

Simian virus 40 may be associated with developing malignant pleural mesothelioma

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Abstract. Malignant pleural mesothelioma (MPM) is associated with a history of heavy, long-term exposure to asbestos. However, MPM may also be associated with simian virus 40 (SV40), a polyomavirus. The association between SV40 and MPM remains unclear. The present study was conducted in order to investigate the proportion of SV40 presence in the histological specimens of Vietnamese patients with MPM. Histological specimens were obtained from 45 patients (19 men and 26 women) with MPM at the Pham Ngoc Thach Hospital in Ho Chi Minh City, Vietnam. The specimens were processed and examined in order to detect the presence of the SV40 large T antigen (SV40 Tag) expression using immunohistochemistry. Of the 45 patients, 23 (51%) were epithelioid, 7 (16%) were biphasic, 6 (13%) were sarcomatoid, 4 (9%) were desmoplastic, 4 (9%) were well-differentiated papillary and 1 (2%) was the anaplastic subtype. In total, 9/45 patients (20%) demonstrated SV40 Tag expression. The proportion of patients that demonstrated SV40 Tag expression was not significantly different between the epithelioid subtype and the other subtypes (22 vs. 18%; $P=1.000$) or between the patients with stage IV disease and other stages (20 vs. 20%; $P=1.000$). The median survival time was not significantly different between the patients with or without SV40 Tag expression (196 vs. 236 days, $P=0.8949$). In summary, a 5th of the Vietnamese patients with MPM were associated with infection with SV40. SV40 may be a potential cause of MPM in Vietnam and this potential association requires additional studies.

Introduction

Malignant mesothelioma is a rare but fatal disease that arises from the epithelial lining of the pleura, peritoneum, pericardium and tunica vaginalis. Malignant pleural mesothelioma (MPM) is the most common form, accounting for 80-90% of malignant mesotheliomas (1,2). A history of heavy and long-term exposure to asbestos is the established cause of MPM (3). However, MPM may result from other factors, including genetics, erionite, radiation and simian virus 40 (SV40), which may work alone or in combination (4). SV40 is a polyomavirus of which the natural hosts are rhesus monkeys. SV40 may infect human mesothelial cells, and may transform the cells using a mechanism whereby the tumor antigens, large T antigen (Tag) and small t antigen (tag), bind and inactivate the cellular tumor suppressors tumor protein p53 and retinoblastoma 1. These interactions may contribute to the development of malignant mesotheliomas by rendering mesothelial cells more susceptible to other carcinogens (5-7).

The role of SV40 in the pathogenesis of MPM remains unclear (1,2,4). Certain studies have detected SV40 DNA sequences or SV40 Tag in mesothelioma cells (8-10), but others have not (11-14). Geographical variation may be one reason for the discrepancy in SV40 detection, as SV40-contaminated polio vaccines, which had varied availability between countries, have been suspected as a major source of human infection (15). The association between SV40 and MPM remains unclear. The interaction between SV40 and asbestos exposure in the pathogenesis of MPM is unknown. The present study was conducted in order to investigate the proportion of SV40 presence in the histological specimens of Vietnamese patients with MPM.

Materials and methods

The present retrospective study was conducted at Pham Ngoc Thach Hospital, a referral chest hospital, in Ho Chi Minh City, Vietnam. The study protocol was approved by the Ethics Committee of the hospital.

Patients. The records of patients that were diagnosed with MPM between January 2008 and June 2012 were searched

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for on the patient database of the Department of Pathology, Pham Ngoc Thach Hospital. The medical records and histological specimens of the patients were archived. The patients or close relatives of the patients were asked to participate in the study, and all participants provided informed written consent. Patients that met the following criteria were enrolled: i) Definitively diagnosed as MPM; ii) the formaldehyde-fixed, paraffin-embedded tissues of the pleural specimens were eligible for additional immunohistochemical analysis; and iii) the patients or close relatives were available for a face-to-face or telephone interview. Patients were excluded for the following reasons: i) The formaldehyde-fixed, paraffin-embedded tissues of the pleural specimens were not eligible for immunohistochemical analysis due to small size or a lack of tumor tissue; and ii) the patients or the close relatives were not available or contactable.

Patients were definitively diagnosed as MPM based on histological examinations and immunohistochemical staining (16,17). In total, 4 positive markers, including calretinin, desmin, monoclonal mouse anti-human mesothelial cell clone HBME-1 and Wilms tumor 1 were used to definitively diagnose MPM. Various negative markers were used to rule out other cancers metastasized to the pleura, including: Keratin 7, carcinoembryonic antigen, transcription termination factor, RNA polymerase I and epidermal growth factor receptor for adenocarcinoma; enolase 2, gamma neuronal, synaptophysin and mouse monoclonal EpCAM antibody for small cell lung cancer; and clathrin, light chain A, cluster of differentiation (CD)3, CD20, CD30, CD68 and myeloperoxidase for lymphoma and leukemia.

Patients or close relatives were interviewed in order to determine a history of asbestos exposure. A history of asbestos exposure was designated to patients that had ever lived in a fiber cement-roofed house or worked in asbestos-associated industries, including the manufacture of fiber cement, ceramic tiles, insulating materials or other construction materials, shipbuilding and mineral mining.

All patients were followed up until August 31, 2013 to determine the survival time. The survival time was measured between the date of clinical diagnosis and mortality or censoring (the last date the patients were lost to follow-up or the last date the patients could be contacted, whether they remained alive or not). The date of clinical diagnosis was defined as the date on which MPM was diagnosed at Pham Ngoc Thach Hospital.

Detection of SV40 Tag expression. The formaldehyde-fixed, paraffin-embedded tissues of the pleural specimens of the patients were immunostained for SV40 Tag expression. The Lab Vision mouse monoclonal antibody pAb101 (dilution, 1:100; catalog no., MS-1832-P; Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to detect SV40 Tag expression. The secondary antibody used was Lab Vision™ biotinylated goat anti-polyvalent anti-mouse/rabbit immunoglobulin G (ready to use; catalog no., TP-125-BN; Thermo Fisher Scientific, Inc.). The detection system used was a Thermo Scientific™ Lab Vision™ DAB Plus Substrate System (Thermo Fisher Scientific, Inc.). The staining procedure was performed according to the manufacturer's protocol. Specimens with nuclear immunoreactive tumor cells were considered to

express SV40 Tag (Fig. 1). The positive results were scored according to the Allred score system as follows: 1-25% of tumor cells demonstrate SV40 Tag expression, 1⁺; 26-50%, 2⁺; and >50%, 3⁺ (18).

Detection of asbestos bodies. A light microscope (ECLIPSE 50i; Nikon Corp., Tokyo, Japan) was used to examine and count the presence of asbestos bodies in specimens of lung tissue or bronchoalveolar lavage fluid. The lung tissue specimens were stained using hematoxylin-eosin (Thermo Fisher Scientific, Inc.). The fluid specimens were stained using the papanicolaou method (19) (Fig. 2), subsequent to breaking down the mucus in the fluid using 10% NaOH (Thermo Fisher Scientific, Inc.). A specimen with >5 asbestos bodies in every 10 examined fields with a x40 magnification was considered to contain asbestos bodies (20).

Statistical analysis. The categorical variables are expressed as frequency and percent. The comparisons of the proportions of SV40 Tag expression between 2 groups were examined using the Fisher's exact test. Comparisons of the survival time between two groups were examined using the Log-Rank test of the Kaplan-Meier analysis. The Cox regression survival analysis was used to examine the effect of chemotherapy on the survival time, adjusting for the clinical stages of MPM. A P-value of <0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using the JMP 9.0.2 statistical software (SAS Institute Inc., Cary, NC, USA).

Results

Study population. Of the patients diagnosed with MPM at Pham Ngoc Thach Hospital between January 2008 and June 2012, 45 patients met the inclusion criteria. The mean (\pm standard deviation) and median age were 59 (\pm 15) and 58 years, respectively. The youngest patient was 25 and the eldest was 88 years old. Over half of the patients were female and 89% were in stage IV disease (Table I). Only 44% of the patients had a history of asbestos exposure. For the definitive diagnosis of MPM, 60% of the patients required a transcutaneous needle biopsy, while 40% required a thoracoscopic biopsy. Epithelioid was the most common histological subtype (51%) of MPM. Only 22 patients had clinical specimens available for the examination of asbestos bodies, 21 of which possessed bronchoalveolar lavage fluid specimens and 1 of which possessed a lung tissue specimen. Asbestos bodies were identified in 10 (45%) out of 22 patients.

Proportion of SV40 Tag expression. In total, 9 (20%) out of 45 patients exhibited SV40 Tag expression in the histological specimens: 2 patients with an Allred score of 1⁺, 4 patients with an Allred score of 2⁺; and 3 patients with an Allred score of 3⁺. However, only 1 (5%) out of 22 patients exhibited SV40 Tag expression and asbestos bodies.

The proportion of SV40 Tag expression was decreased in males compared with females (5 vs. 31%), but this difference was not significant (P=0.0578). Similarly, the proportion of SV40 Tag expression was not significantly different between the patients with and without asbestos bodies (P=0.5940),

Table I. Characteristics of 45 patients with malignant pleural mesothelioma.

Characteristic	No. of patients	% of total
Gender		
Male	19	42
Female	26	58
History of asbestos exposure		
Yes	20	44
No	25	56
Method of pleural biopsy		
Transcutaneous needle	27	60
Thoracoscopy	18	40
Histological subtypes		
Epithelioid	23	51
Biphasic	7	16
Sarcomatoid	6	13
Desmoplastic	4	9
Papillary	4	9
Anaplastic	1	2
SV40 Tag expression		
Positive	9	20
Negative	36	80
Asbestos bodies		
Positive	10	45
Negative	12	55
Clinical stage		
II	3	7
III	2	4
IV	40	89

SV40 Tag, large T antigen of simian virus 40.

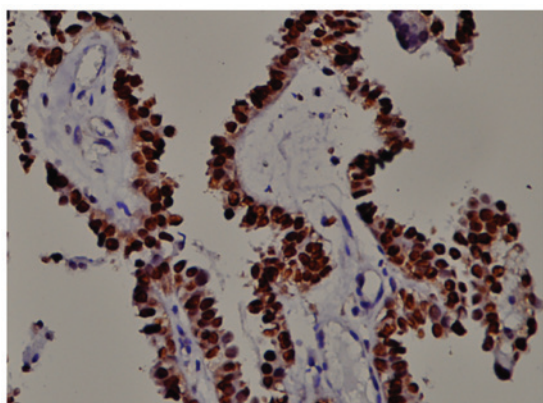


Figure 1. Expression of simian virus 40 large T antigen was assessed by immunohistochemical staining using the pAb101 antibody. The nuclei of the tumor cells are strongly immunoreactive (brown color; original magnification, x100). The specimen in the present figure was provided an Allred score of 3+.

with the epithelioid subtype and other subtypes (P=1.000), or the patients with stage IV and other stages of disease (P=1.000) (Table II). There was no significant difference in

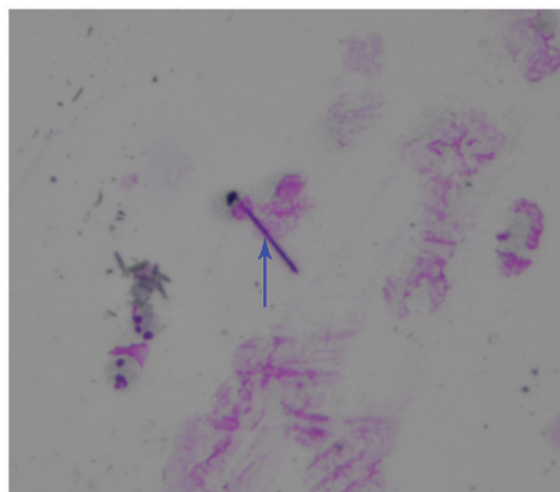


Figure 2. The presence of an asbestos body (blue arrow) in a specimen of bronchoalveolar lavage fluid (papanicolaou stain; original magnification, x100).

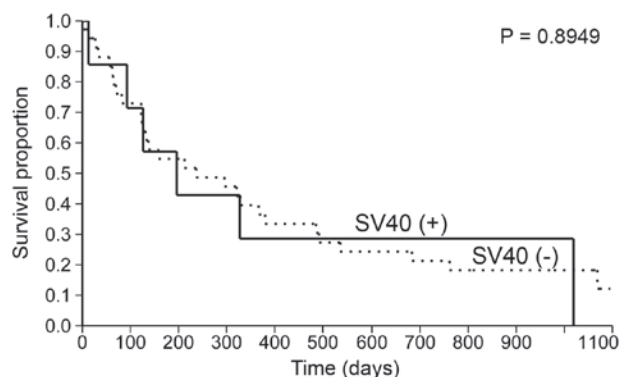


Figure 3. The Kaplan-Meier survival curves of the patients with (solid line) and without (dotted line) SV40 Tag expression. The median survival time was not significantly different between the 2 groups. P = 0.8949

the mean age between the patients with and without SV40 Tag expression (58.8±16.6 vs. 59.1±14.6; P=0.7227).

Survival time. Among the 45 patients, 34 succumbed to the disease, 5 dropped out of the study and 6 survived during the follow-up. The median survival time was 236 days [95% confidence interval (CI), 125-366]. The proportions of patients surviving for 1 and 2 years were 35% (95% CI, 22-51%) and 23% (95% CI, 12-38%), respectively. The median survival time was not significantly different between the patients with or without SV40 Tag expression (196 vs. 236 days; P=0.8949) (Table III; Fig. 3). Similarly, the median survival time was not significantly different between the patients with the epithelioid subtype and other subtypes (327 vs. 131 days; P=0.7803). By contrast, the median survival time was significantly increased in the patients receiving chemotherapy compared with the patients not receiving chemotherapy (435 vs. 196 days; P=0.0397) (Fig. 4A). The mortality in the patients receiving chemotherapy decreased by 52% compared with the patients not receiving chemotherapy (hazard ratio, 0.48; 95% CI, 0.23-0.96) (Table III). However, the Cox regression survival analysis indicated that only the clinical stages of MPM had significant effect on the survival

Table II. Comparison of the proportions of SV40 Tag expression between groups.

Characteristic	SV40 Tag expression		P-value ^a
	Expressed, frequency (%)	Not expressed, frequency (%)	
Gender			
Male	1/19 (5)	18/19 (95)	0.0578
Female	8/26 (31)	18/26 (69)	
Asbestos bodies			
Positive	1/10 (10)	9/10 (90)	0.5940
Negative	3/12 (25)	9/12 (75)	
Histological subtype			
Epithelioid	5/23 (22)	18/23 (78)	1.0000
Other	4/22 (18)	18/22 (82)	
Stage			
Stage IV	8/40 (20)	32/40 (80)	1.0000
Stages II-III	1/5 (20)	4/5 (80)	

^aP-value determined by two-tail Fisher's exact test. SV40 Tag, large T antigen of simian virus 40.

Table III. Comparisons of the survival time between groups. The total number of patients was 45.

Characteristic	No. of cases	No. of mortalities	No. of survivors, n (%)	Median survival time, days (95% CI)	P-value ^a
SV40 Tag expression					0.8949
Expressed	9	6	3 (33)	196 (13-1,019)	
Not expressed	36	28	8 (22)	236 (122-379)	
Histological subtype					0.7803
Epithelioid	23	19	4 (17)	327 (125-491)	
Other	22	15	7 (32)	131 (62-485)	
Management					0.0397 ^b
Chemotherapy	17	12	5 (29)	435 (125-1,019)	
No chemotherapy	28	22	6 (21)	196 (62-327)	

^aBetween two groups by the Log-Rank test. ^bHazard ratio, 0.48; 95% CI, 0.23-0.96. SV40 Tag, large T antigen of simian virus 40; CI, confidence interval.

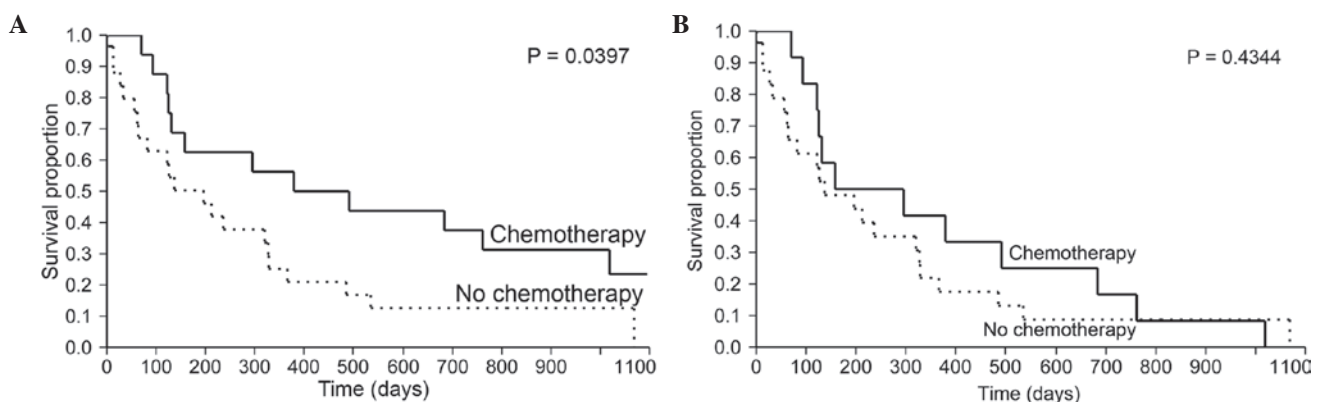


Figure 4. The Kaplan-Meier survival curves of the patients receiving chemotherapy (solid line) and the patients not receiving chemotherapy (dotted line). (A) All patients. Median survival time was significantly increased in the patients receiving chemotherapy compared with patients not receiving chemotherapy. (B) A subset of patients with stage IV disease. Median survival time was not significantly different between the patients receiving chemotherapy and the patients not receiving chemotherapy.

time ($P < 0.0001$), while the chemotherapy did not (adjusted hazard ratio, 0.75; 95% CI, 0.36-1.52; $P = 0.4317$). In a subset of patients with stage IV disease, the median survival time was not significantly different between the patients receiving chemotherapy and the patients not receiving chemotherapy (227 vs. 137 days; $P = 0.4344$) (Fig. 4B).

Discussion

The present study shows that a 5th of the Vietnamese patients with MPM demonstrated SV40 Tag expression in their histological specimens. This finding indicates that a 5th of patients with MPM may be associated with SV40, which may explain why not all patients with MPM are associated with asbestos exposure. Only half of the patients had evident asbestos exposure, indicated by either the history of asbestos exposure or the examination of asbestos bodies in clinical specimens.

To the best of our knowledge, the present study is the first to propose the association between SV40 and MPM in Vietnamese patients and to register Vietnam as one of the countries in which SV40 may potentially affect the pathogenesis of MPM. However, the proportion of patients with SV40 in the present study is decreased compared with previously published studies (8,10,15). This finding may result from the varying prevalence of SV40 infection between countries. The immunohistochemical methods used in the present study may also not be as sensitive as the molecular methods of SV40 detection, used in previous studies (9,11).

For the patients that demonstrated SV40 Tag expression, the means by which they contracted the virus was unknown. In previous studies, SV40 infection was hypothesized to be a result of receiving the SV40-contaminated polio vaccines that were produced before 1961 (8,10,15). In the present study, only 2 patients with SV40 Tag expression were born prior to 1961. Therefore, the remaining patients may have possibly contracted the virus through SV40-contaminated polio vaccines that remained available in Vietnam subsequent to 1961, as was observed in other Eastern European countries (21). Another explanation is that the patients were infected by other unknown sources (22).

In the present study, only half of the patients exhibited evidence of asbestos exposure, which is a lower figure compared with other reports (70-80%) (3). There are several potential reasons for this low prevalence: The method used to detect asbestos bodies may not be sensitive enough (23); only 22 (49%) out of 45 patients had clinical specimens available for asbestos body examination; and there may be a recalled bias regarding the history of asbestos exposure during the interviews of patients or close relatives.

Notably, only 5% of the patients exhibited overlapping results for the presence of asbestos bodies and SV40 Tag expression. This finding may imply that the interaction between SV40 and asbestos exposure is not the prerequisite for the development of mesothelioma in humans, which has been previously demonstrated in hamsters (24). The finding also supports the speculation that SV40 may be an independent carcinogen (25) or a co-carcinogen, and interact with other environmental or genetic factors in the pathogenesis of malignant mesothelioma (6).

The proportion of patients that survived for 1 and 2 years in the present study was similar to other populations (26,27). The finding that only the clinical stages of MPM significantly affected the survival time, whereas chemotherapy did not, partly explains why the prognosis of MPM remains poor, regardless of current therapies for MPM, particularly as the majority of MPM patients are diagnosed at stage IV of disease. In the present study, the median survival time was not significantly different between the patients with or without SV40 Tag expression. This finding may be explained by the lack of significant differences in the mean age, histological subtypes and clinical stages of MPM between the 2 groups of patients (Table II).

There are certain strengths of the present study. First, the present study is the first to propose the association between SV40 and MPM in Vietnam. Second, the present study included a balanced number of male and female patients, making the results more generalizable compared with other studies. Third, SV40 detection was based on immunohistochemical analysis, which avoids the potential false expression that may occur in polymerase chain reaction tests due to the presence of SV40 sequence-contaminated plasmids in pathological laboratories (13).

However, the present study has certain limitations. First, as the present study is retrospective, not all patients had clinical specimens available for asbestos body examination. Therefore, the prevalence of asbestos exposure may be underestimated in the present study. Second, SV40 was only detected using immunohistochemistry, which may not be as sensitive as other molecular methods. Third, immunohistochemistry results may yield false expression due to the immunostaining procedure and result interpretation. However, the immunostaining procedure was performed following the antibody manufacturer's protocols to minimise the risk of false expression. In addition, SV40 expression was strictly defined as the presence of strong immunoreactive tumor nuclei, which indicates the nuclear expression of SV40 Tag. Therefore, the possibility of false expression may be limited. Fourth, since the sample size is relatively small, the power to detect statistically significant differences between groups of patients was not sufficient. Finally, the present study is limited by the lack of published data regarding the general levels of SV40 in the Vietnamese population.

In conclusion, a 5th of the Vietnamese patients with MPM were infected with SV40. SV40 may be another potential cause of MPM in Vietnam and this potential association requires thorough investigation with a larger sample size and more reliable methods of SV40 detection.

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