Chapter 2
Asbestos and Mesothelioma: What Is Recent Advance in Research on Asbestos-Induced Molecular Carcinogenesis?

Marie-Claude Jaurand, Clément Meiller, and Didier Jean

Abstract The relationship between asbestos exposure and malignant mesothelioma is established since the middle of the twentieth century. From this time, scientific researches have progressed investigating the mechanism of action of asbestos on mesothelial cells, and more intensively during the beginning of the twenty-first century the analysis of the molecular changes in mesothelioma. Indeed, asbestos fibers were reported to induce chromosomal and genetic damage in mammalian cells. Mesothelioma is characterized by chromosomal alterations, which include numerous chromosome rearrangements, gene mutations, and gene deletions. Recent studies have enhanced our knowledge of the molecular landscape of mesothelioma, emphasizing mutations targeting more specifically tumor suppressor genes, differential gene expression, and DNA methylation in comparison with normal cells and between mesotheliomas, expression of noncoding RNAs, and alterations of regulatory pathways. Researches also provided knowledge of susceptibility factors in malignant mesothelioma families and relationships with asbestos exposure. It is time to review the recent advances in asbestos-induced molecular changes related to mesothelial carcinogenesis.

Keywords Asbestos · Genetic damage · Genetic susceptibility · Molecular heterogeneity · Pleural mesothelioma

M.-C. Jaurand (✉) · C. Meiller · D. Jean
Centre de Recherche des Cordeliers, Inserm, Sorbonne Université, Université de Paris, Functional Genomics of Solid Tumors laboratory, Paris, France
e-mail: marie-claude.jaurand@inserm.fr

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1 Introduction

The role of asbestos exposure in human mesothelial carcinogenesis is well established, but our knowledge on the mechanism of mesothelial carcinogenesis needs to be enhanced, as well as on the link between the molecular changes in malignant mesothelioma (MM) and the mechanism of action of asbestos on mesothelial cells. Over about 10 last years, progresses have made in the field of MM molecular characterization. Some pathological and molecular changes were ascertained and other established. These findings encouraged us to review the recent advances in asbestos-induced molecular changes related to mesothelial carcinogenesis.

2 Researches on Malignant Mesothelioma

2.1 Molecular Characteristics of Malignant Mesothelioma

Our knowledge of the molecular characteristics of MM and its pleural form has recently progressed. Earlier, chromosome rearrangements and mutations in tumor suppressor genes were reported in MPM. Rearrangements concerned numerous chromosomes, especially chromosomes 9 (9p21), 3 (3p21), and 22q, with more frequent losses than gains. Gene mutations, especially in the tumor suppressor genes *CDKN2A*, *CDKN2B,* and *NF2* mostly occur via partial or complete deletions, and low rates of mutations were detected in *TP53*, one gene frequently mutated in other cancers [1, 2]. Further studies confirmed these findings and increased the list of frequently mutated genes, especially adding *BAP1* (BRCA1-associated gene) and other genes with a lower rate of mutations such as *SETD2* (SET domain containing 2) and *LATS2* (large tumor suppressor kinase 2) [3–6]. A few genes have been inconsistently reported as altered in mesothelioma, *CUL1* [7], or at a lower rate such as *DDX3X*, *ULK2*, *RYR2*, *CFAP45*, *SETDB1* and *DDX51*, or genes from the *SMARC* family (SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily C), *PBRM1*, *COPG1*, *MLRP1*, *INPP4A*, *SDK1*, and *SEMA5B* [4, 8–10].

Gene expression profiles in MPM revealed the differential expression of specific genes in comparison with normal mesothelial cells or lung tissues, or other thoracic cancers and provided a variety of information on the mechanism of mesothelioma carcinogenesis and the prognostic value of the expression level of specific genes [4, 11–15].

Recently, three comprehensive genomic studies demonstrated the molecular heterogeneity of MPM and allowed to distinguish molecular subtypes of MPM according to their gene expression profiles [4, 6, 16]. The molecular classifications were partially related to the histological types. Although MPM is classically defined at the histological level as epithelioid, mixed, and sarcomatoid types, the gene expression profiles allowed to define histology-independent or partly dependent subtypes,

discriminating especially within epithelioid morphologies. Importantly, molecular subtypes were linked to patients' survival [4, 6, 16].

MPM heterogeneity was further investigated by transcriptome analyses using deconvolution methods [17]. This approach allowed to define a set of genes that define epithelioid-like and/or sarcomatoid-like types of MPM. Then, an MPM tumor can be decomposed as epithelioid-like and sarcomatoid-like components and can be defined by an E- and S-score, which refers to the proportion of these components. Interestingly, the S-score is strongly associated with prognosis [17]. Besides, this study also revealed that markers of the adaptive immune response were predominant in tumors with a high S-score, whereas markers of the innate immune response are found in tumors with a high E-score, consistent with an impact of the tumor microenvironment on survival [17]. The interest of associating molecular investigations and histological analysis was later proposed in a review recommending to update the histologic classification of MPM by a more multidisciplinary approach to support clinical practice, research investigation, and clinical trials [18]. An influence of the microenvironment on patients' outcome was further suggested using deep learning based on MPM histology slides [19]. Contribution of histone methyltransferases can be illustrated by the overexpression of *EZH2*, a component of the polycomb complex PRC-2, which silent histone H3 by trimethylation [20]. Recent studies highlighted the strong contribution of epigenetic regulation through DNA methylation or miRNA expression deregulation in MPM. Integration of miRNome and methylome data revealed the contribution of epigenetic regulation in the epithelioid-like and sarcomatoid-like components of the tumors [17, 21]. Some genes such as *WT1* and *PI3KR1*, or *RUNX1* and *PBRM1* were hypermethylated and underexpressed in tumors with a high E-score or S-score, respectively [17]. Next-generation sequencing analyses linked alterations of histone methylation pathway to inactivation of histone lysine methyltransferases, mainly *SETD2* and *SETDB1* [4].

Long noncoding RNAs (LncRNAs) also play a role in epigenetic regulation mechanisms. A number of LncRNAs have been identified as potential regulators of MPM, several of them being involved in EMT [22]. Their expression may be modulated by key genes in MPM, such as *NEAT1*, whose expression is dependent on *BAP1* expression, or *HOTAIR* which regulates E-cadherin expression through the recruitment of PRC2 chromatin remodeling complex [22].

A few data are available on protein expression in MM. Mass spectrometry analyses were carried out to compare differentially expressed proteins in biphasic MM and benign tumors [23]. Pathways analysis revealed a decrease of activation state in pathways of reactive oxygen species (ROS), respiratory system and cell death, and an increase of activation of phagocytes in MM tumors [23]. Großerueschkamp et al. [24] compared epithelioid and sarcomatoid MM using a method integrating FTIR (Fourier Transform InfraRed spectroscopy) imaging and laser capture microdissection, and proteome analysis of the dissected tissue. Laser capture is interesting as it allows the selection of specific regions within the tumor. Epithelioid MM overexpressed calretinin (CALB2) and several cytokeratins (CKs), and collagen A1 was overexpressed in the sarcomatoid form, consistent with the EMT. CKs and CALB2 are markers of epithelioid MM [25].

Proteomic approaches were also used to characterize MM secretome and exosome. MM secretome was analyzed in six cell lines by iTRAQ® mass spectrometry and compared to non-malignant cell lines. Results showed differential expression of proteins involved in metabolic energetic pathways, upregulation of proteins involved in cancer invasion and metastasis, and downregulation of proteins involved in cell adhesion [26]. The protein content of MM-derived exosomes was investigated in the four MPM cell lines studied in the previously quoted paper. A majority of proteins detected are expressed in various types of cancer, but specific proteins were identified in MM, either shared with all MM of differential between the MPM [27]. The proteomic findings correlated with gene expression reported in transcriptomic studies of MPM and identified biomarkers known to be expressed from immunohistochemical studies, as well as immunomodulatory components and tumor-derived antigens [27].

2.2 State of Signaling Pathways in Malignant Mesothelioma

Several signaling pathways are deregulated in human MM, leading to an unmaintained mesothelial cell homeostasis. Pathways analyses from transcriptomic data have revealed alterations in cell proliferation control, apoptosis, differentiation, cell migration, and survival [28, 29]. In cancer, both the MAPKs and PI3K/AKT/mTOR pathways are often affected by activating oncogenic mutations in genes involved in these signaling pathways, but these mutations are rare in MM [30]. In MM, these pathways are activated as assessed with the use of specific inhibitors that reduce cell growth or cell viability, and their activation may result from overexpression of specific growth factors or receptors such as EGFR and MET [29, 30]. Pathway analyses carried out in recent comprehensive integrative genomic studies highlighted P53and mTOR pathways as deregulated in MPM [4, 6, 17]. Other pathways were identified as differentially activated between MPM tumors, depending on the E/S-scores (angiogenesis, EMT, immune checkpoints, and metabolic pathways) [17].

One prominent feature in MM is the deregulation of Hippo, an evolutionarily conserved pathway involved in the development and control of organ size. When turned on, this pathway negatively controls cell proliferation, partly maintaining cell–cell contacts. Protein players of the pathway are merlin (*NF2*), LATS1 and LATS2 that silence YAP and TAZ by phosphorylation, and consequently avoid the transcription of downstream genes such as *CTGF*, *CYR61,* or *c-MYC* [31]. In MPM, several members (*NF2*, *LATS2*, *LATS1*, *SAV1*, etc.) of the Hippo pathway are inactivated due to gene mutations and/or deletions [5, 32]**.** This pathway crosstalks with other pathways, Hedgehog, Wnt, and P53. This last cross is of particular interest regarding the different rates of mutations of *NF2* and *TP53* in MM, with a possible repercussion of alteration of one pathway on the other. A recent review sheds light on the interactions between Hippo and P53 pathways, which show both mutated member genes in MPM [33]. YAP and P53 can bind to the *TP53* and *YAP* promoters, respectively. Moreover, LATS1/2 binds to MDM2, a negative regulator of P53,

and YAP1 can bind to mutant P53 and members of the P53 family [33]. Finally, these two pathways may coordinately maintain genomic stability in response to stress by the modulation of cell senescence, apoptosis, and growth.

2.3 Gene Susceptibility Factors

The possible role of genetic susceptibility in MM was suggested by recurrent familial MPM cases in cancer families. They reported increased susceptibility related to asbestos exposure [34, 35]. Some polymorphisms were found in genes involved in oxidative metabolism such as *GSTM1* or participating in base excision repair (BER) pathway, *XRRCC1* and *XRCC3* [36]. Two genome-wide association studies were carried out to identify the genetic risk factors that may contribute to the development of MPM. In an Australian study, no single nucleotide polymorphisms (SNPs) was of statistical significance when compared to Australian resident controls or asbestos-exposed control population without MM [37]. However, suggestive results for MPM risk were identified in the *SDK1*, *CRTAM,* and *RAS-GRF2* genes, and in the 2p12 chromosomal region [37]. In a case-control Italian study, with a known history of asbestos exposure, SNPs were identified in genes *SLC7A14*, *THRB*, *CEBP350*, *ADAMTS2*, *ETV1*, *PVT1,* and *MMP14* in MPM cases, but without significant threshold [38]. All these genes appeared as low risk-predisposing factors for MPM, with possible synergistic effect with asbestos exposure [39]. In contrast, *BAP1* was reported as a high-risk genetic factor for MPM [39]. Germline *BAP1* mutations were observed in families developing MM [40]. Although not occupationally exposed to asbestos, the family members were exposed in their indoor environment [40].

The frequency of germline mutations was also investigated in 198 MM patients, by targeted capture and NGS. Among 85 cancer susceptibility genes analyzed, mutations were identified in 12% of patients, and in 13 genes. A significant enhancement of the frequency of mutations in *BAP1*, *BRCA2*, *CDKN2A*, *TMEM127*, *VHL,* and *WT1* was found in MM cases in comparison with a non-cancer control population (Exome Aggregation Consortium) [41]. This study, which collected MM from peritoneum, pleura, and tunica vaginalis reported higher germline mutation frequencies in peritoneal MM, in patients with no known asbestos exposure, with a second cancer, and in tumors of epithelioid histology, when compared to pleural MM, definite exposure, no cancer, and biphasic and sarcomatoid histology, respectively. Other studies identified germline mutations in MPM patients in genes such as *PALB2*, *FANCI*, *ATM*, *SLX4*, *BRCA2*, *FANCC*, *FANCF,* and *PMS1* [39, 42–44].

Although germline mutations in *BAP1* are susceptibility factors in the induction of MM in individuals exposed to asbestos, they do not seem to lead to MM in the absence of exposure. This hypothesis is supported by experimental studies using heterozygous $Bap1^{+/-}$ mutant mice not treated with asbestos showing no or a low rate of spontaneous mesotheliomas, despite a high incidence of other types of malignant tumors, and an increased incidence $Bap1^{+/-}$ asbestos-exposed mice in

comparison with their *Bap1*$^{+/+}$ counterparts [45, 46]. Moreover, homozygous conditional knockout mice *Bap1*$^{-/-}$ generated by the injection of Adeno-*Cre* in the pleural cavity also developed a low rate of pleural mesothelioma (1/32 mice) [47, 48].

3 Asbestos Fibers and Mesothelial Carcinogenesis

Literature data have demonstrated that in addition to asbestos fibers, other types of fibers, erionite or fluoro-edenite induce MM due to environmental exposure [11, 49]. Additionally, it should be mentioned that some synthetic fibers were classified as probably (carbon whiskers) or possibly carcinogenic (some type of carbon nanotubes) by IARC [50].

3.1 Global Mechanism of Action of Mineral Fibers

Many papers reviewed the mechanism of action of asbestos fibers. Schematically, they focused either on the physicochemical properties of asbestos that may trigger toxic effects related to their fibrogenic and carcinogenic potency or on the consequences on the cell state in terms of cytotoxicity (cell growth, cell death) and genotoxicity (see for review [51–56]). Important discriminating physicochemical fiber parameters for asbestos effects are dimensions, surface reactivity, and biopersistence [56].

Hypotheses on the mechanisms accounting for the asbestos effects are based on studies with in vitro cell systems and on animal experiments. They will be briefly reminded here. Following asbestos inhalation, the mechanism first includes the clearance mechanism, which eliminates some fibers from the airways, leaving others to deposit in the lung and translocate to the pleura [57–60]. Early effects in the mesothelial microenvironment are suggested to be linked to an inflammatory reaction, as in the presence of foreign particles [58, 61, 62]. As reported in several publications, this reaction produces molecules deleterious for the cells and their microenvironment, and potentially carcinogenic such as ROS and nitrogen–oxygen species (NOS). Endogenous ROS can be also produced by normal cellular metabolism [63]. Asbestos fibers also induce genomic damages such as DNA and chromosome alterations, chromosome missegregation, and mitosis impairment [15]. Accordingly, fiber uptake, inflammation, DNA repair, and cell death are processes that play a role and modulate the effects and the consequences of asbestos–cell interactions on cell homeostasis. At present, one can ask how the molecular features identified in MPM can be linked to the mechanism of action of asbestos. We will briefly suggest some clues.

3.2 Molecular Features of MPM Possibly Related to the Mechanism of Action of Mineral Fibers

3.2.1 Genetic Damage in MPM

Remembering that carcinogenesis is a multistep process, the effects observed on cultured cells, and in short-term animal experiments can tell us on the initial damages from early effects, inflammatory response of cells, and genotoxicity of asbestos fibers. In that context, the production of ROS and NOS play a role, inducing base oxidation and nitration [53]. Inflammation is thought to play a key role in genotoxicity, due to the production of ROS by macrophages and neutrophils. Based on studies of the relationship between dose-dependent inflammation and genotoxicity of particles in animal lungs, no direct experimental evidence suggests that inflammation is a prerequisite for oxidative damage of DNA in the lung, but the association might be due to the use of high doses of particles [53]. In MPM, transversions C > A, which are lesions resulting from unrepaired 8-oxo-7,8-dihydroguanine (8-oxoGua) oxidation by ROS are not the most frequent lesions, but C > T transitions occurring by deamination of 5-methylcytosine in CpG islands [4]. This does not demonstrate a predominant role of ROS to account for gene alterations. It is noteworthy that alterations of genes frequently inactivated in MPM, such as *BAP1*, *CDKN2A*, *CDKN2B*, *SETD2*, consist often in partial or complete large deletions of exons, likely linked to other types of damage and repair systems [6, 32]. DNA alterations may occur in later stages, as a result of chronic inflammation, which can be induced by many physical and chemical [64].

DNA double-strand breaks (DSB) are other forms of DNA damage that can be caused by different sorts of clastogenic agents, by mechanical stress on chromosomes or in case of replication stress, and also promoted by abnormal mitosis [65, 66]. Several experimental works carried out with different types of cultured cells, including mesothelial cells, have shown that asbestos may interfere with mitosis [67–69]. Abnormal mitoses are revealed by various observations including the occurrence of aneuploidy, chromosome and chromatin damages, defects in spindle formation, lagging chromosomes, centrosome amplification, multipolar mitoses, and alterations of cytokinesis [36, 51, 70–74]. Cell cycle investigations have shown an accumulation of asbestos-treated cells in the G2/M phases of the cell cycle, consistent with a protracted mitosis [75–77]. It is known that mitosis impairment may promote chromosome missegregation, rearrangements, and aneuploidy, and delayed mitosis may promote DNA breakage, as shown with agents interacting with microtubule dynamics and other different conditions [66]. Therefore, the impact of asbestos on mitosis, which is due to the fiber internalization and the interaction with cell, is also an important effect to consider in the mechanisms of asbestos-induced carcinogenesis.

Repair processes are very important to resolve DNA damages. They include homologous and non-homologous recombination that may result in error-prone repair [78]. They may play a role in the genesis of MPM. On one hand, asbestos induces DNA breakage, as shown by the genotoxicity data in experimental assays. On the other hand, several publications reported pathogenic variants in DNA repair systems including recombination repair genes [39, 42].

3.2.2 Cell and Molecular Heterogeneity in MPM

A second MPM feature stands in its heterogeneity revealed at the cell and molecular levels. Pathological observations of MPM demonstrated a great morphological heterogeneity of the tumors [79]. This may reflect cell differentiation or different cell origin, as two main types of normal mesothelial cells, flattened and cuboidal, are distinguished and differentially distributed on the pleural sheets [80, 81]. In the same vein, recent data suggested that a tumor can be composed as a combination of epithelioid-like and sarcomatoid-like components, so-called histo-molecular gradients that encompass the tumor morphology and the molecular specificities [17]. This would be compatible with the in situ differences between normal mesothelial cells. Further analyses are needed to determine to what extent in situ normal mesothelial cell heterogeneity is pertinent to account for the origin of tumor heterogeneity.

Molecular heterogeneity of MPM is attested both by mutations and deregulation of signaling pathways. Molecular heterogeneity, in terms of mutations, is likely linked to the polyclonal and sub-clonal evolution of tumor cells, as shown by the intra-tumor heterogeneity [82–84]. Hippo pathway inactivation is a characteristic of some MPM. The role of the Hippo pathway is possibly linked to the structure of the pleura and to the mechanism of action of asbestos fibers. First, normal mesothelial cells form a monolayer at the serosal surface and are joined by junctions, which assure cell–cell and cell–basal membrane contacts [85, 86]. Hippo pathway activity is regulated by mechano-transduction and cell–cell adhesion and controls tight junctions [31, 87]. Its inactivation may abolish control of claudins, which are expressed in tight junctions, and differentially expressed in epithelioid compared to nonepithelioid MPM, and in MPM compared to healthy tissue [4, 17, 88–90]. Second, asbestos fibers provoke numerical chromosome changes and alteration of mitosis, especially the abolishment of cytokinesis, leading to in aneuploid cells including tetraploid cells. Interestingly, the Hippo pathway regulates the proliferation of tetraploid cells and blocks their proliferation. Asbestos fibers avoid cell abscission, and tetraploid and near-tetraploid cells are observed in asbestos-treated mesothelial cells and in MPM [91, 92]. Therefore, knockout of proliferation control may facilitate chromosome instability and the appearance of hypo-tetraploid or hyperdiploid cells, and lead to neoplastic evolution. It may be paradoxical that *NF2* seems more frequently mutated in nonexposed patients than in exposed patients, but *NF2* mutations in asbestos-exposed cells would lead to catastrophic mitosis [32].

Conversely, *BAP1*, the most frequently mutated gene in MPM, might prevent chromosome instability, by the regulation of γ-tubulin ubiquitination in *BAP1* wild-type cells [93, 94].

4 Conclusions

MPM remains thoroughly associated with asbestos fibers exposure in humans. For therapeutic purposes, numerous molecular studies have been carried out on human MPM to identify genomic alterations and activation state of signaling pathways. Experimental studies have been performed in knockout mice to assess the role of genes altered in human MPM. *BAP1* has been identified as a susceptibility gene in asbestos-exposed patients, and the Hippo pathway is the noteworthy pathway in MPM, among other frequently altered pathways in cancer.

Studies on human tumors have shown shared features between MPM tumors characterized by a high rate of chromosome rearrangements and recurrent mutations in a limited number of genes. Oppositely, a heterogeneity was evidenced between MPM at the morphological and molecular levels. Transcriptomic and proteomic studies have defined the MPM heterogeneity by the identification of individual MPM characteristics highlighting acknowledged neoplastic evolution like EMT, but so far without well-established steps of progression. Nonetheless, the original description of a histo-molecular continuum based on transcriptomic data linked to immunologic context and to patients' outcome was established [21].

Toxicology studies have documented the chromosome damage and the occurrence of potentially DNA-damaging inflammatory processes linked to asbestos exposure. The causal relationship between MPM and the mechanism of action of asbestos was consolidated by the occurrence of MPM in asbestos-exposed mice deficient in genes representative of human MPM.

Our present level of knowledge allows us to formulate hypotheses to link the identified MPM features to the mechanism of action of asbestos. In terms of genetics, the generation of abnormal mitoses in asbestos-interacting cells is likely preponderant. Improvement of our knowledge of the inflammatory microenvironment of the tumor cells should precise the role of inflammation in MPM evolution. Concerning heterogeneity, the pleural anatomy may account for the morphological heterogeneity, in addition to the neoplastic evolution. In terms of signal pathways alteration, an involvement of the Hippo pathway is likely related to its role in the regulation of membrane dynamics and growth [95, 96]. At least two elements should be considered. First, Hippo pathway components localize at cell junctions, which are important structures of the mesothelium that is formed by a monolayer of tightly joined mesothelial cell. Second, the Hippo pathway controls membrane junctions and cytoskeleton dynamics, and growth. The presence of solid material inside or near mesothelial cells impairs the chromosome and membrane dynamics during the

mitotic process. Further studies will likely clarify the relationships between mechanisms of action of asbestos and the molecular mechanism of mesothelial carcinogenesis.

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