE-A* family has been divided into several subfamilies and not all tumor cells express the *MAGE-A* gene. Some tumor cells express other *MAGE* family members; therefore, simultaneous determination of *MAGE-A* genes and other markers will better estimate prognosis.

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Disclosure

No authors report any conflict of interest.

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