Chapter 9
Infectious Agents Associated with Mesothelioma

Nguyen Son Lam, Nguyen Van Tho, Tran Dinh Thanh, and Yasutaka Nakano

Abstract Malignant mesothelioma is a rare but fatal disease which arises from the epithelial lining of the pleura, peritoneum, pericardium, and tunica vaginalis. Malignant pleural mesothelioma (MPM) is the most common form. The global incidence of MPM has risen steadily over the past decade and is predicted to peak in 2020. The mechanism of carcinogenesis in MPM is multifactorial. A history of long-term exposure to asbestos is the established cause of MPM. Cytolysins such as Pneumolysin, Streptolysin O, Intermedilysin, Mitilysin, and Lectinolysin secreted by the airways microbiota may create pores through which asbestos can pass through the airways, reach the visceral pleura and cause MPM. However, MPM may result from other factors such as genetics, erionite, chest wall radiation, and simian virus 40 (SV40) that may work alone or in combination. The roles of SV40 in malignant mesothelioma is still controversial. More studies are needed to explain the wide disparity in the prevalence of SV40 in mesothelioma tissues reported by different laboratories or regions. In this chapter we discuss about how infectious agents may be associated with malignant mesothelioma.

Keywords Immunohistochemistry · Malignant mesothelioma · Pleural · Simian virus 40 · Tumor · Viral carcinogenesis · Viral infection

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9.1 Introduction about Malignant Mesothelioma

9.1.1 Epidemiology of Malignant Mesothelioma

Malignant mesothelioma was first recorded in 1870 and the relation between malignant mesothelioma and asbestos exposure was established in 1960 in South African [1]. Malignant mesothelioma is a rare but fatal disease which 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% malignant mesothelioma [2]. Global incidence of MPM has risen steadily over the past decade and is predicted to peak in 2020 [3]. The incidence of malignant mesothelioma is usually underestimated, especially in developing countries. An estimate based on 1994–2008 database suggested an average of 14,200 cases worldwide each year [4]. About 3000 new cases of mesothelioma are diagnosed in the US each year, more often in men, in those aged 65 years and older, and in whites [5].

The mechanism of carcinogenesis in MPM is multifactorial. A history of heavy and long-term exposure to asbestos is the established cause of MPM [6]. However, a history of asbestos exposure have not been found in 20–60% patients with MPM [7]. In these patients, MPM may result from other factors such as genetics, erionite (a mineral found in the rocks of Turkey), chest wall radiation, and simian virus 40 (SV40) that may work alone or in combination [8]. Whatever the etiology, the clinical scenario of MPM is the same.

9.1.2 Histological Sub-Types of Malignant Pleural Mesothelioma

There are four main histological sub-types of MPM: epithelioid (the most common sub-type), sarcomatoid, biphasic, and desmoplastic. A recent study showed that among 45 patients with MPM, 23 (51%) was epithelioid, 7 (16%) biphasic, 6 (13%) sarcomatoid, 4 (9%) desmoplastic, 4 (9%) well-differentiated papillary, and 1 (2%) anaplastic subtype [9]. The sarcomatoid sub-type is associated with the worst outcomes, with a median survival of just 4 months. In contrast, the epithelioid sub-type has the most favourable prognosis with a median survival of 13.1 months [10]. Favorable predictors of overall survival were younger age, female, epithelioid sub-type, well differentiated grade, surgically or radiationally cancer-directed therapy [11]. The median overall survival of patients with MPM in the United State was 8 months [5].

9.1.3 Symptoms of Malignant Pleural Mesothelioma

The clinical onset of MPM is insidious and patients usually have non-specific symptoms. The majority of patients with MPM present with breathlessness, chest pain or both [12]. Dyspnea is the most common in MPM; the level of dyspnea increases

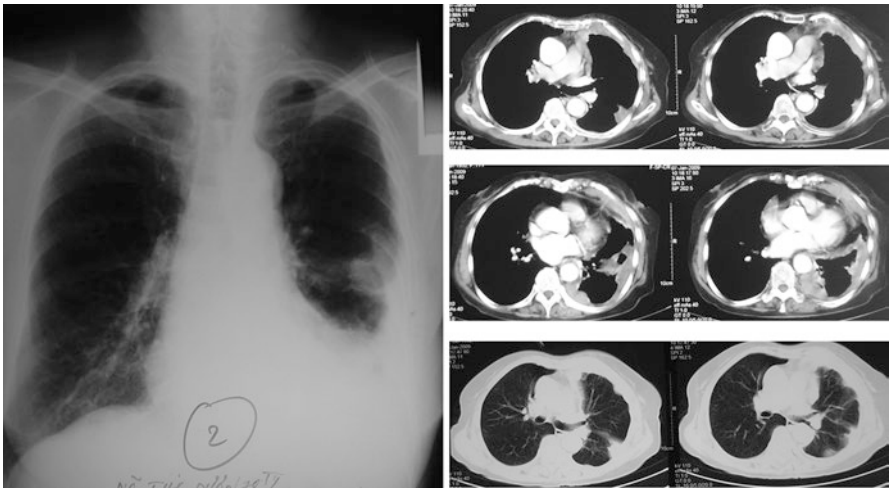

Fig. 9.1 Chest X-ray and CT images of a 77-year-old female patient with pleural malignant mesothelioma. The chest X-ray shows mild left pleural effusion accompanied with mild volume reduction of the left hemithorax. The chest CT images in mediastinal window show mild left pleural effusion and localized pleural thickenings which cause mild volume reduction of the left hemithorax. The chest CT images in parenchymal window show rind-like encasement of the left hemithorax

over time. The pleural effusion is mainly unilateral (95%), especially on the right lung (60%). Patients may present as chest pain, which can be caused by the pleural effusion or the tumor. When the tumor invades the chest wall or ribs, the severity of chest pain increases [6].

Other symptoms of MPM include fatigue, anorexia, weight loss, sweats and malaise which result from circulating cytokines, released by both the tumor and host response [12]. Cough, haemoptysis and lymphadenopathy are less common in MPM than in bronchogenic tumors.

Pleural effusion can be detected by chest X-ray. Most patients with MPM present with large pleural effusion on chest X-ray [1]. Chest CT is more sensitive than chest X-ray in detecting other signs of MPM such as localized effusion, diffuse pleural thickening, rind-like encasement of the entire lung, pleural focal masses (Fig. 9.1). CT is also useful for detecting hilar or mediastinal lymph node enlargement, and mediastinal or chest wall invasion [13].

9.1.4 Diagnosis of Malignant Pleural Mesothelioma

Diagnosing MPM is challenging because cytological evaluation yield of pleural fluid is low with a sensitivity of 26%. Biopsies are usually required to confirm the diagnosis and identify the histological sub-type. Biopsies can be obtained by using a blind Abrams needle method, or under direct vision at thoracoscopy, either as a

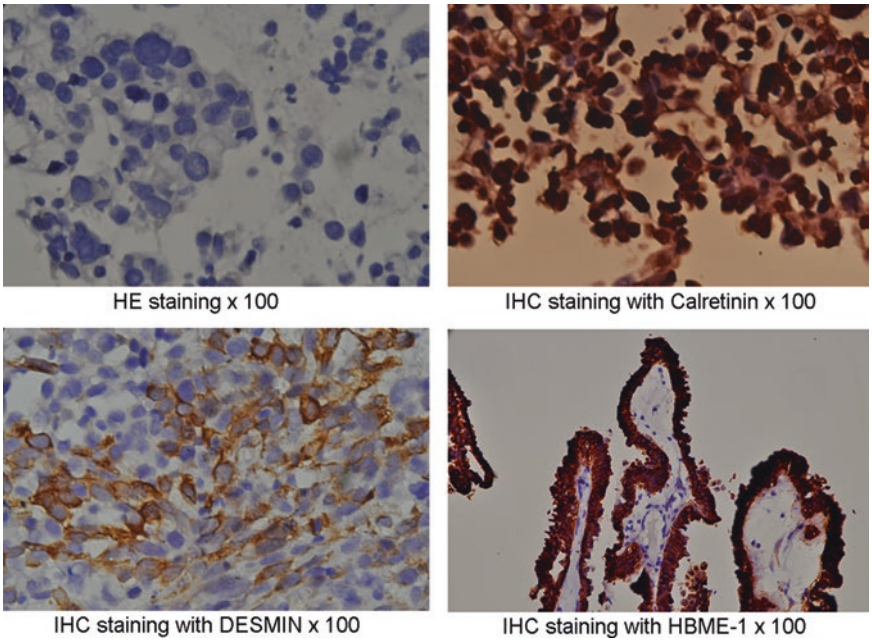

Fig. 9.2 Hematoxylin-eosin (HE) and immunohistochemical (IHC) staining to diagnose malignant mesothelioma. Left upper image: Epithelioid mesothelioma stained by hematoxylin-eosin method (original magnification ×100). Right upper image: Epithelioid mesothelioma stained by IHC method which is positive with Calretinin (original magnification ×100). Left lower image: Sarcomatoid mesothelioma stained by IHC which is positive with DESMIN (original magnification ×100). Right lower image: Well-differentiated papillary mesothelioma stained by IHC which is positive with HBME-1 (original magnification ×100)

medical thoracoscopy or as a video-assisted thoracoscopic surgery [14]. Diagnosis is achieved by needle biopsy in 21% and by thoracoscopy in 98% of patients [15].

Most patients with MPM are diagnosed definitively based on the histological examination of pleural specimens by using hematoxylin-eosin and immunohistochemical staining (Fig. 9.2) [16, 17]. Immunohistochemical panels are integral to the diagnosis of MPM, but the exact makeup of panels employed is dependent on the differential diagnosis and on the antibodies available in a given laboratory. Depending on the morphology, immunohistochemical panels should contain both positive and negative markers for malignant mesothelioma and for lesions considered in the differential diagnosis. Immunohistochemical markers should have either sensitivity or specificity greater than 80% for the lesions in question [18].

Four positive markers including Calretinin, DESMIN, HBME-1, and WT-1 have been used to definitively diagnose MPM (Fig. 9.2). Different negative markers have been used to rule out other cancers metastasized to the pleura such as CK7, CEA, TTF-1, and EGFR for adenocarcinoma; NSE, Synaptophysin, and MOC-31 for small cell lung cancer; LCA, CD3, CD20, CD30, CD68, and Myeloperoxidase for lymphoma and leukemia [18].

9.1.5 Management of Malignant Pleural Mesothelioma

There is no curative treatment for MPM. Systemic treatment options include chemotherapy (pemetrexed and cisplatin/carboplatin), targeted therapy (bevacizumab, an anti-VEGF monoclonal antibody) and radiotherapy, delivered separately or as part of multimodality treatment. Surgery (pleurectomy and decortication) is controversial and limited to patients with early stage and good functional status. Palliative care and symptom management are essential and the control of pleural effusions is an important factor.

A number of novel therapeutic agents are under investigation, and may provide further treatment options for MPM in the future [12]. Mesothelin is a cell surface glycoprotein highly expressed in MPM. Its expression induced matrix metalloproteinase secretion and cell invasion and it was validated as a potential target with both tumor vaccines and antibody-based approaches [19].

Amatuximab is a chimeric monoclonal antibody to mesothelin. It elicits antibody-dependent cellular cytotoxicity against mesothelin-expressing tumor cells and inhibits heterotypic adhesion of mesothelin-positive tumor cells to CA125-expressing tumor cells. A phase II clinical trial of amatuximab with pemetrexed and cisplatin in patients with unresectable MPM showed that this treatment was safe and well tolerated. Although there was no improvement in progression-free survival at 6 months (51%), the median overall survival (14.8 months) was superior to historical controls [20]. CRS-207 is live, attenuated, double-deleted Listeria monocytogenes engineered to express the tumor-associated antigen mesothelin, activating innate and adaptive immunity. The combination of CRS-207 and chemotherapy may act synergistically to alter the tumor microenvironment to potentiate immune-mediated killing. A phase 1b trial in 38 patients with unresectable MPM showed that CRS-207 has been well tolerated. In combination with pemetrexed plus cisplatin, infusions of CRS-207 resulted in a 59% rate of partial response and a median progression-free survival of 8.5 months [21].

9.2 Possible Mechanisms of Mesothelioma Development Associated with Microbiome

In the parietal pleura, where MPM predominantly arises, only ultra-thin and mostly ultra-short fibers of asbestos have been observed. There are two main theories regarding the pathways through which the inhaled fibers reach the pleural surface. Asbestos can either reach the pleural cavity in a mechanical fashion by their extrusion from the alveoli and the lung parenchyma passing through the visceral pleura or through being absorbed by the lung lymphatic system that results in the dissemination throughout the body [22]. For the first pathway, Magouliotis et al. proposed that toxins such as cholesterol-dependent cytolysins (CDC) secreted by the airways microbiota create pores through which asbestos can pass through and reach the visceral pleura [23]. CDCs' action depends on the cholesterol component of the cell

membranes. Therefore, the secretion of a CDC in an individual exposed to asbestos could potentially create the pathway through which an ultrathin fiber can escape the airways and penetrate deeper. The effect of these toxins on the plasma membranes lead to the production of pores with an average diameter of 35–50 nm [24]. The diameter of pores should be bigger than the lower limit of the width of asbestos fibers and the physical flora of the anatomical area near the pleura should contain microorganisms that produce these certain toxins. In fact, Pneumolysin (PLY), Streptolysin O (SLO), Intermedilysin (ILY), Mitilysin (MLY) and Lectinolysin (LLY) are the five main CDC toxins that could take part in the proposed mechanism and all of them are produced by species of the Streptococcaceae family, S. pneumoniae, S. pyogenes, Streptococcus intermedius and S. mitis [23].

Vascular endothelial growth factor (VEGF) plays a key role in tumorigenesis and progression of malignant mesothelioma. Mesothelial cells are unique in preventing fibrosis and adhesive lesions in the body cavities including the pleura, pericardium and peritoneal cavity. Mesothelial cells express VEGF and specific VEGF receptors. VEGF is a mitogen for endothelial cells and enhances vascular permeability [25]. In addition, it also enhances permeability in the mesothelial monolayer. The formation of pleural effusions generally involves the migration of cells and plasma from the systemic circulation to the pleural space across the vascular and mesothelial barriers [26]. VEGF receptors include Toll-like receptor 3 (TLR3), RIG-I and MDA5. TLR3 recognizes dsRNA of viral origin. Activation of TLRs leads to increase of VEGF synthesis [27]. Wornle et al. demonstrated that activation of mesothelial viral receptors leads to a time- and dose-dependent increase of mesothelial VEGF synthesis [28]. This observation could explain how viral infections can lead to pleural effusions and contribute to tumorigenesis and proliferation of malignant mesothelioma.

9.3 The Relation Between Malignant Mesothelioma and Simian Virus 40

9.3.1 Simian Virus 40

Simian virus 40 is a non-enveloped DNA virus and classified as a member of the polyomavirus family, based on the size (about 40 nm in diameter) and morphology of its icosahedral capsid (Fig. 9.3) and on the size of its double-stranded DNA genome [29, 30]. Its genome consists of a single circular double stranded DNA molecule and can be divided into three distinct regions—early, late and regulatory. The early region is expressed soon after entrance into the host cell, while the late region is expressed efficiently only after successful viral DNA replication has begun and it encodes for the capsid proteins (Fig. 9.4). Its closest relatives are two polyomaviruses recovered from humans, JC virus (JCV) and BK virus (BKV). They have shared about 69% genomic similarity at the nucleotide level, with the lowest similarity in the regulatory region sequences. The large T antigens (Tag) of the

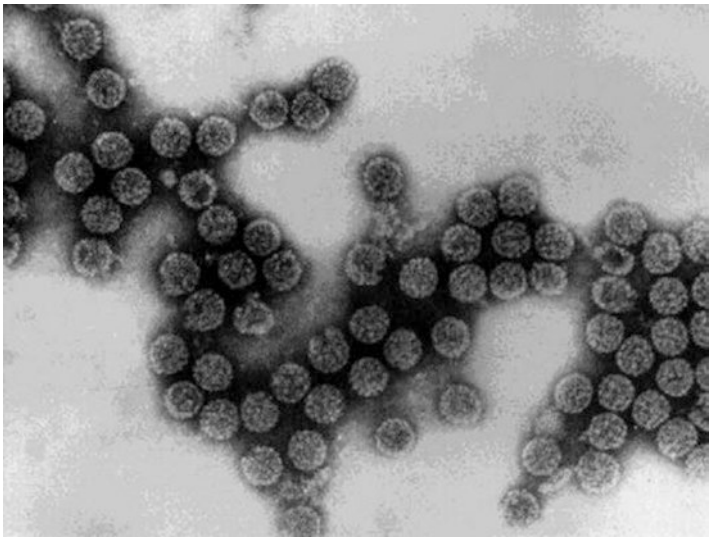

Fig. 9.3 Negative stained Transmission Electron Micrograph (TEM) shows some of the morphological features displayed by a number of Simian virus 40 virions (photo by Dr. E. Palmer, Center for Disease Control, GA, USA; no copyright restrictions under Public Domain—Property of the United States federal government)

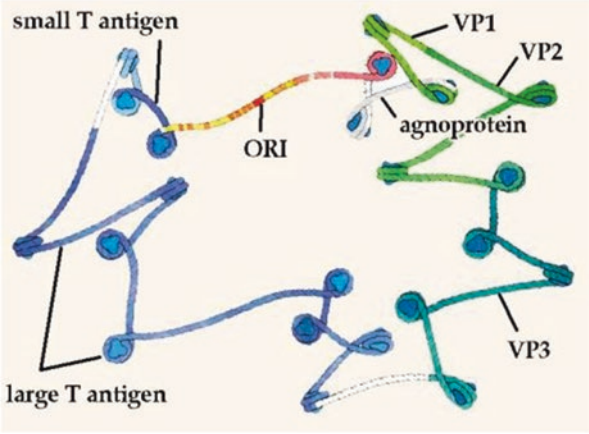

Fig. 9.4 Structural view of the 5243 nucleotide SV40 genome with its characteristic nucleosomes. Blue highlights the early region, while the late region is green. Yellow and red denote the regulatory region of the viral genome (modified from D.S. Goodsell. Simian Virus 40—November 2003 Molecule of the Month. Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank; no copyright restrictions under Public Domain)

polyomaviruses have about 75% amino acid identity [31]. Although they are closely related, they can be distinguished at the level of DNA and protein and can be distinguished by neutralizing serum and hemagglutination inhibition tests. Humans usually have JCV and/or BKV infection, so it is necessary to use specific viral reagents to detect the presence of SV40 in human tissues [32–35].

Common laboratory strains of SV40 were isolated in 1960 from contaminated vaccines or from cultured kidney cells derived from a control group of brown, green, or patas monkeys. Although there is only one known serotype of SV40, different virus strains persist and can be distinguished by changes in the structure of the virus and the designated area of the nucleotide sequence C terminus extreme Tag gene [32, 36]. Distinct nucleotides were used to demonstrate that the viral sequences involving human beings were not resulted from laboratory contamination [18, 32, 34].

9.3.2 Epidemiology of SV40 Infection in Humans

Natural infection by SV40 in humans was rare, restricted to people living in contact with monkeys, the natural hosts of the virus, such as inhabitants of Indian villages located close to the jungle, and persons attending to monkeys in zoos and animal facilities [33, 37]. SV40 can naturally infect rhesus monkey renal cells and is now widespread among the human population. The modes by which the virus has been transferred from monkeys to humans are uncertain, but it may be that the majority of this transmission might had occurred from 1954 to 1963 when hundreds of millions of people in the United States, Canada, Europe, Asia and Africa had been vaccinated with both inactivated and live polio vaccine contaminated with SV40. Barbanti-Brodano et al. showed that people who were vaccinated with the polio vaccine contaminated with SV40 shed the virus in feces for at least 5 weeks after vaccination [32]. This observation suggested that SV40 may be transferred from recipients of contaminated polio vaccine by orofecal route, and raise the possibility that, although human cells are less sensitive to SV40 replication compared with monkey cells, SV40 will spread among people due to horizontal transmission [35, 37].

The history of SV40 has been interwoven with the development of the polio vaccine. Both inactivated and live-attenuated forms of polio vaccine, as well as a number of other viral vaccines, have been prepared in primary cultures of rhesus monkey kidney cells, some of which was naturally infected with SV40 [32]. The contaminating virus escaped detection until African green monkey kidney cells were used and the presence of the virus was recognized by the development of cytoplasmic vacuolizations [30, 34, 36].

All polio vaccines were SV40 free in the United States after 1961 but SV40-contaminated polio vaccines might still be available in several countries after 1961 [38]. When using polymerase chain reaction method (PCR) to test vaccine samples from 13 countries and the World Health Organization seeds, Cutrone et al. found that all the vaccines were SV40 free, except for vaccines from a major eastern European manufacturer. These SV40-contaminated vaccines were produced from

1960s to 1978 and were used throughout the world. The procedure used by this manufacturer to inactivate SV40 in oral poliovirus vaccine seed stocks based on heat inactivation in the presence of $MgCl_2$ did not completely inactivate SV40 [38]. These findings explain possible geographic differences in SV40 exposure and different percentages of SV40-positive tumors detected in some laboratories.

9.3.3 Evidence Supporting Possible Roles of SV40 in Malignant Mesothelioma

Substantial evidence supports a role for SV40 in mesothelioma pathogenesis. SV40 is present in human mesotheliomas, where it is specifically found in the tumor cells and not in the normal surrounding tissues [30]. Mechanistic experiments in human mesothelial cells and animal experiments support a pathogenic role of SV40 in the pathogenesis of some mesotheliomas, including as a co-factor with asbestos [39].

SV40 plays causal role in the induction of malignant mesothelioma in hamsters. In an experiment, 100% Syrian hamsters developed mesotheliomas when wild type SV40 was injected into the pleural space. When SV40 was injected via the intracardiac or intraperitoneal routes, more than 50% of hamsters developed mesothelial tumors [40]. The possibility of mesothelioma induced by SV40 depends on the route of virus injection and types of mesothelial cells.

Why is SV40, not human polyomaviruses JCV and BKV, a carcinogen in malignant mesothelioma? Carbone et al. performed another experiment by culturing four types of human mesothelial cell lines with SV40, JCV, and BKV. They found that JCV did not infect human mesothelial cells. BKV and SV40 infected mesothelial cells, expressed viral oncoproteins, and caused similar alterations of key cell regulatory genes. BKV replicated faster than SV40 and caused mesothelial cell lysis, not cellular transformation. SV40 did not lyse mesothelial cells and caused a high rate of transformation [41].

Experiments in hamsters showed strong cocarcinogenesis between asbestos and SV40. SV40 did not cause malignant mesothelioma, asbestos caused malignant mesothelioma in 20% of hamsters, and asbestos and SV40 together caused malignant mesothelioma in 90% of hamsters. These findings suggested that significantly lower amounts of asbestos were sufficient to cause malignant mesothelioma in animals infected with SV40 [42].

To test the hypothesis that SV40 may contribute to the onset of malignant mesothelioma, Comar et al. conducted a molecular epidemiological study on a series of malignant mesothelioma patients from an area in north-eastern Italy hyperendemic for malignant pleural mesothelioma. They collected 63 mesothelioma samples from incidence cases of patients diagnosed with malignant pleural mesothelioma in the period 2009–2010. SV40 sequence detection and quantification was performed by specific real-time PCR. SV40 was detected in 22% of malignant mesothelioma tumors, with a low viral load. In SV40-positive patients, a threefold increased risk of asbestos exposure was observed, more evident in females (OR 4.32) than in males (OR 1.20) [43].

These findings implied that although asbestos was considered the main risk factor in malignant mesothelioma onset, a role for SV40 could be hypothesized [43].

Jin et al. performed a retrospective study on 18 autopsy samples of Japanese patients with pleural malignant mesothelioma from five hospitals in Japan. In order to detect SV40, PCR for SV40 Tag genome was undertaken following DNA sequence analysis and immunohistochemical staining for SV40 Tag. They found that SV40 Tag genome was detected in 8 amongst 19 malignant mesothelioma cases by one primer PCR. No immunopositive staining for SV40 Tag was found in any of the samples [44]. This study showed that SV40 genome was present in a subset of Japanese malignant mesothelioma patients who were unlikely to have received a contaminated polio vaccine based on their age.

A recent study was conducted to investigate the proportion of SV40 present in the histological specimens of the Vietnamese patients with MPM. Nine (20%) out of 45 patients with MPM in Vietnam were positive with SV40 Tag expression in their histological specimens [9]. This finding implied that SV40 could be another potential cause of MPM in Vietnam and this potential relation needs further investigation.

9.3.4 Potential Mechanism for SV40 to Cause Malignant Mesothelioma

Mesothelial cells of hamsters are more sensitive to SV40 compared to those of humans [45]. Cellular infection by SV40 is divided into several steps: an attachment phase followed by entry of the virus, transport in the cell, then a loss of the protein coating, the production of viral proteins and finally virus replication. The latter step generally induces cell lysis. In mesothelial cells, it has been hypothesized that this last step is limited, and this may be the reason why mesothelial cells are more susceptible to virus infection (Fig. 9.5) [29].

SV40 can transform human mesothelial cells with a “hit and run” type of mechanism. When exposed to SV40, most human mesothelial cells are infected, compared to about 20% of fibroblasts. Then most SV40-infected human mesothelial cells survive infection. When SV40 infects human mesothelial, it replicates; however, fewer viral particles are produced than in human fibroblasts and, therefore, cell lysis is infrequent. Expression of the SV40 tumor antigens (Tag and the small t antigen, tag) in 100% of the infected cells, with minimal cell lysis, causes a very high rate of malignant transformation (around $1/10^3$ cells) (Fig. 9.6) [46].

SV40 produces two oncogenic proteins, Tag and tag. The large Tag is capable of inducing structural and numerical chromosomal alterations. The large Tag also induces insulin-like growth factor expression and inhibits p53 and the pRb family, and it induces c-met activity to stimulate cell proliferation. The small tag inhibits cellular phosphatase 2A, stimulates MAP kinase and AP-1 activity, and works with Tag to bind and inhibit p53 and pRb. The combined activity of both Tag and tag induce Notch-1 and telomerase activity, which are required for malignant transformation and immortalization [30].

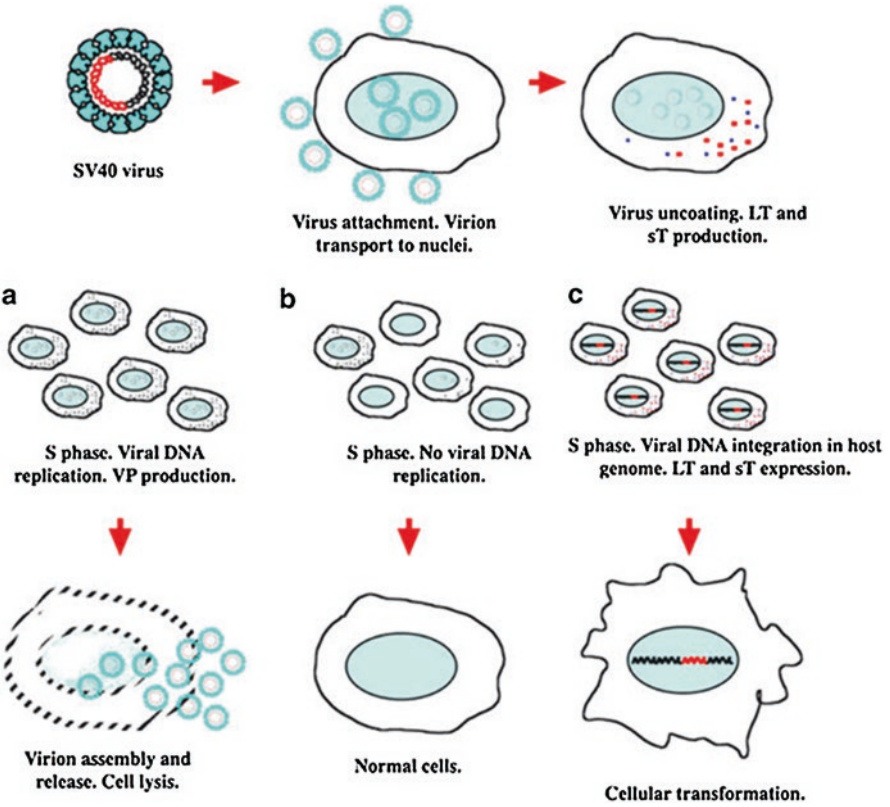

Fig. 9.5 Simian virus 40 (SV40) effects in different cellular environments. (**a**) Infection of permissive cells results in cell death and virion production. (**b**) SV40 infection of rodent cells induces S-phase but does not result in cell death or virus production. (**c**) Integration of viral DNA occurs in a very low percentage of nonpermissive cells, which then become stably transformed. *LT* large T antigen, *sT* small t antigen (Reproduced from D Ahuja et al. Oncogene 2005)

Bocchetta et al. found that p53 is not a passive inactive partner of Tag. Instead the p53-Tag complex promotes malignant cell growth through its ability to bind and activate the Insulin-like Growth Factor-I (IGF-I) signaling pathway [47]. These findings suggested that SV40 could contribute to the development of malignant mesotheliomas that occur in people not exposed to asbestos.

9.3.5 Evidence against the Roles of SV40 in Malignant Mesothelioma

Several arguments about the precise role of SV40 in the pathogenesis of all mesotheliomas remain. First, the possible impact of SV40 on overall mesothelioma incidence has not been determined. This has been limited by the fact that studies comparing

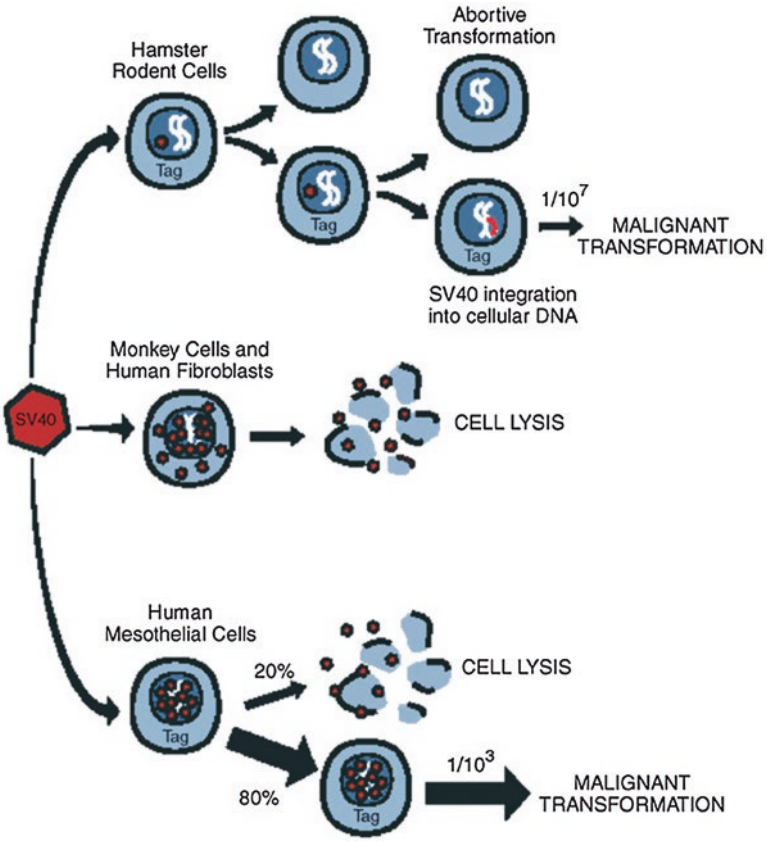

Fig. 9.6 Possible outcomes of Simian virus 40 (SV40) infection. (Top) SV40 infection of nonpermissive rodent cells, no viral particles are produced, malignant transformation is rare; (middle) SV40 infection of permissive monkey or human fibroblasts, many viral particles are produced and the cells are lysed, malignant transformation is very rare; (bottom) SV40 infection of human mesothelial cells leads to limited viral production compared to fibroblasts, limited cell lysis, and frequent malignant transformation. *Tag* large T antigen (Reproduced from M Carbone et al. Oncogene 2003)

mesothelioma incidence in SV40-infected cohorts versus non-infected cohorts are unreliable, because it seems impossible to identify infected and uninfected cohorts. Second, most mesotheliomas develop in people who have been exposed to asbestos, some of whom are SV40-negative. It may be difficult to separate the effect of SV40 and asbestos in individuals exposed to both carcinogens. Third, SV40-infected mesothelial cells should express viral antigens that would be an easy target for the immune system. Why they would not be eliminated before tumor development is unclear, but the immunosuppressive effects of asbestos may play a role. Fourth, SV40 was not found in mesotheliomas in certain countries, which indicates that, like asbestos, it is not always necessary for mesothelioma development [30].

In a retrospective study, Hirvonen et al. tested the presence of SV40-like DNA sequences in frozen tissue samples from 49 Finnish patients with MM who were not exposed to SV40-contaminated polio vaccines. They found that no SV40-specific amplification was observed in any of the mesothelioma tumor samples by PCR [48]. The results suggest that the SV40-like sequences detected in mesothelioma tissue in some previous studies may indeed originate from SV40-contaminated polio vaccines.

In another retrospective study, De Rienzo et al. found SV40 sequences in 4 of 11 mesothelioma samples from the United States but in none of the nine Turkish mesothelioma samples analyzed in the same laboratory under identical conditions using PCR [49]. The findings implied that geographical differences exist with regard to the involvement of SV40 in malignant mesothelioma.

To examine the prevalence of SV40 in malignant mesothelioma specimens in 35 patients in Japan from 1982 to 2002, Aoe et al. found that SV40 infection did not have a major role in the development of malignant mesothelioma. None of the specimens were positive with SV40 using immunohistochemical staining with anti-SV Tag antibody. Only 2 of 34 specimens were positive with SV40 using real-time PCR [50]. Reasons for low prevalence of SV40 in malignant mesothelioma in Japan are low consumption of contaminated polio vaccine in Japan (1961–1963) and ethnic difference in susceptibility to SV40, which is lower in Japanese than in other population with higher rate of SV40 infection.

By using three independent experimental approaches to detect SV40 in 71 frozen mesothelioma samples, *López-Ríos*et et al. did not support a significant role for SV40 in human mesotheliomas [51]. The first two primer sets for DNA PCR gave positive results in proportions similar to those reported in positive studies (56–62%). But these two primers in a region of the Tag gene (nucleotides 4100–4713) that is present in many common laboratory plasmids. Only 6% of specimens showed positive with less-contaminated primers. All 71 mesotheliomas were negative for Tag transcripts by real-time PCR, and lacked Tag positive tumour cells by immunohistochemistry. They suggested that inter-laboratory and geographical variations in PCR positivity for SV40 may be related less to technical factors or geographical differences in the use of SV40-contaminated polio vaccines than to the type of laboratory—i.e., whether groups carrying out the assays were in molecular-biology laboratories (with frequent plasmid work and therefore higher plasmid contamination risk) or in molecular-pathology laboratories (mostly PCR-based work with little or no plasmid work, therefore low plasmid-contamination risk) [51].

A recent retrospective study in South Korea found that SV40 is not associated with the development of malignant mesothelioma in Korea. Immunohistochemical staining demonstrated that all examined paraffin-blocks of 62 patients with malignant mesothelioma were negative for SV40 protein. Sufficient DNA was extracted for real-time PCR analysis from 36 cases. Quantitative PCR of these samples showed no increase in SV40 transcript compared to the negative controls [52].

Another argument against the evidence of supporting SV40 roles in mesothelioma development from previous reports is that the methods used to detect SV40 in those reports are not perfect. These methods include real-time PCR, sanger sequencing, pyrosequencing, and immunohistochemical staining which are used to detect

SV40 sequences or antigens in mesothelioma cells on paraffin-embedded tissues of biopsied specimens. [9, 46, 53]. They may yield false-negative results because of the low viral copy number in infected cells for molecular methods or because of the effect of formalin fixation which may result in absence of immunoreactivity for immunohistochemical staining method [54]. They may yield false-positive results because of SV40 sequences-contaminated plasmids in pathological laboratories for molecular methods [51] or because of the effects of immunostaining procedure and result interpretation for immunohistochemical staining method.

9.4 Conclusions

The mechanism of carcinogenesis in MPM is multifactorial and controversial. MPM may result from the interaction between different factors such as genetics, environmental exposure, airways microbiota and viral infection. There have been many studies supporting the close relation between SV40 and malignant mesothelioma. However, more studies are needed to confirm the potential roles of SV40 in the pathogenesis of malignant mesothelioma.

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