ORIGINAL RESEARCH

Expression and Clinical Significance of MAGE-A Proteins and mRNA in Lung Cancer Patients: A Retrospective Study

Wen Zhu, MD; Hai-Qin Xie, MD; You-Qin Xie, MD; Xue-Dong Lv, MD

ABSTRACT

Objective • This study investigated the expression and clinical significance of Melanoma Associated Antigen (MAGE)-A proteins and mRNA in patients with non-small cell lung cancer (NSCLC).

Methods • A retrospective study was conducted, and we selected a cohort of 88 NSCLC patients treated at our hospital from January 2015 to January 2020. Adjacent tissues were chosen as controls. The expression of MAGE-A proteins in lung cancer and adjacent tissues was assessed via Western blot, while MAGE-As mRNA expression was measured using RT-PCR.

Results • The relative expression levels of MAGE-A proteins and mRNA in NSCLC tissues were significantly higher than those in adjacent tissues ($P < .05$), with values of (0.343 ± 0.101) and (0.728 ± 0.112), respectively. Furthermore, MAGE-As protein expression was significantly higher in stage III - IV lung cancer compared to stage I - II ($P < .05$). No significant differences were observed in MAGE-A protein expression concerning gender, age, tumor diameter, pathological type, and differentiation degree ($P > .05$). The relative expression of MAGE-As mRNA was significantly higher in clinical stage III - IV and moderately differentiated lung cancer tissues compared to stage I - II and well-differentiated tissues ($P < .05$). No significant differences were found in MAGE-As mRNA expression concerning gender, age, tumor diameter, and pathological type ($P > .05$). Patients with high MAGE-As mRNA expression had a significantly shorter median overall survival of 33 months (95% CI: 31.64-34.36) compared to those with low MAGE-As mRNA expression ($P < .05$). However, no significant difference was observed in median overall survival between patients with high and low MAGE-As protein expression ($P > .05$).

Conclusions • In NSCLC, the up-regulation of MAGE-A proteins and mRNA is associated with clinical stage and differentiation degree, warranting further investigation. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Lung cancer, a prevalent thoracic malignant tumor, predominantly comprises non-small cell lung cancer (NSCLC), accounting for over 80% of cases.[1] Currently, lung cancer ranks highest in both the number of new diagnoses and fatalities among malignant tumors.[2] Despite advancements in modern therapeutic approaches, the 5-year survival rate for lung cancer patients remains below 20%.[1-2] Consequently, research efforts are increasingly directed towards biological targeted therapy.[3]

In recent years, melanoma-associated antigen (MAGE) has emerged as one of the few tumor-specific antigens recognized by cytotoxic T lymphocytes, having a substantial 46% total expression rate in lung cancer. However, clinical studies on MAGE tissue expression and gene structure, particularly in NSCLC, exhibit variations.[4] This study analyzed the expression of MAGE-A proteins and mRNA in NSCLC to provide valuable clinical guidance and a robust foundation for further research and therapeutic advancements.

MATERIALS AND METHODS

Study Design

This study employed a retrospective design to investigate the expression and clinical significance of MAGE-A proteins and mRNA in NSCLC. The research cohort included 88 NSCLC patients who received treatment at our hospital between January 2015 and January 2020. Adjacent tissues were utilized as controls for comparative analysis. This cohort consisted of 47 males and 41 females; 43 patients aged < 60

years old and 38 patients aged ≥60 years old; Pathological types: 33 cases of adenocarcinoma, 55 cases of squamous cell carcinoma; Clinical stages: Stage I to stage II in 50 cases, stage III to stage IV in 38 cases; Differentiation degree: 23 cases were highly differentiated, and 65 cases were moderately differentiated, and patients with paracancer tissue (>5 cm) was selected as the control. This study received ethical approval from the Ethics Committee of Nantong First People's Hospital, the Second Affiliated Hospital of Nantong University.

Inclusion and Exclusion Criteria

Inclusion criteria were as follows: (1) all patients were histologically confirmed to have non-small cell lung cancer; (2) no antitumor therapy was administered before surgical intervention; (3) patients with complete clinical follow-up data were included.

Exclusion criteria were as follows: (1) patients with a history of cancer recurrence; (2) individuals with concomitant systemic malignancies; (3) those diagnosed with chronic obstructive pulmonary disease or other lung-related disorders were excluded from this study.

Experimental Procedures

Tissue Sample Preparation. Conventional pathological sections of wax blocks containing non-small cell lung cancer tissue were subjected to HE staining. Using a tissue chip fabricator, recipient wax blocks were punctured to create holes, and the corresponding tissue numbers were carefully documented within these array holes. Subsequently, 4 μm-thick continuous sections were crafted to prepare tissue chip sections.

Immunohistochemistry. Murine monoclonal antibodies against human MAGE-A (6C1), provided by Abcam (UK), were employed. The tissue chip slices underwent an overnight incubation at 67°C. Next, they were subjected to dewaxing with xylene I and xylene II for 15 minutes each, followed by immersion in anhydrous ethanol I for 20 minutes, anhydrous ethanol II for 20 minutes, 95% ethanol for 10 minutes, and 80% ethanol for 10 minutes. Subsequently, the sections were rinsed with tap water three times and soaked in a buffer solution three times for 5 minutes each. Antigen repair was conducted using EDTA antigen repair solution in a pressure cooker for 4 minutes.

The tissue sections were treated with 6% hydrogen peroxide and incubated in darkness at room temperature for 20 minutes. After further rinsing in buffer solution (3 times for 5 minutes each), they were sealed with goat serum and incubated in a humidified chamber at 37°C for 45 minutes. Diluted monoclonal antibodies against MAGE-As (1:200) were added and incubated overnight at 4°C. Following another set of buffer solution soaks (3 times for 5 minutes each), the second-generation biotin-labeled goat anti-mouse secondary antibody working solution was applied and incubated at 37°C for 30 minutes.

Following immersion in the buffer solution, the horseradish peroxidase-labeled streptavidin working solution was introduced and incubated for 30 minutes. After thorough rinsing with the buffer solution, color development was achieved using the DAB solution as per the manufacturer's instructions. Hematoxylin staining was reapplied for 3 minutes, and the sections were briefly immersed in hydrochloric acid and alcohol to induce a color change to pink. Subsequently, the samples were examined under a microscope.

Exosome and DNA Extraction. A total of 4 ml of fasting venous blood was extracted from each patient. After centrifugation at 3000 RPM for 15 minutes, serum was separated, and exosomes in the plasma were isolated using the ExoQuick exosome precipitation solution kit. High-purity exosome DNA was subsequently extracted utilizing an optimized DNA extraction formula. 1 μg of DNA was subjected to vulcanization with a sulfite conversion kit, converting unmethylated cytosine of single-stranded DNA into uracil. The modified DNA was stored at -20°C.

PCR Analysis. MAGE-A gene detection was performed using a PCR test with the following thermal cycle conditions: 95°C for 5 minutes of predenaturation, followed by 30 cycles of 95°C for 15 seconds, 58°C for 15 seconds, and 72°C for 20 seconds. Extension at 72°C was carried out for 6 minutes. After the reaction was completed, the reaction was stored in the refrigerator at -20°C, then the PCR amplification product (7 μl) was loaded onto a 1.2% agarose gel for electrophoresis at a constant pressure of 80 V for 40 minutes. The amplification product of the MAGE-A gene served as the internal reference, and the scanning results were analyzed using Gel-Pro Analyzer 3.1 software. Clear and specific DNA bands were observed and recorded.

Follow-up Methods

Patient hospitalization records, outpatient reviews, and phone calls were carefully documented. Additionally, patient information was tracked through home visits, phone inquiries, and various other methods to gather comprehensive clinical data.

Statistical Analysis

Data analysis was conducted using SPSS 22.0 software (IBM, Armonk, NY, USA). MAGE-A proteins and mRNA data were presented as means with standard deviations ($\bar{x} \pm s$), and differences among all parameters were assessed using the t test. Survival analysis was performed using the Kaplan-Meier method, with a significance level set at $P < .05$.

RESULTS

Comparison of MAGE-As Protein and mRNA Expression in NSCLC and Paracancer Tissue

The relative expression levels of MAGE-As protein and mRNA in non-small cell lung cancer tissue were significantly elevated compared to paracancer tissue ($P < .05$). Refer to Table 1 and Figure 1 for details.

Association Between MAGE-As Protein and Clinicopathological Characteristics of Patients

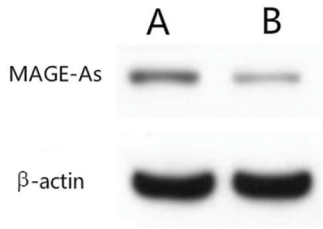
The relative expression level of MAGE-As protein was markedly higher in clinical stage III-IV lung cancer tissues compared to stage I-II lung cancer tissues ($P < .05$). However, no significant differences were observed in the relative expression

Table 1. Comparison of Protein and mRNA Expression of MAGE-As in NSCLC and Paracancer Tissue

Group	n	Relative Expression of MAGE-As protein	Relative Expression of MAGE-As mRNA
Non-Small Cell Lung Cancer Group	88	0.343±0.101	0.728±0.112
Paracancer Tissue Group	88	0.113±0.042	0.215±0.092
t		19.725	33.202
P value		.000	.000

Note: The table presents a comparison of the relative expression of MAGE-As protein and mRNA between the non-small cell lung cancer group and the paracancer tissue group. "n" represents the number of samples, and values for relative expression are presented as mean ± standard deviation. The statistically significant differences in both protein and mRNA expression levels between the two groups ($P < .001$) indicate a substantial up-regulation of MAGE-As in non-small cell lung cancer tissues compared to paracancer tissues.

Figure 1. Western Blot Analysis of MAGE-As Protein Expression.



Note: Western blot analysis comparing the expression of MAGE-As protein in non-small cell lung cancer (NSCLC) tissues (A) and paracancer tissues (B). The analysis reveals significantly higher MAGE-As protein levels in NSCLC tissues compared to paracancer tissues ($P < .05$).

Table 2. Protein Expression of MAGE-As and Clinicopathologic Characteristics of Patients

Clinicopathologic Features	n	Relative Expression of MAGE-As Protein	*t*	*P* value
Gender				
Male	47	0.341±0.100	-0.185	.853
Female	41	0.345±0.102		
Age				
<60	43	0.346±0.104	0.272	.786
≥60	45	0.340±0.103		
Tumor Diameter				
<5 cm	50	0.342±0.101	-0.091	.928
≥5 cm	38	0.344±0.103		
Pathological Type				
Adenocarcinoma	33	0.340±0.105	-0.214	.831
Squamous Cell Carcinoma	55	0.345±0.107		
Clinical Staging				
I~II	50	0.311±0.103	-3.352	.001
III~IV	38	0.385±0.102		
Degree of Differentiation				
Highly Differentiated	23	0.345±0.105	0.119	.905
Medium-Low Differentiation	65	0.342±0.103		

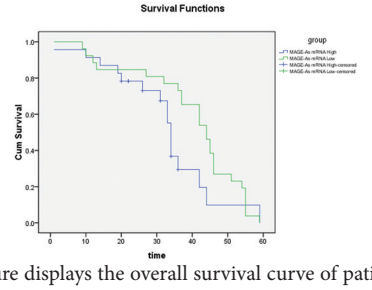
Note: This table presents the protein expression of MAGE-As in relation to various clinicopathologic characteristics of the patients. "n" represents the number of patients in each category, and values for relative expression are presented as mean ± standard deviation. Statistically significant differences are observed in MAGE-As protein expression between clinical stages (I-II and III-IV), with a *P* value of .001, indicating a significant association with the clinical stage. However, no significant differences are observed in protein expression with respect to gender, age, tumor diameter, pathological type, or degree of differentiation.

Table 3. mRNA Expression of MAGE-As and Clinicopathological Characteristics of Patients

Clinicopathologic Features	n	Relative Expression of MAGE-As mRNA	*t*	*P* value
Gender				
Male	47	0.730±0.108	0.176	.861
Female	41	0.726±0.105		
Age				
<60	43	0.724±0.110	-0.343	.733
≥60	45	0.732±0.109		
Tumor Diameter				
<5 cm	50	0.712±0.108	-1.573	.119
≥5 cm	38	0.749±0.111		
Pathological Type				
Adenocarcinoma	33	0.731±0.109	0.205	.838
Squamous Cell Carcinoma	55	0.726±0.112		
Clinical Staging				
I~II	50	0.702±0.108	-2.541	.013
III~IV	38	0.762±0.112		
Degree Of Differentiation				
Highly Differentiated	23	0.688±0.106	-2.085	.040
Medium-Low Differentiation	65	0.742±0.107		

Note: This table presents the mRNA expression of MAGE-As in relation to various clinicopathological characteristics of the patients. "n" represents the number of patients in each category, and values for relative expression are presented as mean ± standard deviation.

Figure 2. Overall Survival Curve Based on MAGE-As mRNA Expression



Note: The figure displays the overall survival curve of patients with varying levels of MAGE-As mRNA expression, indicating a potential association between elevated MAGE-As mRNA expression and reduced overall survival.

level of MAGE-As protein in lung cancer tissues concerning gender, age, tumor diameter, pathological type, and differentiation degree ($P > .05$). Refer to Table 2 for further details.

MAGE-As mRNA and Clinicopathological Characteristics of Patients

The relative expression level of MAGE-As mRNA was significantly elevated in clinical stage III-IV and poorly differentiated lung cancer tissues compared to stage I-II and highly differentiated lung cancer tissues ($P < .05$). However, no significant differences were observed in the relative expression level of MAGE-As mRNA in lung cancer tissues regarding gender, age, tumor diameter, and pathological types ($P > .05$). Refer to Table 3 for further details.

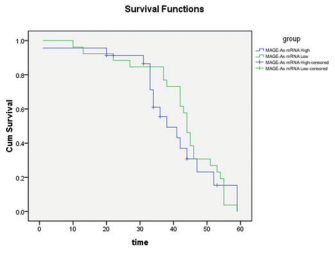
Comparison of Overall Survival Time in Patients with Different MAGE-As mRNA Expression

The median overall survival time for patients with high MAGE-As mRNA expression (≥0.728) was 33 months (95% CI: 31.64-34.36), which was significantly shorter than that of patients with low MAGE-As mRNA expression (95% CI: 41.39-46.61) ($\chi^2 = 4.416$, $P = .036 < .05$), as illustrated in Figure 2.

Comparison of Overall Survival Time in Patients with Different MAGE-As Protein Expression

The median overall survival time for patients with high (≥0.343) and low (<0.343) expression of MAGE-As protein

Figure 3. Overall Survival Curve Based on MAGE-As Protein Expression



Note: This figure illustrates the overall survival curve of patients categorized by their MAGE-As protein expression levels.

was 36 months (95% CI: 34.52-37.38) and 36 months (95% CI: 35.04-36.96), respectively. Notably, the difference between these two groups was not statistically significant ($\chi^2 = 1.003$, $P = .316 > .05$), as illustrated in Figure 3.

DISCUSSION

Lung cancer has emerged as a prevalent disease with high incidence rates, posing a substantial threat to the lives of the Chinese population. Currently, it is one of the leading causes of morbidity and mortality among males.[5] Timely diagnosis and proactive treatment of lung cancer hold significant importance in enhancing patient prognosis.[5,6] In recent years, the rapid advancements in tumor immunology have highlighted that tumor development is not solely attributed to the inherent genetic characteristics of malignant cells. Instead, it is intricately linked with alterations in human immune function.[7,8]

In the past, clinical diagnosis of lung cancer primarily relied on conventional methods such as pathological examination and imaging scans. However, certain patients may exhibit subtle or even no early symptoms, rendering these diagnostic approaches less specific, particularly in the detection of small lesions.[9,10] With the advancements in modern cell biology, specific tumor markers have assumed a crucial role in the diagnosis of malignant tumors.[10] Apoptosis exerts a significant influence on tumor development. Tumorigenesis is intricately linked to the dysregulation of apoptosis, and genetic alterations in this process can trigger cell transformation and, ultimately, contribute to the initiation and sustained progression of malignant tumors.[11,12]

The melanoma-associated antigens (MAGEs) family is a prominent subset of the Cancer/testis antigen (CTA) family. Distinguished by differences in tissue expression patterns and gene structure, the MAGE family is categorized into two subfamilies: type I MAGEs, encompassing MAGE-A, -B, and -C, and type II MAGEs, including MAGE-D, -E, -F, -G, -H, -L, and Necdin.[13] Within the MAGE-A family, consisting of 12 members, these antigens find expression in various tumor tissues, such as laryngeal squamous cell carcinoma, esophageal carcinoma, breast cancer, and head and neck tumors. However, the expression profile of the MAGE-A family in NSCLC remains unclear.[14,15]

This study analyzed the expression profiles of MAGE-As protein and mRNA in NSCLC. The melanoma antigen gene family encompasses genes that encode and yield highly conserved proteins. MAGE protein family members are typically present in eukaryotes and can exhibit aberrant expression patterns in both testicular and multiple cancer tissues. Predominantly, MAGEs find expression in various tumor tissues, with their expression in normal tissues limited primarily to testicular antigens, known as cancer testicular antigens.[13-15]

The current understanding of the clinical biological functions of MAGE proteins remains incomplete. Some researchers have suggested a partial association between the inactivation of the terminal 107 amino acids and the gene-related zinc finger protein 1, which can elevate p53 levels *in vivo*, subsequently inducing cell apoptosis.[16] Conversely, other studies have indicated that MAGE proteins assume a highly invasive role in tumors, participating in early tumorigenesis and regulating cell proliferation and invasion. Notably, their expression may be linked to drug resistance in lung cancer, with higher expression rates correlating with a greater likelihood of drug resistance emerging within a shorter timeframe. These findings collectively emphasize the close relationship between MAGEs and tumor growth.[17]

At present, there is a prevailing acceptance that methylation/demethylation within the gene promoter region plays a pivotal role in influencing the expression and regulation of MAGE-A. Under normal conditions, the MAGE-A gene remains in a silent state. However, upon the formation of malignant tumors, an abnormal demethylation process occurs, leading to an upsurge in MAGE-A expression, thereby influencing tumor progression.[18]

In this study, the clinicopathological analysis of patients revealed that the relative expression level of MAGE-As protein was significantly higher in tissues of clinical stage III ~ IV compared to tissues of stage I ~ II, suggesting a correlation between MAGE-As protein expression and tumor stage. Furthermore, the relative expression level of MAGE-As mRNA in clinical stage III-IV and low-differentiation tissues was markedly elevated in comparison to stage I-II and highly differentiated tissues, indicating an association between MAGE-As mRNA expression and tumor stage as well as differentiation degree.

Our findings suggest a significant correlation between MAGE gene expression and the state of histone acetylation. In terms of histone acetylation, methylated CpG binding proteins play a pivotal role by tightly binding to methylated DNA sequences, thereby enhancing histone deacetylase activity. This activity, in turn, influences the regulation of MAGE expression.[18,19]

In this study, it was observed that the relative expression levels of MAGE-As protein and mRNA were significantly elevated in non-small cell lung cancer tissues compared to adjacent tissues, indicating a high expression of MAGE-As protein and mRNA in non-small cell lung cancer patients. Notably, the MAGE-As protein exhibited pronounced overexpression in non-small cell lung cancer tissues, exerting

a promotive influence on lung cancer progression. Previous investigations have revealed that the accumulation of MAGE-As protein within the nucleus of lung cancer cells inhibits apoptosis and reduces the sensitivity of these cells to chemotherapeutic agents. It suggests that MAGE-As protein serves as a tumor-promoting factor.

MAGE-As undergoes proteasome processing to generate a C-terminal fragment that inhibits apoptosis, ultimately diminishing the activity of p53.[20,21] The expression level of MAGE serves as a diagnostic indicator, distinguishing small recurrent or metastatic tumor foci from other conditions, such as incomplete excised foci or untreated primary foci. Moreover, it can evaluate the sensitivity and specificity of early recurrence and metastasis in lung cancer patients. Additionally, this phenomenon may be linked to the hypomethylation state of the MAGE gene. DNA hypomethylation increases gene instability, inducing the expression of this gene and heightening susceptibility to tumorigenesis.[22]

This study suggests that non-small cell lung cancer patients exhibit a heightened expression of MAGE-As protein and mRNA. Additionally, our analysis of MAGE-As protein and mRNA expression in non-small cell lung cancer tissues among patients has further confirmed the existence of a correlation between the stage of the disease and its differentiation degree. These findings establish a foundation for the early clinical diagnosis and assessment of the stage, type, metastasis, and differentiation degree in lung cancer patients.

Study Limitations

The limitations of this study include the relatively small number of patients and the short follow-up duration. To address these limitations and advance our understanding, future research should aim for more comprehensive investigations. Implementing multi-center studies with larger sample sizes can enhance the statistical robustness of our findings and provide a broader perspective on MAGE-As in non-small cell lung cancer. Moreover, extending the follow-up duration to the long term will help to explore the dynamic changes and potential prognostic significance of MAGE-As over an extended period.

CONCLUSION

In conclusion, our study highlights a significant up-regulation in the expression of MAGE-As protein and mRNA in non-small cell lung cancer patients. Importantly, this increased expression is intricately linked to the clinical stage and differentiation degree of these patients. These findings emphasize the potential significance of MAGE-As as a diagnostic and prognostic marker in lung cancer. Further investigations into the mechanistic implications of MAGE-As in tumor progression and therapeutic resistance are warranted. Overall, this study adds valuable insights to the complex landscape of non-small cell lung cancer, opening doors for future research aimed at utilizing the clinical implications of MAGE-As in the management of this disease.

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

All Authors contributed equally to this work.

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