Original Research Report

EXPRESSION OF MELANOMA ANTIGEN GENES A11 AND A12 IN NON-SMALL CELL LUNG CANCER

Gondo Mastutik[1*], Alphania Rahniayu[1,2], Isnin Anang Marhana[3], Mochamad Amin[4], Heru Fajar Trianto[5], Reny I'tishom[6]

[1] Department of Anatomic Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.
[2]Department of Anatomic Pathology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
[3]Department of Pulmonology, Faculty of Medicine, Universitas Airlangga; Department of Pulmonology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
[4]Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia
[5]Department of Anatomic Pathology, Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia
[6]Department of Medical Biology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

The melanoma antigen gene (MAGE) belongs to the group of cancer-testis antigens that are exclusively expressed in germ cells but may be re-expressed in cancer cells. The highly expressed MAGE-A subfamily in lung cancer may potentially be a diagnostic and prognostic biomarker. This study aimed to identify MAGE-A11 and MAGE-A12 expressions in lung tumors obtained from core biopsy, forceps biopsy, and bronchoalveolar lavage specimens. A cross-sectional observational study was conducted on 90 patients clinically diagnosed with lung tumors. These patients received core biopsy, forceps biopsy, and bronchoalveolar lavage interventions after ethical approval was obtained. The complementary deoxyribonucleic acid (cDNA) quality was assessed by the polymerase chain reaction (PCR) of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The assessment was performed to ascertain if all specimens exhibited positive PCR amplification of the GAPDH gene. MAGE-A11 and MAGE-A12 were identified through a semi-nested reverse transcription PCR. The positive results were detected by measuring the PCR products, with MAGE-A11 and MAGE-A12 at base pairs (bp) of 858 and 496 in the first and second rounds, respectively. The expressions of MAGE-A11 and MAGE-A12 were observed in 3 (3.33%) and 40 (44.44%) out of 90 specimens, respectively. The prevalence rate of non-small cell lung cancer (NSCLC) was 31.11% (28/90). Among these cases, 3.57% (1/28) showed the expression of MAGE-A11, while 32.14% (9/28) exhibited the expression of MAGE-A12. Sixty-two (68.89%) out of 90 patients were diagnosed with no tumor cell malignancy. Out of 62 cases, 2 (3.23%) exhibited the expression of MAGE-A11, while 31 (50%) demonstrated the expression of MAGE-A12. MAGE-A11 and MAGE-A12 were detected in NSCLC and certain specimens with a pathological diagnosis that indicated the absence of malignant cells. In conclusion, MAGE A11 and MAGE A12 have potential markers to improve the pathological diagnosis of lung cancer. Further investigation is necessary to explore the expression of MAGE-A in correlation with lung cancer progression.

Keywords: Cancer; lung cancer; melanoma antigen gene A (MAGE A); mortality; reverse transcription polymerase chain reaction (RT-PCR)

***Correspondence:** Gondo Mastutik, Department of Anatomic Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. Email: gondomastutik@fk.unair.ac.id

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Highlights:
1. In this study, new primers designed using the semi-nested polymerase chain reaction (PCR) method were utilized to identify MAGE-A11 and MAGE-A12 expressions in specimens collected from core biopsy, forcep biopsy, and bronchoalveolar lavage.
2. The histopathological analysis revealed positive expressions of MAGE-A11 and MAGE-A12 in specimens diagnosed with non-small cell lung cancer (NSCLC) as well as in specimens with no malignant cells.
3. This study provides evidence indicating that the detection of messenger ribonucleic acid (mRNA) of MAGE-A11 and MAGE-A12 by nested reverse transcription PCR can improve the accuracy of lung cancer diagnosis.

INTRODUCTION

The melanoma antigen gene (MAGE) is part of the cancer-testis antigens, which are exclusively expressed in germ cells such as the testis, placenta, and ovary. The MAGE family comprises two distinct variations, i.e., type I and type II (Weon & Potts 2015, Õunap et al. 2018). Type I consists of the subfamily members of MAGE-A, MAGE-B, and MAGE-C. The expressions of these MAGE subfamilies are limited to the testis and are rarely found in normal adult cells. Type II includes MAGE-D, MAGE-E, MAGE-F, MAGE-G, MAGE-H, MAGE-L, and necdin genes, which are expressed in normal tissues such as embryonic and adult tissue (Lian et al. 2018).

MAGE-A is a subfamily within type I MAGE. There is a total of 12 subtypes of MAGE-A, specifically MAGE-A1 to MAGE-A12. However, MAGE-A7 is an exception, as it is classified as a pseudogene (Brisam et al. 2016, Mastutik et al. 2021, 2023). MAGE-A is subtly expressed in healthy cells. However, MAGE-A may become reactivated in cancer cells. MAGE-A is highly expressed in various forms of cancer, including thyroid, laryngeal, liver, colorectal, and lung cancers (Lee et al. 2013, Li et al. 2020, Almutairi et al. 2022).

Previous studies have demonstrated that the MAGE-A subfamily is highly expressed in lung malignancies, indicating its potential as a diagnostic and prognostic biomarker of lung cancer. It was found that lung cancer patients who had positive expressions of MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, and MAGE-A6 in their bone marrow had lower survival rates than those who did not exhibit these expressions. Patients with overexpression of all members of the MAGE-A subfamily demonstrated the lowest 10-year survival rate and the worst prognosis (Yi et al. 2017, Sang et al. 2017). The expressions of MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, and MAGE-A6 have been frequently found in patients with distant metastases. It has been found that lymph node metastasis commonly occurs in conjunction with the positive expressions of MAGE-A2, MAGE-A3, MAGE-A4, and MAGE-A6. In addition, the expressions of MAGE-A2, MAGE-A4, and MAGE-A6 have shown a significant association with tumor size changes (Gu et al. 2018). A meta-analysis study revealed that MAGE-A over-expression leads to a low survival rate and unfavorable clinical outcomes. Specifically, increased expressions of MAGE-A1, MAGE-A3, MAGE-A6, MAGE-A9, and MAGE-A10 pose a high risk of mortality and poor clinical outcomes. Furthermore, overexpressions of MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A8, MAGE-A9, MAGE-A10, and MAGE-A12 are associated with a poor prognosis for lung cancer. Therefore, the MAGE-A subfamily has demonstrated its potential as a prognostic indicator for survival in different types of cancer, including lung cancer (Poojary et al. 2020).

MAGE-A11 and MAGE-A12 expressions may have the valuable potential to serve as significant diagnostic and prognostic markers for cancer. MAGE-A11 expression has been associated with advanced stages of tumor and oral cancer, invasion in bladder cancer, and poor overall survival in head and neck cancer cases (Brisam et al. 2016, Jia et al. 2020, Mohsenzadegan et al. 2022). Positive MAGE-A11 expression is commonly observed in breast cancer. The expression has been associated with clinicopathological factors such as estrogen receptor and human epidermal growth factor receptor 2 (HER-2) expression (Hou et al. 2014). Meanwhile, in a study by (Wu et al. 2017), MAGE-A12 over-expression was found to be associated with late-stage gastric cancer, poor prognosis, and a low survival rate of the disease. MAGE-A11 and MAGE-A12 overexpressions were also found to be associated with a poor prognosis and low survival rate in lung cancer. MAGE-A11 and MAGE-A12 may suggest their potential abilities to act as diagnostic and prognostic markers of lung cancer. Therefore, the aim of this study was to identify the MAGE-A11 and MAGE-A12 expressions in lung tumors obtained from core biopsies, forceps biopsies, and bronchoalveolar lavage specimens.

MATERIALS AND METHODS

This cross-sectional observational study was conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The Health Research Ethics Committee of the hospital issued the ethical approval for this study, with reference No. 497/Panke.KKE/VIII/2017 on 25/8/2017. The samples were obtained via core biopsies, forceps biopsies, and bronchoalveolar lavage. This study involved 90 patients with a diagnosis of lung tumors between August 2017 and August 2018. The inclusion criteria were those aged 20–80 years who had a measurable tumor or lesion, underwent a certain intervention (i.e., a core biopsy, a forceps biopsy, or bronchoalveolar lavage), had not received any therapy, and provided informed consent as proof of their voluntary participation in this study. Patients who had a primary tumor in other organs other than the lungs, were unwilling to participate, and had arrhythmia, hypoxemia, hypercapnia, or unstable hemodynamics were excluded from this study (Keung et al. 2020).

Ribonucleic acid (RNA) was extracted according to the instructions of the RNeasy Plus Mini Kit

(Qiagen, batch no. 74134, Hilden, Germany). Afterward, reverse transcription (RT) was performed using the RT-qPCR Master Mix (Toyobo, batch no. FSQ-301, Osaka, Japan) in accordance with the protocols outlined in our previous study (Mastutik et al. 2021). The quality of complementary deoxyribonucleic acid (cDNA) was assessed through a polymerase chain reaction (PCR) for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. The GAPDH primer and PCR protocol were set in a consistent manner with our earlier study (Mastutik et al. 2021). Once all specimens were confirmed as positive for the GAPDH gene, PCR was then performed to identify MAGE-A11 and MAGE-A12.

The semi-nested reverse transcription polymerase chain reaction (RT-PCR) was performed to identify MAGE-A11 and MAGE-A12. The forward and reverse primers used for the identification of MAGE-A11 in the first round were MF11: 5'-GGA GGA GAA CAA GTG CTG TGG-3' and MR11: 5'-CAC CAG GTA CTT TTC CTG CAC-3', respectively. In the second round, the forward and reverse primers for the identification of MAGE-A11 were MF11 and MR12: 5'-CCA GYA TTT CTG CCT TTG TGA-3', respectively. Additionally, the forward and reverse primers used for the identification of MAGE-A12 in the first round were MF12: 5'-CCA AGC ATC CAG GTT CTG AGG-3' and MR10: 5'-CTC CAG GTA STT YTC CTG CAC-3', respectively. Meanwhile, the forward and reverse primers used for the identification of MAGE-A12 in the second round were MF12 and MR12. The PCR protocol was implemented following the same method as outlined in our previous study on the MAGE-A family (Mastutik et al. 2021). MAGE-A11 and MAGE-A12 were identified by assessing the positive results, which were indicated by PCR products of 858 base pairs (bp) and 496 bp for the first round and second round, respectively. After the data were analyzed, they were tabulated and presented in figures and tables.

RESULTS

This study was conducted on 90 patients with a clinical diagnosis of lung tumors. The patients underwent core biopsy, forceps biopsy, or bronchoalveolar lavage procedures. The youngest patient was 21 years old, while the oldest was 79 years old. Most of the patients fell within the age group of 51–50 years old, with the following highest proportions in the age groups of 41–50 years and 61–70 years. A total of 60 (66.67%) out of 90 patients were male. The pathological diagnosis showed that 28 (31.11%) out of 90 patients had non-small cell lung cancer (NSCLC) types, including adenocarcinoma and squamous cell carcinoma. A majority of the specimens, specifically 62 (68.89%) out of 90, did not exhibit any presence of malignant cells. This was particularly evident in the specimens obtained from bronchoalveolar lavage, as shown in Table 1.

Out of the 90 specimens examined, MAGE-A11 expression was detected in 3 specimens (3.33%), yet MAGE-A12 expression was observed in 40 specimens (44.44%) (Table 2). Out of the 28 samples diagnosed with NSCLC, 1 sample (3.57%) displayed MAGE-A11 expression, and 9 samples (32.14%) demonstrated MAGE-A12 expression. In addition, among the 62 samples that did not contain malignant cells according to the pathological analysis, 2 (3.23%) demonstrated MAGE-A11 expression, and 31 (50%) exhibited MAGE-A12 expression (Table 3).

Table 1. Characteristics of the patients diagnosed with lung tumors.

Variables		n	%
Age (years)			
21-30		5	5.55
31-40		4	4.44
41-50		23	25.55
51-60		34	37.78
61-70		18	20
71-79		5	5.55
Mean±SD:	53.82±11.34		
Min-max:	21-79		
Sex		60	66.67
Male		30	33.33
Female			
Specimens		31	34.44
Core biopsy		19	21.11
Forceps biopsy		40	44.44
BAL			
Histopathological diagnosis			
NSCLC, adenocarcinoma		25	27.78
NSCLC, squamous cell carcinoma		3	3.33
No cancer cells found		62	68.89

Notes: SD=standard deviation; NSCLC=non-small cell lung cancer; BAL=bronchoalveolar lavage.

Table 2. Distribution of MAGE-A11 and MAGE-A12 expressions.

Gene expression	Positive		Negative	
	n	%	n	%
MAGE-A11	3	3.33	87	96.67
MAGE-A12	40	44.44	50	55.55

Table 3. Distribution of MAGE-A11 and MAGE-A12 expressions according to the pathological diagnosis.

MAGE-A Expression	NSCLC		No malignant cells found	
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
MAGE-A11	1 (3.57)	27 (96.43)	2 (3.23)	60 (96.77)
MAGE-A12	9 (32.14)	19 (67.86)	31 (50)	31 (50)

Note: NSCLC=non-small cell lung cancer.

DISCUSSION

Lung cancer is the most common form of cancer and has a high mortality rate worldwide. The 2020 data from the Global Cancer Observatory (GLOBOCAN) revealed that the estimated mortality rate for lung cancer was 1.8 million. In the United States, lung cancer ranks as the second most prevalent cancer and causes the highest number of deaths in both men and women (Sung et al. 2021, Siegel et al. 2023). In Indonesia, the disease is the third most common cancer after breast and uterine cervical cancers. There were 34,783 new cases, accounting for 8.8% of all cancer cases. Lung cancer is the most common type of cancer in men, with 25,943 new cases (14.1%). Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the most prevalent histopathological types of lung cancer (Gu et al. 2018). Lung cancer is frequently diagnosed after the disease has progressed to an advanced stage. This is because of the unclear symptoms presented during the early stages of the disease, leading to a high mortality rate (Cainap et al. 2020). Symptoms that appear once the cancer has progressed to an advanced stage typically result in a poor prognosis for patients. Moreover, the five-year survival rate is around 16%. Meanwhile, the approximate five-year survival rate for patients with stage IV lung cancer is below 10% (Ning et al. 2021). Therefore, it is important to carry out screening and be aware of the early symptoms of cancer in individuals with major risk factors for lung cancer, such as tobacco smoking. Appropriate methods for collecting specimens in diagnostics and identifying certain biomarkers have attracted the interest of experts to improve diagnosis accuracy (Malhotra et al. 2016).

This study investigated specific biomarkers, i.e., MAGE-A11 and MAGE-A12, by using several methods to collect specimens through core biopsy, forceps biopsy, and bronchoalveolar lavage procedures. Specimens from the periphery of the chest cavity were obtained by core biopsies using ultrasonography or computed tomography (CT) guidance, while specimens from the central chest cavity were acquired by forceps biopsies and bronchoalveolar lavage. The aforementioned methods are considerably safe and feasible to use in collecting specimens for pathological or molecular diagnosis (Marhana et al. 2022). However, these methods are quite invasive and can result in difficulties, such as too few specimens or insufficient exfoliated cells for pathological diagnosis. In addition, there is a potential increase in the risk of bleeding (Goel et al. 2022). Hence, the use of a polymerase chain reaction to detect MAGE-A11 and MAGE-A12 may support pathological examination in molecular diagnosis.

This study identified MAGE-A11 expression in 3 (3.33%) out of 90 specimens, while MAGE-A12 expression was detected in 40 (44.44%) out of 90 specimens. According to the pathological diagnosis, 28 patients were found to have NSCLC, while 62 patients showed no presence of malignant cells on the slides. It is interesting that the investigation in this study could detect MAGE-A11 and MAGE-A12 expressions in specimens that were pathologically determined to be devoid of malignant cells. The results showed that 2 (3.23%) out of 62 specimens were positive for MAGE-A11 expression, while 31 (50%) out of 62 specimens exhibited positive MAGE-A12 expression. Pathological examination is considered the gold standard for diagnosing malignancy. Accurate diagnosis is crucial for the patient because the selection of the appropriate treatment, such as targeted therapy, is contingent on the type of cancer (Ning et al. 2021). Pathological diagnosis relies on the examination of cell morphology, necessitating a sufficient number of observable cells on the slide. However, cancer tissue is fragile, and its cells are easily lysed, making them prone to damage and destruction during the bronchoscopy process. This can cause the cells to be undetectable on the pathology slide. This study showed that the expressions of MAGE-A11 and MAGE-A12 were detected even in specimens that were pathologically defined as devoid of cancer cells. Therefore, the utilization of semi-nested RT-PCR to detect MAGE-A11 and MAGE-A12 can serve as an additional method for identifying cancer cells from core biopsy, forceps biopsy, and bronchoalveolar lavage specimens.

The MAGE-A subfamily genes, including MAGE-A11 and MAGE-A12, are silenced in normal cells

by the process of deoxyribonucleic acid (DNA) methylation. However, MAGE-A11 and MAGE-A12 have been known to undergo reactivation in cancer cells, particularly in the early stages of carcinogenesis, due to epigenetic changes such as demethylation or histone acetylation. The overexpression of MAGE-A12 promotes the degradation of p21, a tumor suppressor gene (Zhao et al. 2019). In normal cells, p21 regulates cell cycle arrest together with retinoblastoma protein (pRB). The activation of p53 leads to an increase in p21 expression, which in turn causes the formation of the retinoblastoma-E2F transcription factor (RB-E2F) complex. This complex then downregulates a number of genes related to the cell cycle, resulting in cell cycle arrest (Engeland 2022). The MAGE-A12 gene promotes cell cycle progression, immortality, and anti-apoptosis in cancer cells by causing the degradation of p21. In addition, the increased expression of MAGE-A11 was associated with DNA hypomethylation in the promotor region. In other conditions, the increased MAGE-A11 expression was found to be related to both DNA hypomethylation and histone acetylation (James et al. 2013, Yanagi et al. 2017). In their study, Su et al. (2013) reported that MAGE-A11 promotes carcinogenesis by specifically affecting the retinoblastoma (RB) pathway. The study additionally showed that MAGE-A11 interacts with the p107-RB-related protein. MAGE-A11 was found to be associated with retinoblastoma-like 1 (p107), resulting in the stabilization of p107. This interaction can inhibit the ubiquitination or degradation of p107 and result in the hypophosphorylation of E2F transcription factor 1 (E2F1). The hypophosphorylation of E2F1 causes the stabilization and activation of E2F1, which subsequently leads to cell cycle progression and anti-apoptosis.

MAGE-A11 and MAGE-A12 expressions are upregulated in several types of cancer. MAGE-A11 was identified in cases of squamous cell carcinoma in both head and neck cancer and lung cancer. An elevated expression of MAGE-A11 has been related to poor overall survival. The study conducted by Jia et al. (2020) revealed that MAGE-A11 expression was higher in cancer tissue than in the surrounding tissue. Moreover, this increased expression was found to be associated with the occurrence of lymph node metastasis. It was found that MAGE-A11 and MAGE-A12 are associated with the progression of oral cancer to advanced stages. Furthermore, increased MAGE-A11 expression was found to be associated with invasive and advanced-stage bladder cancer. The progression of gastric cancer to advanced stages was also found to be related to increased MAGE-11 expression (Wu et al. 2017, Mohsenzadegan et al. 2022). The study conducted by Sang et al. (2017) used tissue microarray immunohistochemistry to demonstrate that the overexpression of MAGE-A11 and MAGE-A12 was associated with the lowest ten-year survival rates in patients with lung adenocarcinoma, indicating a poor prognosis. The expression of MAGE-A, specifically MAGE-A11 and MAGE-A12, has been associated with the lowest survival rate in lung cancer patients. MAGE-A11 and MAGE-A12 may potentially serve as prognostic markers in certain types of cancer, such as lung cancer. Additionally, it has been proposed that MAGE-A12 may be useful as an additional marker for early detection of oral cancer (Brisam et al. 2016, Poojary et al. 2020). Hence, in cases where cancer cells are absent but MAGE-A11 or MAGE-A12 expressions are detected, it is necessary to obtain new specimens for a reevaluation of the histopathological diagnosis. This is due to the potential failure of the specimen collection or lysis, resulting in the absence of cancer cells during the initial diagnosis.

Strength and limitations

This study detected the presence of MAGE-A11 and MAGE-A12 expressions in small tissue samples obtained from core biopsies and forceps biopsies, as well as in fluid obtained from bronchoalveolar lavage during bronchoscopy. MAGE-A11 and MAGE-A12 expressions were observed in specimens diagnosed with NSCLC as well as in specimens without any malignant cells. Thus, this study suggests that using nested RT-PCR to detect the mRNA of MAGE-A11 and MAGE-A12 can improve the accuracy of lung cancer diagnosis. However, this study has limitations as it solely evaluated MAGE-A11 and MAGE-A12 expressions in lung tumors obtained from core biopsy, forceps biopsy, and bronchoalveolar lavage procedures without establishing any relationship with the stage and invasion of the malignancies. Further investigation is required to examine the relationship between MAGE-A11 and MAGE-A12 expressions and the progression and prognosis of lung cancer.

CONCLUSION

MAGE-A11 and MAGE-A12 expressions are detectable in non-small lung cancer (NSCLC). These expressions can be identified in specimens that have been pathologically diagnosed with NSCLC, as well as in those that do not contain malignant cells. Therefore, MAGE-A11 and MAGE-A12 have potential as markers that can support the pathological diagnosis of lung cancer.

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Conflict of interest

None.

Ethical consideration

This study received ethical approval from the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, with reference No. 497/Panke.KKE/VIII/2017 on 25/8/2017.

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Author contribution

GM contributed to the conception and design, the analysis and interpretation of the data, the drafting of the article, critical revision of the article for important intellectual content, final approval of the article, and the acquisition of funding. AR contributed to the analysis and interpretation of the data, the drafting of the article, the critical revision of the article for important intellectual content, and the final approval of the article. IAM contributed to the final approval of the article, the provision of study materials or patients, and the collection and assembly of data. MA contributed to the analysis and interpretation of the data, the final approval of the article, and the provision of administrative, technical, or logistic support. HFT contributed to the final approval of the article, the provision of administrative, technical, or logistic support, and the collection and assembly of data. RI contributed to the analysis and interpretation of the data, the final approval of the article, and the collection and assembly of the data.

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