Journal of Carcinogenesis

BioMed Central

Review

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Pathogenesis of malignant pleural mesothelioma and the role of environmental and genetic factors

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Published: 28 July 2008

Journal of Carcinogenesis 2008, **7**:3 doi:10.1186/1477-3163-7-3

Received: 13 September 2007

Accepted: 28 July 2008

This article is available from: http://www.carcinogenesis.com/content/7/1/3

Abstract

Malignant pleural mesothelioma (MPM) is a rare, aggressive tumor for which no effective therapy exists despite the discovery of many possible molecular and genetic targets. Many risk factors for MPM development have been recognized including environmental exposures, genetic susceptibility, viral contamination, and radiation. However, the late stage of MPM diagnosis and the long latency that exists between some exposures and diagnosis have made it difficult to comprehensively evaluate the role of risk factors and their downstream molecular effects. In this review, we discuss the current molecular and genetic contributors in MPM pathogenesis and the risk factors associated with these carcinogenic processes.

Introduction

Malignant pleural mesothelioma (MPM) is a solid, locally aggressive tumor of the pleura that encases and invades the lung parenchyma in late stages of the disease (see figure 1) causing clinically significant morbidities such as dyspnea and chest pain [1,2]. Without treatment, MPM is associated with a poor median survival, ranging from 4 to 12 months [2].

The single term MPM can be misleading in that these tumors present with substantial phenotypic variability and are, therefore, classified according to the relative proportions of epithelial and spindle cells. The three major histological types include epithelial (see figure 2), sarcomatous (see figure 3), and mixed types. In all of these types of malignant mesothelioma, the cells are more frequently bi- or multi-nucleated, arranged in clumps, and the nuclear and nucleolar sizes are proportionally larger [3].

Epithelium-derived mesothelioma consists of large spherical cells arranged in solid masses and columns that form mainly within the lymphatics. These cells may also form glandular structures that resemble adenocarcinoma (see figure 2). The epithelial cells contain large numbers of desmosomes, tonofilaments, and long, slender branching microvilli that may have contact with extracellular collagen because the basement membranes are incomplete [4,5]. The sarcomatoid type originates from the deep connective tissue of the mesothelium. These tumors are characterized by ovoid-to-spindle-shaped cells similar to cells seen in fibrosarcomas (see figure 3) [6]. Epithelial mesothelioma is the most prevalent type, followed by mixed/biphasic, sarcomatous, and, rarely, desmoplastic types [7]. In a large study, epithelial cell type was observed in 61.5% of specimens (n = 930), followed by biphasic in 22% (n = 334) and sarcomatous in 16.4% (n = 247) [8]. Additional subtypes of epithelia histology include: tubular, papillary, solid, large/giant cell, small cell, clear cell,

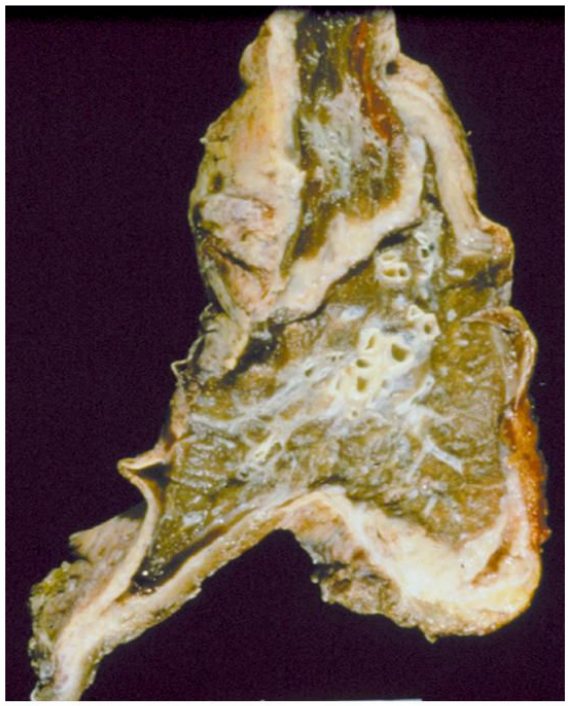

Figure 1
Gross specimen of malignant pleural mesothelioma. Presented here is an advanced case of malignant pleural mesothelioma encasing the lung and limiting lung compliance. Diffuse growth into the lung parenchyma is also present. (Personal photo of Neragi-Mianoab).

signet cell, glandular, microcystic, myxoid, and adenoid cystic [9,10].

Unlike some other tumors, there is no evidence for the existence of a premalignant, non-invasive phase for MPM [11]. This has made it difficult to determine which risk factors and molecular changes are responsible for the initiation and further progression of MPM development. However, through the use of cell culture, animal models, and epidemiology, an active field of research and debate has uncovered many players in MPM pathogenesis. The first part of this review considers the genetic and molecular changes that have been identified in the development of MPM. The second section will provide a more detailed review of the known risk factors that may cause these molecular changes and the controversies that surround these risk factors.

Genetic and molecular processes in MPM

In the development of cancer, a cell acquires alterations in gene expression and protein function that allow the cell to surpass its normal growth limits. One way of acquiring these alterations is through changes to the genome itself.

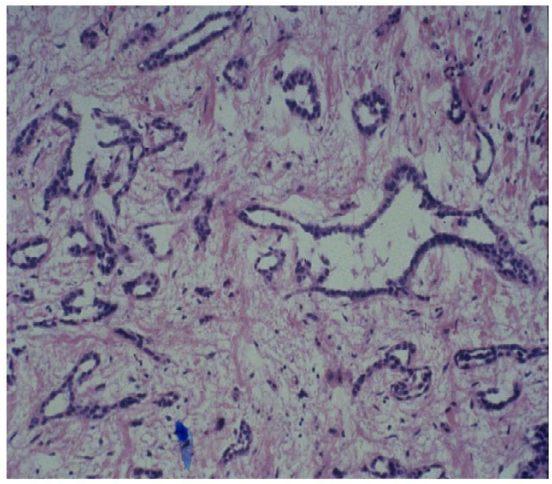

Figure 2
Malignant pleural mesothelioma of epithelial type. Epithelial MPM derives from the mesothelial cells and consists of glands and tubules that resemble adenocarcinoma. Large spherical cells are arranged in solid masses and columns mostly within lymphatics. There may also be glandular formation as demonstrated in this photograph. (Personal photo of Neragi-Mianoab).

Abnormal karyotypes, often with extensive aneuploidy and structural rearrangements, have been described for a number of genetic loci in MPM [12,13]. The effects of these mutations and additional effects from environmental risk factors have begun to explain how malignant mesothelioma cells form malignant tumors. Additional

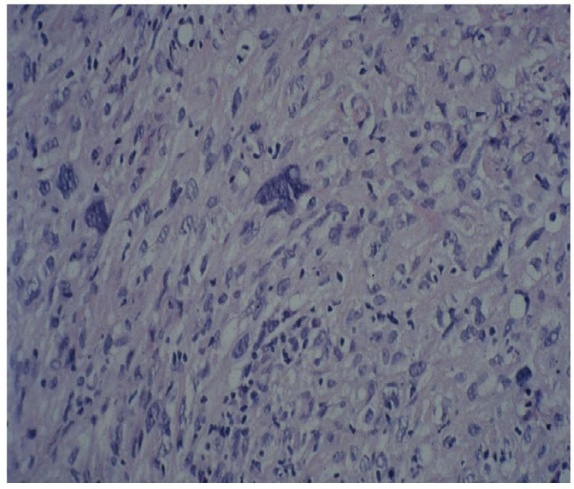

Figure 3
Malignant pleural mesothelioma of sarcomatous type. Sarcomatous MPM originates from the deep connective tissue of the mesothelial surface and resembles fibrosarcoma. (Personal photo of Neragi-Mianoab).

details about many of these pathways can be found in earlier reviews [14-16].

Tumor Suppressors

Tumor suppressor genes (TSGs) play vital roles in regulating the cell cycle in response to DNA damage and other stressors. The loss of TGS function is one of the fundamental events in tumorigenesis [17]. Loss of heterozygosity (LOH) seems to be a consistent feature of MPMs, which commonly lead to the loss and/or inactivation of multiple TSGs. Pylkkanen et al. [18] demonstrated frequent deletions at specific sites within chromosomal arms 1p, 3p, 6q, 9p, 13q, 15q, and 22q. *p16/CDKN2A*, *p15/CDKB2B* and *p14ARF* at 9p21, *FHIT* gene at 3p and *neurofibromatosis 2* (NF2) at 22q12 are frequently altered TSGs that account for some of these deleted sites in MPMs [18-22]. Epigenetic methods may also contribute to TSG inactivation. Promoter hypermethylation has been demonstrated for *p16/CDKN2A* in MPM tumor samples and cell lines, and for another TSG candidate *RASSF1A* located at 3p21 in cell lines [23,24]. Wilms tumor suppressor gene (WT1), another TSG associated with MPM, will be discussed in the section on pediatric mesothelioma.

A surprising finding in mesothelioma research is the lack of frequent mutations in the two most notorious TSGs: p53 and pRb. Although p53 mutations have been found in MPM cell lines [21,25], there is a general sense that the contributions of p53 mutations in MPM pathogenesis are minor [21,26-28]. However, the importance of p53 deregulation is well-recognized. p53 is essential for cell cycle arrest in response to DNA damage and in genomic instability. When Vaslet et al. [29] induced mesothelioma growth in heterozygous $p53^{+/-}$ mice with crocidolite asbestos fibers, the mice that had lost the functioning allele of p53 had shorter latent periods and more aggressive tumors than the mice that still maintained one copy. Outside of this model, p53 function is more commonly affected by mutations in upstream and downstream members of the p53 pathways [16,25]. The most well-known mechanism is the inactivation of p53's upstream regulator p14ARF [14]. With the loss of p14ARF, the cell loses its ability to inhibit MDM2. This allows MDM2 to inhibit p53, which can no longer induce cell cycle arrest and apoptosis.

The loss of pRb function occurs in a similar manner to p53. This time *p16/CDKN2A* is mutated. In brief, p16/CDKN2A can no longer inhibit cyclin-dependent kinases 4 and 6, which are responsible for the G1-S phase transition of the cell cycle. Left unchecked, these kinases can phosphorylate and thereby inactivate pRb, which allows an uncontrolled entry into S phase [14]. Homozygous deletion of *p16/CDKN2A* has been reported in >70% of malignant mesotheliomas, and has been associated with poor prognosis [30,31]. In addition to proteins within the p53 and pRb pathways, the role of a viral protein has also been shown to inactivate these TSGs. The large tumor antigen protein (SV40Tag) of the SV40 virus can bind to and inactivate both p53 and pRb [32-37]. However, the role of SV40 virus in mesothelioma is still a matter of debate, as discussed below.

Not only must malignant tumors continue their growth, but they must also be able to move and invade. A recent paper by Poulikakos et al. [38] proposes a mechanism through which merlin, the product of *NF2*, may help mesothelioma cells acquire this ability. Through the use of *in vitro* assays, Poulikakos et al. [38] found that the re-expression of merlin in two human malignant mesothelioma cell lines significantly decreased cell motility and invasion. Furthermore, merlin may mediate its effects through the phosphorylation of focal adhesion kinase. Further evidence is needed in order to determine if merlin functions in this particular way *in vivo*.

Oncogenes

While the loss of TSGs allows the cell to grow in light of aberrant changes in cellular DNA and function, it is the activation of oncogenes that inspires cell growth and proliferation. AP-1 and β-catenin transcription factors are implicated in MPM pathogenesis. The AP-1 family of transcription factors is known for mediating a wide range of processes including proliferation, apoptosis and transformation in response to a variety of stimuli [39]. The activation and expression of AP-1 proteins are regulated by different mitogen-activated protein kinases (MAPK) [40]. Fra-1, a member of the Fos family of AP-1 transcription factors, has been implicated in MPM pathogenesis. Ramos-Nino et al. [41] found a high level of AP-1 DNA binding activity and an increased expression of Fra-1 in mesothelioma cell lines. In addition, rat mesothelial cells exposed to either asbestos or epidermal growth factor (EGF) showed an increase in Fra-1 expression. This expression was abrogated when cells were pretreated with an inhibitor to the MAPK, extracellular-signal-regulated kinase (ERK). When mesothelioma cell lines were treated with either the ERK inhibitor or were transfected with a vector carrying a dominant negative fra-1, there was a reversal of the transformed phenotype of the cells [41]. Previous work also examined the role of asbestos and the EGF receptor in ERK activation [42]. Zanella et al. [42,43] hypothesized that asbestos may activate the EGF receptor itself or may also induce the ERK pathway through the formation of reactive oxygen species. Hepatocyte growth factor (HGF) and signaling through its receptor, c-Met, may also activate ERKs in addition to the Akt pathway [44]. Furthermore, the small tumor antigen (SV40tag) protein of the SV40 virus may activate ERK. SV40tag is known to inhibit protein phosphatase 2A (PP2A). Due to this inhi-

bition, PP2A can no longer dephosphorylate and inactivate members of the MAPK family [32,41].

The transcription factor β-catenin is regulated by an ubiquitin ligase pathway. In the absence of Wnt signaling, glycogen synthase kinase 3B (GSK3β) phosphorylates adenomatous polyposis coli (APC) and axin, which increases their affinity for β-catenin. GSK3β can then phosphorylate β-catenin, marking it for destruction. When Wnt is present, GSK3β phosphorylation is inhibited, allowing β-catenin to escape ubiquination, and enter the nucleus where it can bind to T-cell factor/lymphoid-enhancer factor (Tcf/Lef) transcription factor proteins thereby activating transcription of downstream effectors [45]. The role of this oncogenic pathway has been previously reviewed by Lee et al. [14] Here we briefly discuss the concerns surrounding the activation of β-catenin in MPM. It is interesting that activating mutations in the GSK3β phosphorylation sites of β-catenin have not been detected [46] since mutations in the p53 and pRb tumor suppressors also do not appear to play an important role in MPM pathogenesis as discussed above. Just as with these TSGs, we may need to look to upstream regulators in order to find our answer as to how β-catenin is activated. For example, an increase in disheveled expression has been observed in patient samples and mesothelioma cell lines, [47] and a lack of staining in some mesotheliomas for the C-terminus of APC has led to the hypothesis that inactivating mutations of APC may be involved [46]. A group of upstream negative-regulators of the Wnt pathway have also been implicated. Wnt inhibitory factor 1 (WIF-1) [48], Dickkopf-1 [49], and secreted frizzle-related proteins (sFRP) [50,51] have the ability to inhibit Wnt signaling at the level of cell membrane receptor activation. Thus far, data has demonstrated a possible role for promoter methylation in the inactivation of WIF-1 and sFRPs [48,50], a mechanism also described for TSG inactivation. Lee et al. [49] has also hypothesized that some of these mediators may have a role outside of the canonical Wnt-β-catenin pathway. Lastly, SV40tag may also be involved in the activation of the Wnt pathway, in addition to its role of ERK activation [32]. In this case, the inhibited PP2A cannot inactivate the transcription factor β-catenin, making entrance to the nucleus possible.

Landscapers

The role of growth factors in carcinogenesis has already been suggested above in the case of MAPK pathway activation by EGF. Growth factors can stimulate proliferative pathways through their contact with membrane receptors. They may also play a role in the process of tumor invasion and metastasis, which has been demonstrated by their ability to stimulate chemotactic and/or chemokinetic motility in mesothelioma cell lines [52]. Moreover, they can act on stromal cells in order to provide an environment favorable to tumor growth. For example, endothelial cells proliferate during the process of angiogenesis, which supplies the growing tumor with necessary oxygen and nutrients. Another important family of landscaper genes is the matrix metalloproteases (MMPs), which help to degrade the extracellular matrix that surrounds the tumor [53-57]. This process is also important in angiogenesis as well as in tumor migration and invasion. Here we also mention that the expression of cyclooxygenase 2 (COX-2) has been recognized as a prognostic factor in MPM as reviewed elsewhere [15,16]. It is possible that COX-2 may play a role in angiogenesis and/or resistance to apoptosis [58].

Many growth factors, such as insulin-like growth factor-1 (IGF-1) [16,59], hepatocyte growth factor (HGF) [44,60], basic fibroblast growth factor (b-FGF) [61], TNF-α [62], EGF [14,63], VEGF [14,64-68] and platelet-derived growth factor (PDGF) A and B [69], have been implicated in the development and progression of malignant mesothelioma. Growth factors may come from a variety of sources during the course of MPM pathogenesis such as the surrounding lung parenchyma [70], macrophages [62], and from the mesothelial cells themselves [44,62] in response to a number of stimuli including inflammatory cytokines [62,70], asbestos [62], and SV40 infection [64,65]. Some of these stimuli may also induce ectopic signaling through growth factor receptors without the need for ligand stimulation [42] and/or activating mutations of these receptors may result in signaling [44]. The role of growth factors in oncogenesis has become a provocative subject in cancer therapy since the development of cytotoxic drugs that target these growth factors offer fresh potential for the treatment of mesothelioma [67,71]. Here we discuss the role of VEGF, HGF, and TNF-α in more detail. Additional growth factors are reviewed elsewhere [14-16].

VEGF is an angiogenic peptide, which is an independent prognostic factor in MPM [66]. Higher VEGF levels were found in the pleural effusions of patients with MPM compared to its level in the effusions of patients with non-malignant pleural disease [68]. While VEGF is a fairly specific angiogenic factor, it has also been shown to increase the growth of mesothelioma cells *in vitro* [68]. The use of antisense oligonucleotides (ODN) to inhibit the production of both VEGF and VEGF-C slowed mesothelioma cell growth. In addition, antibodies to VEGF receptor (VEGFR-2) and VEGF-C receptor (VEGFR-3) had a synergistic effect in inhibiting mesothelioma cell growth [67]. One mechanism that may increase the production of VEGF in MPM is SV40 infection. Production and release of VEGF was greater in SV40Tag-positive MPM cells than in MPM cells that did not show evidence of SV40 infection [65]. It appears that this effect is mediated through more than just

SV40Tag, since mesothelial cells transfected with the full length SV40 genome produced higher levels of VEGF as compared to cells transfected with SV40Tag only [65].

HGF has many possible roles in MPM pathogenesis. It may stimulate morphological changes [64], promote cell growth and migration [44,64] and induce angiogenesis by itself or through an increase in the production of VEGF [72]. Some of these effects may be mediated by downstream signaling via Akt and ERK pathways [44]. The mechanisms by which HGF and its receptor are activated are not fully understood. Cacciotti et al. proposes an SV40-mediated activation that is dependent on SV40Tag binding to pRb. The HGF that is produced in response to SV40 infection signals through autocrine and paracrine mechanisms [64]. HGF may also come from neighboring lung tissue that has been damaged by asbestos exposure and inflammation [70]. Lastly, signaling through c-Met may be caused through activating mutations [44].

TNF-α may help to explain the survival of mesothelial cells after exposure to asbestos [62]. Human mesothelial cells are sensitive and often die after phagocytosis of asbestos fibers [73]. The dilemma caused by this finding is well stated by Yang et al. [62]: "How can asbestos cause MM [malignant mesothelioma] if HM [human mesothelial cells] exposed to asbestos die?" They show *in vitro* that asbestos induces the expression of TNF-α and its receptor in human mesothelial cells, and that cell survival may be mediated by the NF-κB pathway downstream of receptor activation. They also propose that other cells, macrophages in particular, may be important contributors of TNF-α *in vivo* [62]. More research is needed in order to test this hypothesis.

Apoptosis Genes

Apoptosis frequently occurs in response to signals from outside the cell. For example, TNF-related apoptosis-inducing ligand (TRAIL), Fas ligand and a lack of growth factor stimulation may result in programmed cell death [74]. However, it is a couple of intracellular mediators of apoptosis that have thus far been implicated in MPM. The over-expression of BCL-2 helps to protect the cell from apoptosis as reviewed elsewhere [14-16]. MPM cells may also be protected from apoptosis by the ectopic expression of telomerase [75], and SV40 infection may be one way mesothelial cells activate this protein [76]. In the absence of telomerase, telomeres located at the terminal segments of chromosomes shorten with each cell division. At first, these segments protect the coding regions of chromosomal DNA from degradation. When the telomeres become too short, apoptosis ensues. The activation of telomerase allows MPM cells to escape this mechanism of cell death and to perpetuate mutations that might have otherwise been discarded in the normal process of "cell aging."

Risk factors of MPM: Their contributions and controversies

Many risk factors have been identified as contributors to MPM pathogenesis. Above, we began the discussion surrounding the contribution of genetics, asbestos, and SV40 infection. Here we will complete our discussion of these factors and further introduce concerns surrounding the relationship of MPM to radiation and to the pediatric population.

Asbestos Exposure

Asbestos' contribution to the pathogenesis of MPM is multifaceted with effects ranging from direct to indirect, genetic to molecular. Asbestos induces mutations in mesothelial cells. The more direct mechanism of injury includes deposition of asbestos fiber in the pleura. Longer fibers can penetrate deeply into parietal pleura and have a high likelihood of causing cancer [77]. Asbestos fibers may also damage the mitotic spindle of cells and thereby disrupt mitosis, resulting in aneuploidy and DNA damage [78,79]. In a less direct fashion, asbestos can lead to the formation of reactive oxygen species (ROS). The production of ROS can be catalyzed by the iron content of the fibers or can occur through additional reactions on the fiber surface [80,81]. Macrophages that have phagocytosed asbestos fibers release ROS and lymphokines, which can damage DNA and possibly suppress the immune system [82,83]. Asbestos and/or the resulting ROS may also directly activate cell-signaling pathways [42,43].

The response of mesothelial cells to asbestos and ROS is an important factor in MPM pathogenesis. Above, we described the possible role of TNF-α in protecting mesothelial cells from death after asbestos exposure [62]. The ability to manage oxidative damage may be another mechanism mesothelial cells use to protect themselves [84]. Ferritin heavy chain (FHC) is a subunit of ferritin involved in iron sequestration. Aung et al. [84] found that when two mesothelioma cell lines were treated with asbestos, the cells that expressed higher levels of FHC had a smaller percentage increase in hydrogen peroxide generation and experienced less apoptosis as compared to cells that expressed lower levels. In addition, polymorphisms in some genes of important free radical scavenging enzymes such as mitochondrial manganese superoxide dismutase (MnSOD), glutathione-S-transferase M1 and mEH have been associated with MPM [85,86].

The Asbestos Controversy: Which Fiber is Responsible?

The use of the single term "asbestos" to describe at least five unique fibrous silicate minerals (see table 1) hides the underlying controversy as to which fibers truly carry carci-

Table 1: Classification of asbestos fibers

Type	Subtype	Known Chemical Formula
Serpentine		
	Chrysotile	Mg6Si4O10(OH)8
Amphibole		
	Crocidolite	Na2(Fe3+)2(Fe2+)3Si8O22(OH)2
	Tremolite	Ca2Mg5Si8O22 (OH)2
	Anthophyllite	(Mg,Fe)7Si8O22(OH)2
	Amosite	
Actinolite		Ca2(Mg, Fe)5Si8O22(OH)2
Fluoro-edenite		
Erionite		
Zeolite		

nogenic potential. The asbestos fibers, with regard to their bio-persistence and dimensional properties, have been stratified into two main groups: serpentine fibers (mainly chrysotile), and amphibole fibers (consisting of crocidolite, tremolite, anthrophylite and amosite). Nearly 95% of asbestos used internationally is chrysotile, and only 5% is amosite and crocidolite [87], although these groups of fibers are commonly found mixed together. New members of the asbestos family and new fiber species have been found to have carcinogenetic potential [88,89]. Exposure to these fibers may help to explain why some communities experience a high incidence of MPM without any known evidence of asbestos exposure [89].

The two different theories dealing with carcinogenicity of asbestos fibers are the Amphibole Hypothesis and Stanton's Theory. The Amphibole Hypothesis claims that only amphibole fibers can cause cancer, since chrysotile fibers are broken down and cleared too quickly to provoke carcinogenesis [90]. This is in contrast to amphibole fibers, which persist in the body for a longer period of time as a result of their durability and biopersistance [91]. The Stanton Theory suggests that long and thin fibers ($\geq$ 8 micrometer in length and $\leq$ 0.25 microm in width) are strongly carcinogenic regardless of their physicochemical nature [92], since they can penetrate further into the pleura [77].

There is data that both supports and opposes these theories. In his recent reviews [93,94], Yarborough concludes that the epidemiological evidence for the role of chrysotile fibers in MPM pathogenesis is weak and that chrysotile may not play a significant role in this disease process. However, he does admit that a threshold for chrysotile most likely exists though it has not yet been adequately detected by epidemiological research [93]. Although Yarborough's conclusions mainly support the Amphibole Hypothesis, they do not completely rule out a role for chrysotile fibers in MPM pathogenesis. Conclusions concerning the Stanton Hypothesis are also mixed. A recent expert panel concluded that longer fibers have more carcinogenic potential [95] although an earlier review of animal and *in vitro* studies by Jaurand [96] did not find this correlation. The role of the different fiber types and the quantity of exposure necessary to cause MPM are still controversial. The data are complicated by the variability in study designs and definitions as well as by the role of other risk factors such as genetics, industrial hygiene, and concomitant smoking [96-99]. However, the general consensus holds that longer fibers and amphibole fibers have more carcinogenic potential than their shorter and chrysotile counterparts in regards to MPM.

Radiation

Recent advances in cancer treatment involve multimodality approaches that include surgery, chemotherapy, and irradiation. Although these combined therapies increase survival in certain types of cancer, they can also cause the development of new malignancies [2,100,101]. Ionizing radiation, in particular, has been shown to play an important role in secondary benign and malignant tumors [102,103]. Post-radiation malignant mesothelioma has been reported after radiation therapy for breast cancer [102,104,105], Hodgkin's disease [106], cervical cancer [107]. Wilm's tumor [108-110], and seminoma [111]. In order to rule out previous asbestos exposure as a risk factor in these cases, Wissman [112] measured the levels of ferruginous bodies in the lung of a patient who developed MPM after radiation for Hodgkin's disease. The lung tissue showed 250 ferruginous bodies per gram of lung tissue, which is consistent with no significant prior asbestos exposure. In addition, Cavazza et al. [113] reviewed the National Cancer Institute's SEER data for 30 patients who developed malignant mesothelioma after radiation therapy. According to Cahan's criteria, these cases of mesothelioma may be considered as treatment-related post-radiation mesothelioma or sarcoma [114]. Radiation seems to contribute to MPM development in a small percentage of patients after radiation therapy. This low incidence may be explained by a multifactorial cause of secondary malignancies. In addition to radiation, exposure to chemotherapy, genetic predisposition, environmental cocarcinogens and other factors may be needed in their development [115]. As the frequent use of radiation therapy has raised concerns about future increases in the incidence of these secondary malignancies [116-118], future research to recognize these additional risk factors may be useful in identifying and modifying the treatment of patients who are likely to develop these secondary cancers.

SV40 virus

SV40 is a poliomavirus with double-stranded circular DNA [33,119]. The virus has two regions, early and late. The early region encodes SV40Tag, SV40tag, and 17 KT.

The late region encodes structural proteins of the virus [120]. Important roles of the early proteins in TSG, oncogene and growth factor regulation were described above. Briefly, SV40Tag can bind to and inhibit p53 and pRb TSGs, and SV40tag has been shown to inhibit PP2A, which may lead to the activation of Wnt and ERK signaling pathways. SV40 infection may also increase autocrine and paracrine signaling through a variety of growth factor pathways and induce the expression of telomerase [32]. Another possible role of SV40 in MPM that was not stated above is that SV40 infection may increase the transcription and activation of Notch-1, which may have an important role in mesothelial cell transformation and proliferation [121]. Lastly, SV40 has been hypothesized to work as a cocarcinogen with asbestos [73,122-125]. SV40 may provide another mechanism by which mesothelial cells escape asbestos' cytotoxic effects [122]. Cacciotti et al. [122] found that mesothelial and mesothelioma cells infected with SV40 were more resistant to asbestos treatment. They concluded that this effect may be mediated by the activation of the phosphatidylinositol-3 kinase/Akt (PI3K/Akt) pathway. This pathway may become activated downstream of SV40's ability to induce growth factor signaling pathways or through other mechanisms. They provided *in vivo* evidence from mesothelioma samples. 10 out of 11 tumors with detectable SV40Tag expression also stained positively for activated Akt. After helping mesothelial cells survive asbestos exposure, SV40 may also work with asbestos to cause DNA damage [125] and to transform cells [73,122,124]. When Bocchetta et al. [73] expressed SV40Tag and SV40tag in human mesothelial and fibroblast cells, cells that had been treated with asbestos showed a larger number of transformed foci as compared to cells that only expressed SV40.

Controversy about SV40: Does SV40 really play a role?

Not only does the above data supply strong mechanistic support for the role of SV40 infection in MPM pathogenesis, but *in vitro* experiments have also demonstrated a high susceptibility of mesothelial cells to develop stable infections by SV40 as compared to human fibroblasts, which quickly lyse after only semipermissive infection [73]. Despite this collection of mainly *in vitro* data, a role of SV40 infection in MPM pathogenesis has not been established due to the conflicting results of epidemiological studies.

From 1955 to 1963, the polio vaccine supplied to the United States, Canada, Europe, Asia and Africa was contaminated with SV40. Furthermore, the possibility of horizontal transmission may have enlarged this exposure [32]. Many studies [123,126-130] have found evidence of SV40 infection. A meta-analysis conducted by Vilchez [131] reviewed molecular, pathological, and clinical data from 1,793 cancer patients. He concluded that there is significant data to support a role for SV40 infection in human brain cancers, bone cancers, malignant mesothelioma, and non-Hodgkin's lymphoma. However, whether these results can be attributed to polio virus contamination is not completely clear [128,129,132,133]. Populations in Finland and Turkey were not exposed to the contaminated vaccine. In these cases, SV40 contamination was not observed in MPM as would be expected if the source of infection was primarily vaccination. On the other hand, Engels et al. [134] reported that although a contaminated poliovirus vaccine was administered to most children in Denmark from 1955 to 1961, there was no increase in malignant mesothelioma incidence [134]. Additional studies argue against the significance of SV40 infection [135,136]. In a study by Manfredi et al. [135], SV40Tag DNA was not detectable in tumor tissue of 69 mesothelioma patients. SV40Tag protein was also undetectable in tumor samples and mesothelioma cell lines by immunohistochemistry. Perhaps the standardization of SV40-detection techniques and more comprehensive studies will determine if SV40 will be a worthwhile target for preventative measures in the future [32].

Pediatric mesothelioma, familial cases and genetic predisposition

Mesothelioma is very rare in childhood with an estimated 2%–5% of all cases occurring in the first two decades of life. The diagnosis may be challenging because of its rarity and its pathologic similarities to other papillary or spindle cell neoplasms in the pediatric age group [137,138]. To date, little is known about the pathogenesis of MPM in children. Frair et al. [139] reviewed the risk factors in a series of 80 pediatric patients with malignant mesothelioma. Only four of the 80 children had exposure to known risk factors (two had history of exposure to asbestos, one had received radiation therapy, and one was exposed to isoniazid *in utero*). A causal relationship between malignant mesothelioma and asbestos exposure, radiation, and/or isoniazid could not be established. As far as asbestos is concerned, the long latency of asbestos-related mesothelioma makes a role for this risk factor in pediatric MPM unlikely [140,141] unless the natural history of asbestos-related cancer in children is very aggressive and rapidly progresses [140].

Pediatric and familial cases may open an avenue for the study of genetic contributions to MPM. Small case studies have found that pediatric patients with Wilm's tumor who were treated with radiation may be at an increased risk for mesothelioma [110,142]. This risk may represent a role of *WT1* as a TSG in MPM pathogenesis. However, this picture is complicated by data that reports a lack of inactivating mutations in MPM [143-145]. Also, one study has reported a de novo activating-mutation in *WT1* in a 45-year old woman with a perotineal mesothelioma [145],

and many studies have demonstrated the expression of WT1 in mesothelioma as reviewed by Whitson et al. [16]. It may be possible that the WT1 plays different roles in pediatric versus adult mesotheliomas, or that the association of Wilm's tumor with mesothelioma is confounded by the exposure to radiation and other risk factors. The study of pediatric patients who have not been exposed to known risk factors may provide a key opportunity to evaluate WT1 involvement in MPM in addition to the role of other genetic contributors.

Familial cases also support the role of genetic predisposition to MPM [146,147]. However, the interpretation of these studies may be complicated by the presence of common environmental exposures. Ascoli et al. [146] found that most of the familial cases in their study had been exposed to asbestos. One might conclude that this common exposure outweighs any contribution of genetics. On the other hand, these cases provide a resource to examine genetics-asbestos interactions in MPM pathogenesis, and may help to explain why less than 10% of people exposed to asbestos develop mesothelioma [130]. Already, various studies have begun to apply modern genetic association techniques to the study of mesothelioma [31,85,86,148,149]. However more rigorous studies with larger sample sizes will be needed in order to make the most out of these techniques. Ohar et al. [150] has also offered other demographic information that may be taken into consideration when planning and analyzing genetic studies.

Conclusion

MPM has a complex etiology in which asbestos, ionizing radiation, viruses, genetic factors, and even diet [151] may act alone or in concert to activate the molecular processes necessary for carcinogenesis. A multi-step process is supported by the observation that numerous chromosomal deletions accumulate in most malignant mesotheliomas, many of which result in the loss and/or inactivation of TSGs [18,22]. However, uncovering the temporality of these steps has been difficult. Although many experimental techniques have been employed, the study of MPM is complicated by its late stage at diagnosis and its rarity. The long latency between asbestos exposure and MPM diagnosis exemplifies these problems. It is unclear if the time lag between asbestos exposure and diagnosis reflects a slow-growing tumor after early genetic mutations, or if the accumulation of genetic changes reaches a threshold of malignant transformation [11] since the late stage of diagnosis makes it difficult to determine the temporality of various genetic and molecular events. In addition, the long latency and the rarity make asbestos-induced MPM a poor candidate for comprehensive cohort studies. Through the application of innovative animal models [21,124,152], *in vitro* studies, and epidemiology, we may be able to gain a better understanding of which risk factors and molecular targets are the most important for future preventative and therapeutic measures.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SJW and SNM both contributed to the research and writing. All authors read and approved the final manuscript.

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