RESEARCH ARTICLE

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Expression of *MAGE A1* to *MAGE A10* in the Forceps Biopsy and Bronchoalveolar Lavage Specimens from Patients with the Central Lung Tumor

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Abstract

Objective: The objective was to evaluate the expression of the *MAGE A* subtypes family in the central lung tumor patients from the forceps biopsy (FB) and bronchoalveolar lavage (BAL) specimens and to analyze its association with the histopathological examination. **Methods:** An observational study was conducted on 32 FB and 43 BAL specimens from patients with central lung tumors. All samples were assessed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression by reverse transcription (RT) polymerase chain reaction (PCR) and samples showing a positive result were examined for *MAGE A* subtypes family expression by nested-RT PCR. **Result:** The *MAGE A1* to *MAGE A10* genes were highly expressed in the FB and BAL specimens from patients with central lung tumors. The *MAGE A1* to *MAGE A10* gene and *MAGE A1* to *MAGE A6* gene were expressed in 60/75 (80%) and 16/75 (21.3 %), respectively. *MAGE A8*, *MAGE A9*, and *MAGE A10* were the most commonly expressed. In FB specimens diagnosed without malignant cells, *MAGE A1* to *MAGE A10* and *MAGE A1* to *MAGE A6* were positive in 16/18 (88.9 %) and 1/18 (5.6 %), respectively. In all BAL specimens were diagnosed with no malignant cells, but *MAGE A1* to *MAGE A10* and *MAGE A1* to *MAGE A6* showed positive results in 36/43 (83.7%) and 9/43 (20.9%) %), respectively. There was a significant association between *MAGE A1* to *MAGE A6* expression with histopathological diagnosis. **Conclusion:** The *MAGE A* subtype family genes are highly expressed in central lung tumor patients from FB and BAL specimens, even in specimens that were diagnosed with no malignant cells. All BAL specimens were diagnosed as no malignant cells, but expression of the *MAGE A* subfamily genes was found in more than 80% of the specimens. These observations suggest that combining histopathological and molecular examination could improve the diagnosis of lung malignancy.

Keywords: Lung cancer- cancer cell- *MAGE A1-10*- Forceps Biopsy- Bronchoalveolar Lavage

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Introduction

Lung cancer is currently the most common cancer with a high incidence and mortality rate in the world [1]. In the United States, lung and bronchial cancer is estimated to be the second most common cases after prostate cancer in men and breast cancer in women, with an incidence of up to 238,340 new cases in 2023. In addition, the cancer is also the highest cause of death both in women and men, with an estimated incidence of 127,070 deaths [2]. Most of the lung cancer patients are diagnosed at an advanced stage, making it difficult to treat. Unfortunately, it is a low success rate of therapy, causing the patient's death [3, 4]. It is because patients with lung cancer do not show clear symptoms at an early stage. The new symptoms appear and can only be felt after cancer has reached the advanced stage and patients have a poor prognosis [3, 5]. In addition, the life expectancy of lung cancer sufferers is very low, only about 16% survive for up to 5 years despite receiving treatment, including surgery [6]. The five years of survival for late stage such as stage IV was only less than 10% [3]. Therefore, appropriate screening and diagnosis methods with a molecular approach to detect gene expression might improve the accuracy of early diagnosis and patient prognosis.

Determining the type and stage of cancer histopathologically is crucial as a consideration in choosing the appropriate treatment that affects survival rates [6]. Currently, histopathological examination is the gold standard for the diagnosis of lung cancer [3]. The

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examination is based on cell morphology observation of tissue or fluid containing exfoliating cells from patients suspected of lung cancer. Biopsy using computed tomography (CT) guided is generally considered to be a useful technique to collect tissue from lung tumors as a diagnostic procedure for histopathology. However, this technique may increase the risk of complications such as pneumothorax and intrapulmonary hemorrhage [7]. In addition, CT-guided is commonly used to diagnose peripheral lung cancer [7]. For central lung tumors, lung tissue collection is usually carried out using bronchoscopy, such as forceps biopsy (FB) or bronchoalveolar lavage (BAL) [5]. The FB has been performed to collect the lung specimens to diagnose malignancy with minimal complications such as minor bleeding that could be spontaneously resolved or treated using ice-cold saline or an agent for vasoconstriction [8]. In general, bronchoscopy with FB and BAL is a safe technique and allows for obtaining specimens for histopathological examination [4]. However, this method is invasive [6, 9] and sometimes encounters obstacles [5], such as inadequate cell number for a histopathological diagnosis. In addition, there is also an increased risk of bleeding in the very fragile cancerous tissue [4, 5]. Thus, molecular examinations are required to support histopathological examination.

Melanoma-associated antigen A (*MAGE A*) belongs to the group of cancer/testis antigens presented on the cell surface by the major histocompatibility complex (MHC) class I molecule [10]. There are 12 subtypes of *MAGE A* which share a conserved MAGE homology domain (MHD) [11]. The subtypes including *MAGE A1*, *MAGE A2, MAGE A3, MAGE A4, MAGE A5, MAGE A6, MAGE A7* (pesudo gene), *MAGE A8, MAGE A9, MAGE A10, MAGE A11, MAGE A12* [11, 12]. Expression of these genes are usually restricted to germline cells such as the ovary, testis, placenta, and fetus that may relate to germ cell development [10, 11, 13]. Interestingly, several studies showed that those genes were also highly expressed in many cancers such as melanoma, ovarian cancer, endometrial cancer, prostate cancer, bladder cancer, oesophageal cancer, gastric cancer, colon cancer, liver cancer, brain cancer, as well as in lung cancer [10, 11, 13, 14]. Expression of *MAGE A* subtypes in a variety of human cancer may relate to tumorigenesis through various mechanisms that result in cancer progression, metastasis, and cancer recurrence [11, 13, 14]. Therefore, expression of *MAGE A* subtypes indicates a poor cancer prognosis and expression profiling of these genes holds a potential marker for prognostic and therapeutic in cancer [14].

Meta-analysis studies suggested that expression of *MAGE A* genes is associated with cancer [13]. Another study showed that the expression *MAGE A* genes in lung sputum is correlated with the presence of lung cancer cells or pre-cancerous cells in specimens [15]. In lung cancer, specimens for histopathological diagnosis could be obtained using minimally invasive methods such as bronchoscopy FB or BAL [6]. Identification of 6 subtypes of *MAGE A1* to *MAGE A6* in bronchial washing specimens from the peripheral lung tumor suggested more sensitive detection than conventional cytology [16]. It was indicated that expression profiling of *MAGE A1* to *MAGE A6* genes is a useful tool for the diagnosis of lung cancer [15]. Therefore, profiling of 10 subtypes of *MAGE A1* to *MAGE A10* genes might improve the accuracy of diagnosis of lung tumors compared with the gold standard, i.e. histopathological diagnosis. Here, we report the expression profile of *MAGE A* subtypes family in the FB and BAL specimens collected from the central lung tumor patients, including 10 subtypes of *MAGE A* from *MAGE A1* to *MAGE A10*, 6 subtypes of *MAGE A* from *MAGE A1* to *MAGE A6*, and the expression of the single gene of *MAGE A.* The expression profile is further analyzed for its association with the histopathological examination both in specimens where malignant cells were found and in specimens where no malignant cells were found.

Materials and Methods

An observational study with a cross-sectional approach was conducted in Dr. Soetomo General Academic Hospital Surabaya, Indonesia, and approved by the ethical commission, number 497/Panke.KKE/ VIII/2017. Samples were collected from patients with the clinical diagnosis of central lung tumor who received intervention by FB and BAL in the Lung Intervention Room, Diagnostic Center Building from August 2017 to August 2018.

Sample collection

Samples were divided into two parts, for pathological diagnosis and *MAGE A* examination by reverse transcription PCR (RT PCR). The inclusion criteria were age 20–75 years, having at least one measurable tumor or lesion in the center of lung, being able to collect specimens from bronchoscopy by FB or BAL, having a Karnofsky score >70%, never receiving systemic therapy, and willing to participate in the study by signing an informed consent. The patients were excluded if they have a primary tumor in other organs and those who were not in optimal condition to undergo invasive diagnostic procedures, such as hypercapnia, hypoxemia, arrhythmias, hemodynamic instability, and uncooperativeness.

Histopathological diagnosis of the FB specimens showed carcinoma, malignant tumor, and non-small cell lung cancer (NSCLC), which was then classified as a found malignant cell and a no-found malignant cell. While the samples from BAL, all showed no malignant cells. All specimens were used for PCR examination.

The RNA extraction and cDNA synthesis

RNA was extracted using RNAeasy Plus Mini Kit (Qiagen, Hilden, Germany) according to the protocol instructions. Total RNA was used as a template for reverse transcription (RT) polymerase chain reaction (PCR) using the RT PCR Master Mix (Toyobo, Osaka, Japan) and followed by nested PCR. For cDNA synthesis, a total of 25 µl of RNA template were mixed with 12 µl 4× DN master mix and 3 µl random primers. The mixture was incubated at 37° C for 5 minutes, for genomic DNA removal. After addition of 10 µl 5×RT master mix II, incubation was continued at 37° C for 15 minutes and followed by 50°C for 5 minutes. Finally, the reaction was stopped by

incubation at 98° C for 5 minutes. The resulting cDNA was stored at 40° C or 20° C [12, 17]. To monitor the quality and integrity of the cDNA, RT PCR was conducted to detect the housekeeping gene, i.e., the glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The samples with positive GAPDH were used to examine expression of *MAGE A* subtypes family by nested-RT PCR.

The MAGE-A identification

Expression of *MAGE A* genes were detected by conducting nested PCR. The primers of the single gene of *MAGE A*, the group of *MAGE A1* to *MAGE A10*, the group of *MAGE A1* to *MAGE A6*, and GAPDH were performed as in the previous studies [12, 17]. The PCR was performed using the PCR Master Mix from Promega (Madison, USA). In the first round, a total 20 µl PCR mixture was set to consist of 10 µl master mix, 2 µl primer forward and reverse, 5 µl nuclease-free water, and 3 µl cDNA template. PCR experiment was carried out for 1 cycle pre-denaturation step at 94° C for 5 minutes, followed by 40 cycles that consist DNA denaturation at 94° C for 30 seconds, DNA annealing at 55° C for 45 seconds, and DNA extension at 72° C for 45 seconds. The experiment was finalized by 1 cycle of the final extension at 72° C for 7 minutes. The second PCR round was performed using 3 µl template from the first round. The PCR mixture and condition were performed as in the first PCR round. PCR products were visualised on 2 % agarose gel electrophoresis [12, 17].

Statistical analysis

The association between the expression of *MAGE A1* to *MAGE A10, MAGE A1* to *MAGE A6*, and the single gene of *MAGE A* from *MAGE A1* to *MAGE A10* with pathological data were analyzed with Fisher's Exact Test 2-sided.

Results

Patients consisted of 52 males and 23 females, minimum age of 24 years and a maximum of 74 years, with an average of 54.39 ± 11.18 (mean ± standard deviation). There were 75 samples from the central lung tumor biopsies which consist of 32 samples from FB and 43 samples from BAL (Table 1). The histopathological diagnosis was shown Figure 1.

PCR for the GAPDH gene from all specimens used in this study showed positive results. It showed that the quality and integrity of cDNA from the specimen were still adequate for PCR examination. Although histopathologically no malignant cells were found, the specimen still contained sufficient DNA for PCR.

The *MAGE A1* to *MAGE A10* was the most frequently expressed in FB and BAL samples. *MAGE A1* to *MAGE A10* was expressed on 60/75 (80%), followed by *MAGE A8* was 30/75 (40 %), *MAGE A9* was 27/75 (36 %), and *MAGE A10* was 18/75 (24 %), while *MAGE A1* to *MAGE A6* was expressed on 16/75 (21.3 %) (Figure 2, Table 2).

In the FB specimens showed that *MAGE A1* to *MAGE A10* and *MAGE A1* to *MAGE A6* were found positive in 24/32 (75 %) and 7/32 (21.9 %), respectively (Table 2, Figure 2). The single of *MAGE A* from FB showed that *MAGE A5* was the most common found, it was found positive in 8/32 (25 %) samples. Thus, it was followed by *MAGE A1* and *MAGE A3* respectively in 7/32 (21.9 %) samples, *MAGE A9* was 6/32 (18.8 %) samples, *MAGE A8* was 5/32 (15.6 %) samples, *MAGE A2* was 4/32 (12.5 %) samples, and *MAGE A4* was 1/32 (3.1 %) samples. The *MAGE A6* and *MAGE A10* were negative (Table 2).

In this study, *MAGE A* showed positive on specimens that not contain malignant cells based on histopathological examination. All BAL specimens showed that these did not contain malignant cells, but *MAGE A* was found positive in most specimens except four samples. *MAGE A1* to *MAGE A10* were found positive in 36/43 (83.7 %) and *MAGE A1* to *MAGE A6* were found in 9/43 (20.9 %). The individual *MAGE A* showed that *MAGE A8* was found in 25/43 (58.1 %), *MAGE A9* was 21/43 (48.8 %), *MAGE A10* was 18/43 (41.9 %), *MAGE A5* was 11/43 (25.6%), *MAGE A3* was 7/43 (16.3 %), *MAGE A1* was 6/43 (14 %), and *MAGE A2* was 5/43 (11.6 %) samples (Table 2). Moreover, in FB samples that did not contain malignant cells showed that *MAGE A5* was found positive in 6/18 (33.3 %) specimens, *MAGE A9* was 4/18 (22.2 %) specimens, *MAGE A1* was 3/18 (16.7 %) specimens, *MAGE A3* and *MAGE A8* were 2/18 (11.1 %) specimens

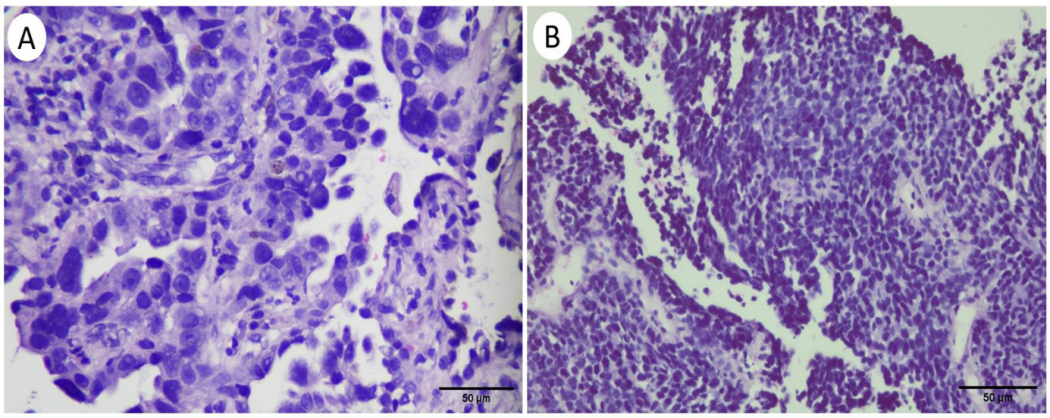

Figure 1. Histopathological Diagnosis from the Central Lung Tumor Confirmed by Hematoxylin Eosin (HE) staining. Non-small cell lung cancer type adenocarcinoma (A) and non-small cell lung cancer type squamous cell carcinoma (B), magnificent 100x.

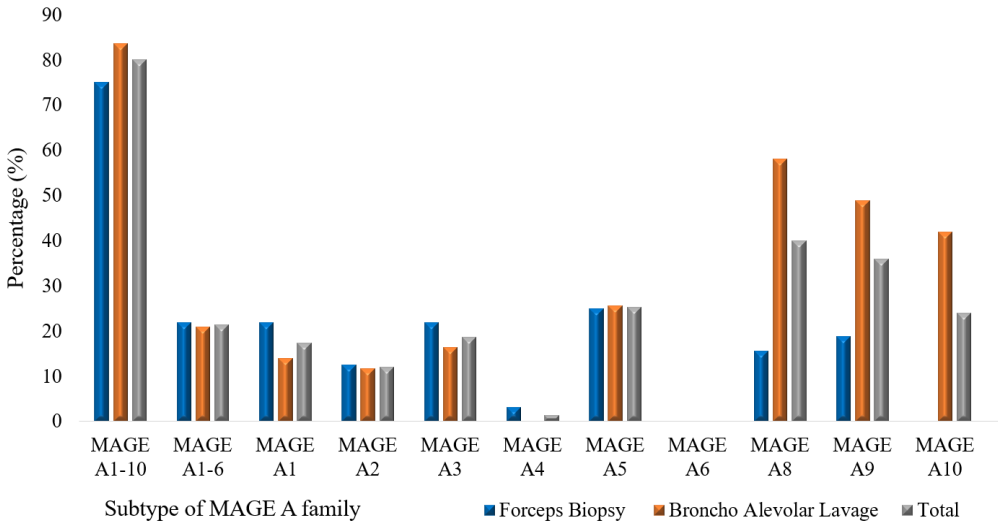

Figure 2. Expression of *MAGE A* Family in the Forceps Biopsies and Bronchoalveolar Lavage of Central Lung Tumors

respectively, and *MAGE A2* was 1/18 (5.6 %) specimens (Table 3).

The Fisher's Exact test showed that there was a significant association between *MAGE A1* to *MAGE A6* expression with histopathological examination with p value, 0.027 ($p < 0.05$) in moderate relationship strength (contingency coefficient 0.409). There were no significant association between *MAGE A1* to *MAGE A10*, *MAGE A1*, *MAGE A2, MAGE A3, MAGE A4, MAGE A5, MAGE A8, MAGE A9*, and *MAGE A10* expression with histopathological examination ($p > 0,05$) (Table 3).

This study found that several subtypes of *MAGE A* were co-expressed or at least one subtype was expressed. There were 54/75 (72 %) samples expressed the *MAGE A* gene and 21/75 (28 %) samples were negative. There were 17/75 (22.7 %) samples that expressed one of *MAGE A* gene subtype, 12/75 (16 %) samples expressed two subtypes of *MAGE A* gene, and 20/75 (20 %) samples expressed three subtypes of *MAGE A* gene, 8/75 (20 %) samples expressed four subtypes of *MAGE A* gene, 2/75 (2.7 %) samples expressed five subtypes of *MAGE A* gene, and 2/75 (2.7 %) samples expressed six subtypes of the *MAGE A* gene (Figure 3).

Table 1. Characteristics of Patients

Characteristic Patients	N (%)
Age (mean ± SD)	
54.39 ± 11.18	
Age range (years)	
21-74	
Age (years)	
21-30	3 (4)
31-40	4 (5.33)
41-50	17 (22.67)
51-60	30 (40)
61-70	16 (21.33)
71-80	5 (6.67)
Sex	
Forceps Biopsy	
Male	24 (75.0)
Female	8 (25.0)
Total	32 (100.0)
Bronchoalveolar Lavage	
Male	28 (65.1)
Female	15 (34.9)
Total	43 (100)
Histopathological Diagnosis	
Forceps Biopsy	
Carcinoma	1 (3.1)
Malignant tumor	2 (6.3)
Non-small cell lung cancer	11 (34.4)
No-found malignant cell	18 (56.2)
Bronchoalveolar Lavage	
No-found malignant cell	43 (100)

Discussion

The expression of *MAGE A* is limited in reproductive tissues but its expression is found to be aberrant in several types of cancer [14]. The subfamily of *MAGE A* gene consists of 11 genes including *MAGE A1* to *MAGE A12*, while *MAGE A7* is a pseudogene [12]. This study observed the expression of *MAGE A1* to *MAGE A10* in patients with nodules in the lung. It showed that the *MAGE A1* to *MAGE A10* were highly expressed. Identification of MAGE A individuals indicated that *MAGE A8, MAGE A9*, and *MAGE A10* were the most common expressed in patients with suspected lung cancer. Other studies showed that in bladder cancer, *MAGE A8* was found overexpression in 17/23 (74 %) of clear cell carcinoma and no expression in the normal bladder and ureter [18]. In breast cancer, *MAGE A9* is expressed higher in cancer tissue compared to non-cancerous tissue in adjacent tumor and related to histopathological grade and distant metastasis [19]. *MAGE A9* in ovarian cancer showed high expressed and related with stage, high grade, metastasis, and worse overall

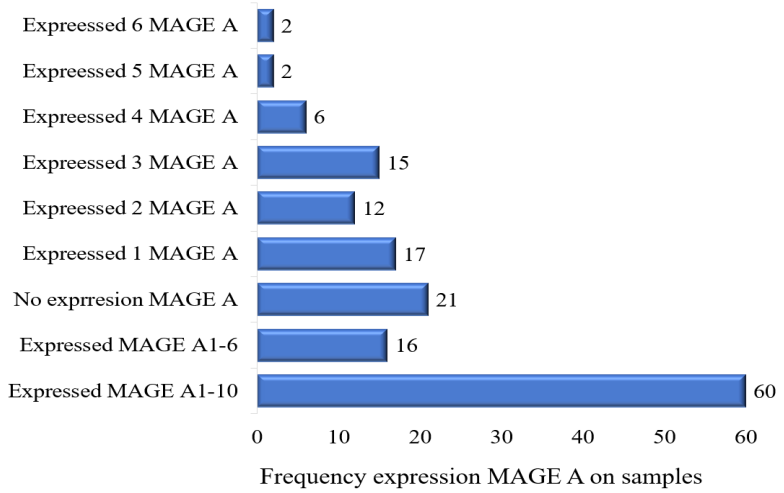

Figure 3. Specimens Expressed the Subtype of *MAGE A* Gene Family from Forceps Biopsies and Bronchoalveolar Lavage of Central Lung Tumors

Table 2. Expression of *MAGE A* Gene Family from Forceps Biopsies and Bronchoalveolar Lavage of Central Lung Cancers

Subtype of MAGE-A	Forceps Biopsies N (%)	Bronchoalveolar Lavage N (%)	Total N (%)
MAGE A1 to *A10*			
Positive	24 (75)	36 (83.7)	60 (80)
Negative	8 (25)	7 (16.3)	15 (20)
MAGE A1 to *A6*			
Positive	7 (21.9)	9 (20.9)	16 (21.3)
Negative	25 (78.1)	34 (79.1)	59 (78.7)
MAGE A1			
Positive	7 (21.9)	6 (14)	13 (17.3)
Negative	25 (78.1)	37 (86)	62 (82.7)
MAGE A2			
Positive	4 (12.5)	5 (11.6)	9 (12)
Negative	28 (87.5)	38 (88.4)	66 (88)
MAGE A3			
Positive	7 (21.9)	7 (16.3)	14 (18.7)
Negative	25 (78.1)	36 (83.7)	61 (81.3)
MAGE A4			
Positive	1 (3.1)	0	1 (1.3)
Negative	31 (96.9)	43 (100)	74 (98.7)
MAGE A5			
Positive	8 (25)	11 (25.6)	19 (25.3)
Negative	24 (75)	32 (74.4)	56 (74.7)
MAGE A6			
Negative	32 (100)	43 (100)	75 (100)
MAGE A8			
Positive	5 (15.6)	25 (58.1)	30 (40)
Negative	27 (84.4)	18 (41.9)	45 (60)
MAGE A9			
Positive	6 (18.8)	21 (48.8)	27 (36)
Negative	26 (81.3)	22 (51.2)	48 (64)
MAGE A10			
Positive	0	18 (41.9)	18 (24)
Negative	32 (100)	25 (58.1)	57 (76)

Table 3. Association of *MAGE A* Expression with Finding on Histopathological of Forceps Biopsies from Central Lung Tumour

Subtype of *MAGE-A*	Histopathological examination		P Value	Contingence coefficient
	Found malignant cell N (%)	No-found malignant cell N (%)		
MAGE A1 to *A10*				
Positive	8 (57.1)	16 (88.9)	0.096	
Negative	6 (42.9)	2 (11.1)		
MAGE A1 to *A6*				
Positive	6 (42.9)	1 (5.6)	0.027	0.409
Negative	8 (57.1)	17 (94.4)		(p = 0.011)
MAGE A1				
Positive	4 (28.6)	3 (16.7)	0.669	
Negative	10 (71.4)	15 (83.3)		
MAGE A2				
Positive	3 (21.4)	1 (5.6)	0.295	
Negative	11 (78.6)	17 (94.4)		
MAGE A3				
Positive	5 (35.7)	2 (11.1)	0.195	
Negative	9 (64.2)	16 (88.9)		
MAGE A4				
Positive	1 (7.1)	-	0.437	
Negative	13 (92.8)	18 (100)		
MAGE A5				
Positive	2 (14.3)	6 (33.3)	0.412	
Negative	12 (85.7)	12 (66.7)		
MAGE A6				
Negative	14 (100)	18 (100)	-	
MAGE A8				
Positive	3 (21.4)	2 (11.1)	0.631	
Negative	11 (78.6)	16 (88.9)		
MAGE A9				
Positive	2 (14.3)	4 (22.2)	0.672	
Negative	12 (85.7)	14 (77.8)		
MAGE A10				
Negative	14 (100)	18 (100)	-	

survival [20]. In addition, *MAGE A9* was expressed in 77/180 (42.7 %) of NSCLC type adenocarcinoma and 21/94 (22.3 %) in tumor-adjacent tissues and associated with poor differentiation, large tumor diameter, and lymph node metastasis [21]. As well as related to shorter survival time [21]. *MAGE A9* was expressed in 111/213 (52.1 %) of NSCLC and was commonly present in squamous cell carcinomas. It was related to large tumor diameter, lymph node metastasis, late stage TNM classification [22]. In overall survival, it showed a high expression of *MAGE A9* related to poor survival in NSCLC [22]. *MAGE A10* was frequently expressed in lung cancer and the *MAGE A10* protein was expressed in more than 50 % of tumor cells [23] and bladder cancer showed *MAGE A10* was expressed in 7/36 (19.4 %) of non-invasive and 21/67 (31.3 %) of invasive [23].

This study found that one specimen expressed one or more subfamily of *MAGE A* genes. A subfamily of *MAGE A* has a highly conserved region which is more than 80 % and it consists of approximately 170 amino acids [10, 14]. This region shares a common homology domain that has the same function to bind the targeted protein [10, 14]. The expression of one or more of *MAGE A* subfamily has the worse prognose. The computer analysis using the KM plotter database showed that the *MAGE A* gene was significantly associated with poor prognosis of some malignancies such as head and neck, esophageal, kidney, liver, ovarian, pancreatic, bladder, breast, stomach, and cervical cancer, as well as in lung cancer [13]. In NSCLC, individual *MAGE A1*, *MAGE A2, MAGE A3, MAGE A4, MAGE A9, MAGE A10,* and *MAGE A12* were significant in lung squamous cell carcinoma, while *MAGE A1*, *MAGE A3, MAGE A8,* and *MAGE A9* were significant in lung adenocarcinoma [13]. The meta-analysis study showed that *MAGE A3* and *MAGE A9* were significantly associated with poor clinical outcomes of lung cancer [14].

In addition, high expression of *MAGE A* was associated with poor survival outcomes in some malignancies, including breast cancer, stomach cancer [13] and epithelial ovarian carcinoma [13, 20] as well as in lung cancer [13]. In addition, the expression of *MAGE A* was related to resistance to chemotherapy in melanoma [10] and NSCLC [21].

MAGE A expression is regulated by epigenetics such as DNA demethylation and histone acetylation, resulting in the activation of the *MAGE A* gene to the transcript and translate to *MAGE A* protein [10, 24]. DNA hypomethylation has been proven to induce the aberrant expression of *MAGE A* gene [25]. The MAGE homology domain of *MAGE A* protein interacts with TRIpartite Motif 28 (TRIM28) which is also known as Krüppel-associated box (KRAB)-associated protein 1 (KAP1) or Transcription Intermediary Factor 1 (TIF1) [24, 26]. *MAGE A* role as a scaffold for the complex of RING-TRIMS28/KAP1/TIF1-E3 ubiquitin ligase to activate the ubiquitination with its substrate, such as p53, and result in the degradation of p53 and tumorigenesis [10]. In addition, *MAGE A* directly interacts with the DNA binding domain of p53 and block its interaction with chromatin resulting in the downregulation of its target genes [27]. Further, *MAGE A* binds to the DNA binding domain of p53 and down regulates the expression [13]. It suggests an association of *MAGE A* with tumorigenesis and a poor prognosis.

The sample in the study was obtained from patients suspected of lung cancer in the central region and specimens were taken by FB and BAL. In fact, histopathological examination demonstrated that 56.2 % of FB samples and all samples from BAL showed no malignant cells, but these samples were positive for *MAGE A.* Several studies have shown that tissue sampling using FB and BAL is one way to establish a histopathological diagnosis of lung cancer [6, 28, 29]. In addition, the FB is a procedure to collect specimens in patients with pulmonary nodules. It is feasible to perform and relatively safe for the patient [6, 29]. When compared with open lung biopsy, the FB has fewer complications, such as pneumothorax and bleeding [30]. Meanwhile, BAL is a useful procedure for the diagnosis of lesions in the lung, both due to infections such as tuberculosis and fungal infections as well as in the malignancy diagnosis [31]. Furthermore, BAL is a safe and minimally invasive method to collect specimens from the respiratory tract for cytological or microbiological examination [8, 30, 31]. However, histopathological finding of FB compared to surgical specimens showed low sensitivity (20%) and high specificity (100%), with diagnostic accuracy (36%) [32]. While the histopathological examination of BAL compared to transbronchial biopsy showed low sensitivity (47%) and high specificity (92%) with diagnostic accuracy (70%) [30]. In addition, the specimen obtained from FB is a small piece of tissue, while BAL obtains an exfoliating fluid that does not necessarily contain cells [30, 29]. Therefore, it may explain that in this study more than half of the samples from FB and all the samples from BAL were not found malignant cells.

Based on histopathological findings among the specimens where no cancer cells were found, *MAGE A* expression was still found, both in specimens from FB and BAL. Histopathological examination is the gold standard for determining the diagnosis of malignancy in lung lesions [30, 31]. This examination was based on the presence of cells in specimens. In the FB specimen, use very small tissue so it was possible not to find malignant cells. Furthermore, the BAL sample was an exfoliated liquid that was the result of brushing or washing, so it was possible that it did not contain cells or only a few cells that can lyse during the specimen collecting and handling process. Therefore, these cells were not found in the cytology process [29, 30]. All specimens in this study were already positive for GAPDH which showed specimens have adequate RNA although cytologically no cells were found. In addition, *MAGE A* examination was based on molecular examination using nested RT PCR technique and using primers that were specific to the target mRNA area, so even though no malignant cells were found histopathologically, it did not rule out the possibility of *MAGE A* mRNA being found in these specimens [12]. Therefore, combining cytological examination, both from FB and BAL specimens and molecular examination can increase the sensitivity in diagnosing lung malignancy.

The *MAGE A1* to *MAGE A10* was highly expressed in FB and BAL specimens from patients with a clinical diagnosis of central lung tumor, and it was also expressed in the specimen with no found malignant cells based on the histopathological examination. *MAGE A8, MAGE A9*, and *MAGE A10* were the most common expressed. Therefore, if the results of the histopathological evaluation do not find malignant cells and PCR targeting the *MAGE A* gene is found to be positive, then it is possible that there are cancer cells in the patient. Consequently, it is necessary to review or re-collect the specimen to confirm the type of lung cancer in the patient.

The limitation of this study is evaluating *MAGE A* expression based on the origin of specimens obtained by bronchoscopy, either from FB or BAL. Therefore, further research can evaluate *MAGE A* expression based on histopathological diagnosis, either in small cell lung cancer or in non-small cell lung cancer.

In conclusion, the *MAGE A* subtype family is highly expressed in central lung tumor patients and also expressed in specimens where histopathologically no malignant cells are found. In addition, all BAL specimens were not found with malignant cells histopathologically, but more than 80% of the specimens expressed the *MAGE A* subfamily. Therefore, combining histopathological examination from the FB and BAL specimens and molecular examination could improve in diagnosing lung malignancy.

Author Contribution Statement

Mastutik G: the concept idea, the writing, editing, and reviewing of the manuscript, laboratory work, and data analysis. Rahniayu A: Histopathological diagnosis and reviewing the manuscript. Marhana IA: Samples collection and reviewing of the manuscript. Ruslan SEN and Amin M: laboratory working and reviewing the manuscript. Trianto HF: Data collecting, statistical

analysis, Histopathological diagnosis, and reviewing manuscript.

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Ethical Declaration

The ethical clearance of this study was obtained from the Ethics Committee of Doctor Soetomo Hospital Surabaya, Indonesia with ethical number 497/Panke. KKE/VIII/2017.

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