



**HAL**  
open science

## Malignant Mesothelioma: Mechanism of Carcinogenesis

Agnes B Kane, Didier Jean, Sakari Knuutila, Marie-Claude Jaurand

► **To cite this version:**

Agnes B Kane, Didier Jean, Sakari Knuutila, Marie-Claude Jaurand. Malignant Mesothelioma: Mechanism of Carcinogenesis. Occupational Cancers, Springer International Publishing, pp.343-362, 2020, 10.1007/978-3-030-30766-0\_19 . inserm-02486806v2

**HAL Id: inserm-02486806**

**<https://inserm.hal.science/inserm-02486806v2>**

Submitted on 21 Feb 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Malignant Mesothelioma: Mechanism of Carcinogenesis

**Agnes B. Kane MD, PhD<sup>a</sup>, Didier Jean PhD<sup>b</sup>, Sakari Knuutila PhD<sup>c</sup> and Marie-Claude Jaurand DrSc, PhD<sup>d\*,1,2</sup>**

<sup>a</sup> Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, USA

<sup>b</sup> INSERM UMRS1138-EQUIPE 28. Functional Genomics of Solid Tumors, Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France

<sup>c</sup> Department of Pathology and Genetics, HUSLAB, Helsinki University Central Hospital, Haartmaninkatu 3, 400, Helsinki, HUS, 00029, Finland

<sup>d</sup> UMR-S1162. Functional Genomics of Solid Tumors, Institut National de la Santé et de la Recherche Médicale (INSERM), 27, Rue Juliette Dodu, Paris, 75010, France

<sup>1</sup> The updated version of this chapter has been made by Didier Jean and Marie-Claude Jaurand

<sup>2</sup> E-mail: [marie-claude.jaurand@inserm.fr](mailto:marie-claude.jaurand@inserm.fr)

## Keywords

Malignant mesothelioma • Mesothelial carcinogenesis • Asbestos • Pleural carcinogenesis • Genomic changes • Epigenetic changes • Signaling pathways.

## **Abstract**

Almost 60 years ago, malignant mesothelioma (MM) was acknowledged as a specific cancer related to the inhalation of asbestos fibers (1). Its strong association with asbestos exposure triggered the development of researches. They consisted in epidemiological studies to know the risk factors that explain MM occurrence in the population, and of experimental studies to understand MM biological development as a neoplastic disease. Since that time, MM remains a rare and highly aggressive cancer that prompts researches to better manage patients with MM and to offer efficient therapies. To achieve this goal, a solid knowledge of the mechanisms of mesothelial carcinogenesis is needed and deserves basic researches to progress. So far, our knowledge is based on pathophysiological and toxicological researches, and from biological and molecular studies using MM tissue tumor samples and cell lines from humans and experimental animals. Most experimental studies have been based on the cellular and/or animal responses to asbestos fibers, and in genetically modified mice, demonstrating the genotoxic effect of asbestos and relationship with MM induction. The development of large-scale analyses allowing global integration of the molecular networks involved in mesothelial cell transformation should increase our understanding of mesothelial carcinogenesis. In human, MM tumors appeared as heterogeneous entities, based on morphological patterns and molecular specificities including gene mutations. The recent development of high throughput methods allowed classification of MM according to their histological type, genomic and epigenomic characteristics and deregulated pathways. The aim of the present review is to propose a potential mechanism of mesothelial carcinogenesis by integrating data, underlying the mechanisms that may be shared with other types of fibres that may pose current health issue.

## **Introduction**

Our present knowledge of the mechanisms of mesothelial carcinogenesis originates from pathophysiological and toxicological research carried out *in vivo* in rodents and in mammalian cells in culture. The development of analytical tools allowed biological and molecular studies of malignant mesothelioma (MM) tissue tumor samples and cell lines from humans and experimental animals. Most experimental studies have been based on the cellular and/or animal including genetically modified mice responses to asbestos fibers. These investigations have provided a body of data on the cellular and molecular effects of asbestos fibers on mesothelial cells and the mesothelium, including genomic and genetic changes and alterations of regulatory and signaling pathways. Human MM has been characterized at the genomic, genetic, epigenetic, and physiological levels, with the development of large-scale analyses allowing global integration of the molecular networks involved in the transformation of the mesothelial cell.

As this volume is devoted to occupational cancer, the studies reported here will focus on asbestos, the only known human etiological factor widely used in the occupational environment associated with MM. Although epidemiological studies have clearly linked mesothelial carcinogenesis with both occupational and non-occupational asbestos exposure, no history of exposure can be found in about 10–20 % of MM cases (2-5). Some MM may be related to other fiber exposure or to other causes (6). Indeed, other types of natural fibers are associated with MM following environmental exposure, and other fibers used for industrial or commercial applications have been found to produce MM in animals, including man-made mineral fibers and more recently CNT. In 2014, IARC reviewed the classification of other fibrous materials, fluoro-edenite, silicon carbide fibres and whiskers, and CNT. Fluoro-edenite, a fibrous amphibole, was classified as carcinogenic (Group 1) as asbestos and erionite, silicon carbide whiskers as probably carcinogenic (Group 2A) and a type of CNT as possibly carcinogenic (Group 2B) (7).

CNT are of particular interest because of similarities with asbestos, which are discussed in several reviews (8-10). Recent studies investigating the effects of other elongated particles such as carbon nanotubes (CNT), and asbestos fibres as controls, have brought additional information on the mechanism of action of asbestos. In the field of investigation of the toxic potency of nanoparticles, the relation between the biological effects of asbestos and their properties led to the concept of high aspect-ratio nanoparticles (HARNs). In some parts of this review, we will mention CNT, which are a type of engineered HARNs that induces mesotheliomas and lung cancer in animal experiments (11). The aim of the present chapter is to update the data on potential mechanisms of mesothelial carcinogenesis by integrating data based on cellular and molecular effects of asbestos fibers on mesothelial cells with data obtained on altered physiological and molecular features of MM (12)

## **Deposition and Translocation of Asbestos Fibers**

The initial route of entry of asbestos fibers is by inhalation. Fibers deposit in the tracheobronchial regions, distal airways, and alveolar spaces of the lungs (13). The major deposition mechanisms are by impaction, interception, sedimentation and diffusion, and are dependent on the physical characteristics of the particles (14, 15). It results that asbestos and other elongated mineral particles have a greater inhalability than spherical particles having the same mass or volume (16). While particles and fibers are readily cleared from the tracheobronchial airways by mucociliary transport, clearance from distal airways and alveoli is slower and mediated by phagocytosis by alveolar macrophages. Fiber length impairs macrophage-mediated clearance, especially for fibers that exceed the diameter of alveolar macrophages (10–25 µm). Impaired clearance may result in penetration of fibers through the

alveolar epithelium and subsequent translocation to the pleura and distant sites (17). Fibers that enter the interstitium may cross the visceral pleura by paracellular migration or by direct penetration (18). An alternative route of translocation to the pleural space is transport via lymphatics or the bloodstream (19).

The parietal pleura lines the chest wall and the superior surface of the diaphragm, and the visceral pleura covers the lungs. The pleural space in humans is lined by a single layer of mesothelial cells approximately 1  $\mu\text{m}$  thick resting on a basement membrane and underlying connective tissue and blood vessel (20). The major route of drainage of fluid, protein, particulates, and cells from the pleural space is lymphatic stomata that open between mesothelial cells on the parietal pleural lining. Lymphatic stomata are communication holes between the pleural cavity and the parietal pleura lymphatics, where the particles are not cleared and concentrate, depending on their shape and dimensions (21-23). The diameter of lymphatic stomata ( $\sim 10\text{--}12\ \mu\text{m}$ ) limits the clearance of long fibers from the pleural space (19). The translocation of asbestos fibres in the lymph nodes and in the pleura has been reported in animal experiments, a process also found in CNT-exposed animals (8, 24-27). Asbestos persists in the lung regional lymphatics of mice one year after pharyngeal aspiration and giant cells formation is present in lymph nodes (28).

Systemic dissemination of fibers through lymphatics and the bloodstream has been described in humans following autopsy (29-31). Asbestos fibers and asbestos bodies have been noted in the liver, mesentery, spleen, and abdominal lymph nodes (32, 33). Several studies have demonstrated the presence of asbestos fibers in the human pleura (30, 31, 34). The translocation of asbestos to the pleura is also suggested by the presence of pleural plaques that develop in the parietal pleura in asbestos-exposed subjects. Parietal pleura is also the location of early MM, although MM does not seem arise from pleural plaques. However, a statistically significant association was observed between mesothelioma and pleural plaques, consistent with the role of asbestos in these pathologies (35).

Diffuse peritoneal malignant mesothelioma is also associated with exposure to asbestos fibers (36, 37). Fibers might reach the peritoneal mesothelial lining via diaphragmatic lymphatics that connect the pleura and peritoneal spaces or following systemic vascular and lymphatic dissemination. Another route of entry may be via swallowing of expectorated mucus and penetration of fibers through gastrointestinal wall. A bioavailability of asbestos fibers may account for the occurrence, not only of MM and lung cancer, but of other types of cancers, larynx, ovary, and possibly pharynx, esophagus, stomach colon and rectum (38-42).

### **The Mesothelial Cell In Situ**

The mesothelium consists of a monolayer of mesothelial cells lying on a basement membrane and supported by connective tissue containing fibroblasts and macrophages. It provides a protective barrier for frictionless interface for the free movement of apposing organs and tissues, and in fluid transport across the pleura (43). Mesothelial cells may have specialized functions at different anatomical sites, as demonstrated by morphological studies at the ultrastructural level (44). Mesothelial cells play a role in the resolution of inflammation and tissue repair after pleural injury (45). Fibrosis is a potential outcome of chronic inflammation. These processes are of particular interest in investigating the mechanism of action of asbestos fibers in the pleura.

So far, the mechanism of mesothelial cell regeneration remains poorly understood, mostly in the context of serosal injury following dialysis. However, some controversial hypotheses, have been formulated. Comprehensive reviews summarize our present knowledge of these potential mechanisms (46, 47). The regeneration process has been studied experimentally following mechanical, chemical, or heat injury of the peritoneal serosa. Briefly, six

mechanisms have been suggested to replace the injured mesothelial cells: (1) centripetal migration of adjacent mesothelial cells, (2) exfoliation of mature or proliferating mesothelial cells that replicate on the wound surface, (3) preexisting free-floating serosal cells having the capability to differentiate into new mesothelium, (4) macrophage transformation into mesothelial cells, (5) submesothelial mesenchymal precursors that migrate to and differentiate at the mesothelium surface, and (6) bone marrow-derived circulating precursors (47). The origin of these new mesothelial cells has not yet been confirmed, but according to Mutsaers et al. (47), mesothelial regeneration is not dependent on subserosal cells, but more likely results from implantation, proliferation, and incorporation of free-floating mesothelial cells (48). Recently, floating mesothelial cells were identified in pleural fluid after lung surgery in human, in the vein of this hypothesis (49).

### **Effects of Asbestos Fibers in Wild-Type Animals**

The relationship between mesothelioma and exposure to asbestos, or to other fibers, erionite and fluoro-edenite, has been well demonstrated by numerous experimental studies carried out in rodents. Some samples of asbestos fiber substitutes, refractory ceramic fibers (RCF) and glass fibers, have induced MM after inhalation by rats or hamsters. These data have been described in detail in several IARC monographs (14, 39, 50). Other routes of exposure by intracavitary pleural or peritoneal injection have illustrated the carcinogenic potency of these mineral fibers. Both types of exposure have been used to assess fiber parameters modulating the oncogenic response in the pleura. It can be emphasized here that fiber-induced MM show similar morphological features in rodents as in humans (51-54).

Some studies have investigated the pleural responses to asbestos fibers following deposition in the lung. An inflammatory reaction characterized by the recruitment of inflammatory cells and the presence of growth factors in the pleural fluid was demonstrated. These growth factors were able to induce proliferation of mesothelial cells in culture (55). An inflammatory response may be triggered by fiber translocation to the pleura as demonstrated in rodents exposed to glass fibers, RCF or CNT (56-59). The pleural reactivity to asbestos was observed in mechanistic studies using CNT, and asbestos as control fibers. Shvedova et al (28) reported the occurrence of pleuritis and mesothelial hyperplasia and/or atypia one year after pharyngeal aspiration of crocidolite in mice (28).

### **Chromosome and DNA alterations**

Chromosomal and molecular alterations have been studied in mesothelial tissue and in MM developed in rats exposed to asbestos by intraperitoneal injection. Chromosomes losses and rearrangements were observed in rats exposed to crocidolite and chrysotile (60). Significantly enhanced mutation rate of *lacI* gene from omenta in Big Blue rats (a model to detect mutation potency) was found 12 and 24 weeks post-exposure to crocidolite, and significant enhanced level of 8-Oxo-2'-deoxyguanosine (8-OHdG), a major product of DNA oxidation, in DNA from 10-20 weeks post treatment of Wistar rats (61, 62). 8-OHdG in DNA was also enhanced in rats and hamsters after intratracheal instillation 1 day after the exposure to crocidolite (63).

The type of mutations has been poorly investigated in animals. No mutations were found in *Trp53* (exons 5-8) or in *Kras* (exons 1, 2) (60, 64, 65). Additionally, no hot spot point mutation in *Kras* were detected, one year after pharyngeal aspiration of crocidolite in mice (28). In MM from Big blue rats, transversions G>T were predominant (29%) followed by deletion (26%), G>A (20%), G>C (12%), A>T (6%), A>G and insertion (3%), while in controls spontaneous mutations were G>T (19%), deletion (5%), G>A (57%), G>C (14%), A>T and A>G (0%) and insertion (5%) (61). Recently mutational signature was investigated in human malignant pleural mesothelioma (MPM) (66). The authors found a highest rate of mutations

C>T, which C>T mutations can be generated by spontaneous deamination of methyl-cytosine bases in 5'-CpG, and by APOBEC-catalyzed deamination of cytosine bases to uracil (67). One of the signature in human MPM may be associated to reactive oxygen species (ROS) but no significant difference in the mutational signature was found between asbestos-exposed and non exposed patients (66).

DNA mutations in asbestos-exposed cells may occur through generation of ROS by surface reactivity of particles, by asbestos uptake, or by inflammation. Oxidative DNA damage has been reported in several studies (68-71). Moller et al (72) reported a critical assessment of the association between pulmonary exposure to particles, considering carcinogens carbon-derived particles, quartz and asbestos and levels of oxidatively damaged DNA in lung tissues from animals (72). The authors mentioned that the results show that asbestos can generate genotoxicity in a dose-dependent manner and without a clear threshold, and that measurements of oxidatively damaged DNA, as marker of particle-induced genotoxicity in animal tissues, did not show evidence that inflammation is a prerequisite for generating DNA oxidation (72).

### **Inflammation**

Inflammation plays a role in cancer. Asbestos-related MPM pathogenesis is associated with fibroproliferative response (73). This process partly involves IL-1, as reported in a study comparing inflammation in wild-type (WT) and IL-1 $\beta$ /KO mice following injection of crocidolite or carcinogenic CNT fibers in the pleural cavity (74). Both types of mice developed mesothelial cell hyperplasia, leucocyte infiltration, granulomas and fibrotic responses, but fibrosis specific genes were downregulated in the IL-1/KO mice in comparison to WT mice (74).

Induction of inflammation was confirmed by transcriptomic and proteomic analyses. Inflammatory response to asbestos, crocidolite and tremolite, was studied at the acute (1 day) and subacute (7 days) phase in lung of mice exposed by oropharyngeal aspiration (75). Gene expression demonstrated inflammatory response (increased cytokine and chemokine release) and tissue damage (LDH release in the broncho-alveolar fluid) (75). Fifty six days post exposure perivascular and parenchymal inflammation, granulomas and fibrosis were moderate to severe (75). A proteomic analysis was carried out in lungs of mice exposed to crocidolite, single-walled CNT and ultrafine carbon black by pharyngeal aspiration (76). The overall pattern of protein changes was similar across treatments, and GO functional categories were related to inflammation/immune response, fibrosis and tissue remodeling (76).

### **Global Gene Expression**

In the previously mentioned transcriptomic study (75), apart from genes of the inflammatory response, differentially expressed genes were involved in several other pathways regulating cell movement, death and survival, growth and cell proliferation, in comparison to control. In another study where amosite asbestos fibers and CNT were instilled into the pleural cavity of mice, transcriptomic microarrays analysis showed a common molecular signature of inflammatory lesions, and antibody-based array analysis showed activation of pro-oncogenic signaling pathways, including Src family kinases, Akt, mTOR, ERK1/2, and STAT3 (77). Progression of fiber-induced lesions is characterized by increased proliferation and oxidative DNA lesions (77).

Gene mutations and signal pathway dysregulation were studied in 15 MM cell lines obtained from crocidolite-induced murine MM in three different mice strains, BALB/c, CBA and C57BL/6 (78). Whole exome analysis reported homozygous deletions in *Cdkn2a* in 14/15 cell lines, and deletion in *Trp53*, *Setd2* or *Lats2* in 1 to 3 cell lines, as well as a frequent

amplification of *Myc*(78). The genes significantly mutated belonged to pathways Wnt, Mapk and Jak/Stat, and mutations were also detected in genes from the Hedgehog and Notch pathways (78). A differential response depending on the mice strain must be noted, as the BALB/c MM cells had higher average number of mutations than MM cells from the other strains, and only mutation in one sample in the Mapk signaling pathway(78).

Exposure of laboratory mice to carbon nanotubes mimics exposure to asbestos, from initial and chronic inflammation, through loss of the same tumour-suppressor pathways and eventual sporadic development of MM. These data support that fibers of a similar nature may pose significant health risks to MM (79).

### **Immunological Effects**

Pathogenesis of asbestos, also shared by carcinogenic CNT, may be linked to their immunosuppressive effects, as reported in different studies (80-82).

### **MM induction in GEM**

To investigate the role of specific genes in MM development, several models of MM have been developed using genetically modified mice (GEM) unexposed or exposed to mineral fibers. A recent review analyzes the different studies (83).

#### **GEM Unexposed to Asbestos Fibers**

A few studies investigated the development of MM in conditional mutant mice carrying either heterozygous (Htz) or homozygous (Hom) inactivated genes in the absence of asbestos exposure (84-87)(see (83) for review). Gene inactivation was carried out by injection of AdCre (Adenovirus expressing *Cre* recombinase) in the pleural or peritoneal cavity of mice carrying floxed relevant genes. All targeted genes were tumour suppressors, *Nf2*, *Cdkn2a/Ink4a*, *Cdkn2a/Arf*, *Trp53*, *Rb*, *Tsc1*, *Pten* or *Bap1* alone or in combination. A high rate of thoracic MM was observed after injection of AdCre in the pleural cavity of double mutants *Nf2* and *Cdkn2a*, *Trp53* or *Rb* and in triple mutants *Nf2*, *Trp53* and *Ink4a* (almost 100%) (85). After injection of AdCre in the peritoneal cavity or in the bladder of double Hom *Trp53/Tsc1* mutants a high rate of MM developed, but none in Htz/Hom mutants showing a higher contribution of *Trp53* (84). Involvement of *Pten* was also reported in the occurrence of pleural MM, as Hom *Pten* mice developed a frequency of 7% MM, but when coupled with Hom *Trp53*, 56% of mice developed pleural MM (87). Of note, genetic alterations in *Tsc1* and *Pten* are found at very low frequency in MM. Kadariya et al (86) investigated the role of *Bap1*, a gene predisposing to the development of MM and frequently mutated in human MM (88, 89). Interestingly, the authors generated mice with point mutations in *Bap1* identical to germline mutations found in two human families with a *BAP1* cancer syndrome, and presenting mesothelioma in several family members (86). They also studied Htz mice (knockout in exons 6 and 7). The results showed that Htz mice developed numerous types of cancers, but few or no MM (86). The tumour type with the highest incidence was ovarian sex cord stromal tumours, found in 63% *Bap1* mutant mice (86).

#### **GEM Exposed to Asbestos**

Several studies investigated the development of MM in mice carrying Htz mutation in genes homologous to the most frequently inactivated in human MM, *NF2*, *CDKN2A/INK4A*, *CDKN2A/ARF*, *BAP1* and *TP53* (83). Studies were carried out on mice Htz for one tumor suppressor *Trp53*, *Nf2*, *Cdkn2a/Ink4a*, *Cdkn2a/Arf* or *Bap1*. A higher level of MM was found in asbestos-exposed Htz mice (crocidolite) in comparison with asbestos-exposed WT mice. No MM was observed in untreated mice (54, 86, 90-93).

Interestingly MM cells obtained from ascites in *Trp53*<sup>+/-</sup> mice exhibited *Trp53* LOH and polyploidy (94). A loss of heterozygosity (LOH) of the *Nf2* gene was found in *Nf2*<sup>+/-</sup> mice, suggesting a common mechanism for loss of the WT allele (54, 91). Moreover, in *NF2*<sup>+/-</sup> mice, two other TSG, *Cdkn2a/Ink4a* and *Cdkn2a/Ink4b*, were deleted at a high rate, while *Trp53* was mutated at a much lower rate similar to human MM, (91, 92). A loss of the WT allele was also observed in MM Htz *Bap1* mice exposed to asbestos (86).

Gene alteration and expression were studied in mesothelioma cells from asbestos-treated MexTAg transgenic mice carrying SV40 large T Antigen (SV40Tag), in comparison with WT mice (95). Analysis of the *Cdkn2* locus revealed deletion in WT animals, but not in MexTAg mice (95). As SV40Tag protein targets and impairs the p53 protein, this is consistent with different pathways of mesothelial cell transformation involving *Cdkn2a/b* and *Trp53*. Differentially expressed genes were involved in cell cycle regulation and DNA replication (95).

Murine MM closely mimics the human disease characterized by peritoneal ascites, a long latency between fiber injection and MM appearance, and histological subtypes, epithelioid, sarcomatoid, and biphasic, similar to human MM. The results obtained with GEM show that in most cases, MM progression could follow several routes involving different TSG with *Cdkn2a* and *Trp53* as independent key players. This is consistent with the specific clinical features and molecular alterations in human MM.

Collectively, results obtained in the different GEM experiments with or without asbestos exposure show that the most frequently altered murine genes homologous to the human genes, *NF2*, *CDKN2A* and *TP53* are important in the neoplastic transformation of mesothelial cells, consistent with findings in human MM. The potential of other genes, *Rb* and *Pten*, is dependent on the inactivation of other key MM genes. *Bap1* plays a role as cancer predisposing gene and is not specifically linked to the development of MM. The data obtained with asbestos-exposed mice are consistent with this observation.

### **Effects of Asbestos Fibers on Mesothelial Cells in Culture**

While early studies have been carried out with cells of different species and tissues, human and rodent normal mesothelial cells have been most widely used to study the response of mesothelial cells to asbestos fibers (96).

#### **Genotoxicity**

In cultures of normal rat pleural mesothelial cells, asbestos induces chromosome alterations and abnormal mitoses (97-102). DNA breaks, base oxidation and stimulation of DNA repair were also evidenced (68, 103-108). Furthermore, DNA breakage and cell cycle arrest were detected in rabbit pleural mesothelial cells exposed to crocidolite (68). Interestingly DNA breakage was related to the phagocytosis of fiber by mesothelial cells as reduction of phagocytosis reduced the level of DNA breakage (68). When incubated in the absence of serum or in low levels of serum concentration, cell proliferation was observed (109, 110). However, in proliferating mesothelial cells, asbestos provoked a p53- and p21-dependent cell cycle arrest consistent with the induction of a DNA damage-induced response (102). P53 was also induced in serum-deprived G0-synchronized mesothelial cells exposed to asbestos, but failed to block cell cycle progression (111). Comparison between different studies showed that significant effects were found with doses of 0.5–1 µg/cm<sup>2</sup> (71).

To summarize, studies on genotoxicity of asbestos fibers demonstrate that asbestos fibers are genotoxic for mesothelial cells. DNA repair processes are stimulated in asbestos-treated mesothelial cells. The consequences of DNA damage will be dependent on the efficiency and

fidelity of repair. When genomic damage is extensive, an apoptotic program should be induced. Life-or-death decisions may be at the heart of malignant transformation, and defective mechanisms of arrest or apoptosis may be critical to the development of malignancy (112). Several studies with mesothelial cells in culture have emphasized the occurrence of apoptosis (68, 102, 113). However, some cells can survive with genetic alterations that can be inherited in daughter cells. In that context, it is remarkable that mesothelial cells show both cell cycle arrest and mitotic abnormalities, suggesting that some cells could pass through cell cycle checkpoints with unrepaired DNA and chromosomal damage. Recent findings suggest that BAP1 could play a role as reducing apoptosis (114).

### **Inflammation**

The ability of mesothelial cells to interact and internalize asbestos fibers is an important feature that is linked to the deleterious effects of asbestos, especially production of inflammatory factors by these cells, and interaction with the dynamic of mitosis. Activation of the Nalp3 inflammasome that triggers inflammation, is observed in mesothelial cells exposed to asbestos (73, 115).

### **Epigenetic Changes**

Recently, some data on epigenetic changes in asbestos-exposed cells in culture were reported. DNA methylation profiling and gene expression were studied in Met5A cell line exposed to asbestos (chrysotile and crocidolite) (116). Only 26 CpG sites were differentially methylated after treatment by both asbestos types and methylation changes were the same for 15 of them (116). Results did not show correlation between methylation and gene expression, except for *DKK1*, an inhibitor of Wnt signal pathway, whose expression is upregulated by chrysotile treatment. With chrysotile, differential methylation occurred in genes involved in cell response to stimuli, cell adhesion and cellular matrix (116). With crocidolite, several genes from the DNA damage response were downregulated and upregulated genes were involved in metabolic process (116).

### **Effects on Signaling Pathways**

Two studies investigated the response of human mesothelial cells to crocidolite asbestos by transcriptomic analyses (117, 118). Gene expression was investigated in normal pleural human mesothelial cells and in LP9, an h-TERT immortalized human mesothelial cell line exposed to crocidolite by transcriptomic analysis (118). Several genes were upregulated (*ATF3*, *PTGS2*, *FOSB*, *IL8*, *NR4A2*, and *TFPI2*). Among them the transcription factor *ATF3* regulated levels of asbestos-induced inflammatory cytokines, IL-1b, IL-13, G-CSF, and the growth factor, PGDF-BB, in LP9/TERT-1 cells (118). *ATF3* silencing by specific siRNA reduced cytokines and PGDF-BB expression levels (118).

The response of Met-5A cells to crocidolite was investigated using a Protein Pathway Array, which assesses proteins and phosphoproteins functionally linked to proliferation, apoptosis, cell cycle regulation, DNA repair, signaling, and transcription activity (119). Three pathways were only affected by crocidolite, ILK signaling, PPARa/RXRa and G1/S phase checkpoint regulation (119). Interaction between pathways, investigated by Ingenuity Pathway Analysis identified several proteins regulating the networks, P53, CCND1, RB1 and CTNNB1 in asbestos-treated Met-5A cells in comparison with untreated cells (119). These results confirm the effects of asbestos on cell cycle progression. Concerning the role of P53, it must be noted that Met-5A are SV40-transformed cells, which show a basal accumulation of nuclear P53(120).

An upregulation of genes involved in invasion, including *MMP2*, was reported in a transcriptome microarray analysis of Met-5A mesothelial cells exposed to CNT and crocidolite at subcytotoxic concentrations (121). Gene signaling network analysis found other genes

involved in the asbestos- or CNT-induced invasion network as potential regulators of *MMP2* (121).

### **Fiber Properties in Relation to the Biological Effects and Carcinogenic Potency**

These paragraphs summarize the biological mechanisms leading to the development of diffuse malignant mesothelioma, focusing on the physiochemical properties of asbestos fibers, and other carcinogenic natural mineral fibers known to induce MM in human. Several recent mechanistic studies have been carried out with CNT providing new perspectives to account for the mechanism of action of elongated particles. The reader is referred to comprehensive reviews for a details on the fiber properties in relation to the biological effects and carcinogenic potency (8, 10, 11, 19, 122). Several fiber parameters are of importance in the mechanism of asbestos toxicity.

### **Physico-chemical Properties of Asbestos Fibers and Elongated Particles.**

Asbestos fibers are fibrous silicates and are classified into two groups based on their crystal structure and chemical composition: serpentine asbestos which is called chrysotile and amphibole asbestos which includes crocidolite, amosite, tremolite, actinolite, and anthophyllite (123, 124). Fluoro-edenite is a fibrous amphibole not used in the industry, but naturally occurring present in quarry stones (125). Erionite fibers are a form of the mineral zeolite characterized by a high internal surface area. They are associated with the development of diffuse malignant mesothelioma in epidemiological studies (126-128). These naturally occurring fibrous minerals are variable with respect to chemical composition, associated minerals, and trace contaminants depending on their geographic origin (129). Asbestos fibers may contaminate other mineral deposits, for example, talc (126, 130) and vermiculite from Libby, Montana (130, 131), and exposure to these mixed materials has also been linked with diffuse malignant mesothelioma (128, 132). The physiochemical properties of mineral fibers associated with biological activity include shape and dimensions, surface chemistry and reactivity, and biopersistence (8).

### **Shape and dimensions**

Shape and dimensions are fiber parameter modulating the biological effects of asbestos and elongated fibres. Fiber length and diameter determine the respirability and site of deposition in the lungs, and clearance mechanisms. Short fibers are taken more easily by macrophages than long fibers and can be eliminated by the clearance mechanisms. In experimental studies, it was generally found that the fiber dimensions are important, with long and thin fibers more active than shorter fibers on cultured cell and with a greater carcinogenic potency in animals.

Phagocytosis is an important function of macrophages and other cells as it determines the intracellular availability of the fibers and possible interactions with cell components (73, 115). A recent study investigated phagocytosis of CNT according to their geometry and demonstrated that geometry and volume influence the efficiency of phagocytosis (133).

Fiber length has been associated with the induction of aneuploidy and chromosomal damage due to direct physical interference with the mitotic apparatus or by binding to cell cycle regulatory proteins (134, 135) (136). Chromosome damage and mitosis impairment is also a feature of CNT as observed in several types of rodent and human cells (137-139).

### **Surface chemistry**

Surface chemistry determines interactions between the fiber and the molecules present in the fiber vicinity. Fibers may interact with macromolecules in the biological fluids (proteins,

phospholipids...) (128). Surface iron, especially on amphiboles surface may be released, which could catalyze the formation of reactive oxygen species (ROS), and may be associated with biological effects of mineral fibers including lipid peroxidation, oxidative DNA damage, and activation of intracellular signaling pathways (140-143).

### **Biopersistence**

Biopersistence is considered as a major determinant of fiber pathogenicity in the lungs (144). It is dependent on fiber clearance and on the ability of fibers to be broken, splitted, or attacked by the biological medium in the lungs (144). Differences in biopersistence of asbestos fibers have been linked with carcinogenic potency, as biopersistent fibers could sustain a local inflammatory response (145). Amphibole asbestos fibers are more potent than chrysotile asbestos fibers due to their increased biopersistence in the lungs (8). However, chrysotile fibers are detected in autopsic lungs several years past-exposure to asbestos, and their biopersistence and effects could be linked to the surface modification of the fibers (146-148). Additionally, these fibers should be stable regarding the lung pH (149). Fiber biopersistence in the pleura is not documented; in particular, there are no data on the relationship between biopersistence in the lung and translocation of fibers from the lung to the pleura, nor on the pleural clearance of fibers following inhalation (150, 151).

High aspect ratio and biopersistence have been hypothesized to be important properties of engineered nanomaterials that raise concern about their potential to be translocated to and retained in the pleura following inhalation (19, 152). A long-term study, after intratracheal instillation of CNT in rats, reported that pulmonary lung burden did not decrease significantly over time up to more than one year after instillation (153).

### **Summary Hypotheses on the Mechanism of Action of Asbestos Fibers to Generate MM**

The development of diffuse malignant mesothelioma is a complex, multistage process that is governed by the physicochemical properties of crystalline mineral fibers and their propensity to migrate to the pleural and peritoneal linings. The most important properties of asbestos fibers related to carcinogenicity are fibrous shape and dimensions, surface chemistry and reactivity, and biopersistence (39).

Interactions between mesothelial cells and fibers can cause genetic and chromosomal changes. There is a great body of evidence (1) that asbestos fibers can directly interfere with chromosomes and the mitotic spindle and (2) that they induce the formation of reactive ROS resulting in DNA breaks, oxidation, and mutations (154-157). Further, (3) the physical interaction of fibers with target cells causes persistent inflammation and, consequently, modulation of inflammatory and immune responses. ROS have been clearly indicated to cause genetic damage including chromosomal breaks and mutations and they are well known to initiate signal transduction pathways that are, in turn, linked to inflammation, proliferation, and apoptosis (157, 158). Free radical scavengers decrease genotoxic endpoints such as micronucleus formation induced by fibers and antioxidant enzymes can protect cells against genotoxicity induced by chrysotile fibers (159, 160).

Prolonged interaction between pleural inflammatory cells and adjacent mesothelial cells causes persistent release of chemokines and cytokines, inflammatory mediators, ROS and reactive nitrogen species, and growth factors, that trigger repeated episodes of inflammation resulting in mesothelial cell injury, death and/or proliferation (161). This may be also linked to altered gene methylation patterns and to epigenetic gene silencing identified in human MM (162-164). Genomic instability and acquired gene and chromosomal alterations in mesothelial cells may lead to altered cell cycle and growth regulation, resistance to apoptosis, impaired repair of DNA and chromosomal damage, activation of oncogenes and inactivation

of tumor suppressor genes (134, 135, 163, 165). This persistent inflammatory microenvironment in combination with oxidative stress and cell division impairment generates a strong selective force for mesothelial cells that have acquired genetic and epigenetic changes that promote their survival, proliferation, and tumor progression (164).

### **Molecular Alterations in Human MM**

Carcinogens provoke several types of somatic gene mutations, consisting of DNA and chromosome alterations. Some mutations are the signature of past exposure to given carcinogens. Somatic mutations in tumors are of interest both to determine the mechanism of action of carcinogens and to elucidate their adverse consequences on cellular homeostasis.

### **Chromosomal imbalance**

Structural and numerical chromosomal abnormalities are numerous and complex in MM. A detailed review can be seen in Chapter 19 (166). It can be summarized here that one of the most frequent alterations are losses in the 3p21 region including the frequently inactivated gene *BAP1*, and other less frequently altered gene *SETD2* (167). Frequent losses also occur in 9p21, which encloses the *CDKN2A(INK4A/ARF)* locus, encoding both the P16<sup>INK4A</sup> and the P14<sup>ARF</sup> proteins, and the *CDKN2B* locus, encoding P15 protein, and in 22q12 which encloses the *NF2* locus, which encodes the protein merlin.

### **Gene mutations**

In MPM, there are a limited number of genes known to be recurrently mutated in a high percentage of MM.

Inactivation of *CDKN2A* and *CDKN2B* TSG are mostly due to large deletions (168-170). *CDKN2A* deletions have been considered as a marker of asbestos exposure in a study of non-small cell lung carcinomas (171). In MM, DNA methylation of *CDKN2A* and *CDKN2B* have been reported at a frequency of 13 % (nine patients) and 4 % (three patients) respectively, and positively correlated with asbestos body counts in the lung (172, 173). The average methylation frequency of these genes in the literature is about 10 % (92, 172, 174-178). It was also suggested that mesotheliomas express microRNA (miRNA) that could inhibit *P16/CDKN2A* expression, based on an in silico analysis for miRNA target gene prediction (179). Interestingly, a recent experimental study of instillation of either long asbestos fibers (amosite) or long CNT showed hypermethylation of *Cdkn2a(Ink4a/Arf)* in early lesions that precedes mesothelioma (77). Both P16<sup>INK4A</sup> and P15<sup>INK4B</sup> are inhibitors of the kinase function of cyclin/cdk complexes involved in cell cycle progression. The protein P14<sup>ARF</sup> has an indirect function on cell cycle regulation, by positively regulating the level of P53 through interaction with P53 inhibitors. Consequently, cells with damaged DNA can proliferate and survive in the absence of P14<sup>ARF</sup>. In murine models of asbestos-induced mesothelioma, the orthologous genes, *Cdkn2a/Ink4a* and *Cdkn2b*, are also inactivated by deletion (91, 92, 180)(83).

*TP53* mutations occur at a lower rate in comparison with other human cancers, they are mainly due to non- or missense substitutions (66, 168, 170, 181, 182). Different frequencies of 7.4% and 16.3% are reported in 2 studies respectively(66, 170). None *TP53* mutation was reported in the epithelioid molecular MM subtype, in a whole exome analysis of 202 MPM (66), but *TP53* mutations were found in MM of epithelioid histologic type in other studies (170, 183). The protein P53 is activated in response to DNA damage and is a regulator of senescence, apoptosis and autophagy. In animal models of MM (see above), the mutated status of *Trp53* was investigated in mice exposed to mineral fibers by intraperitoneal inoculation. In C57Bl/6 p53<sup>+/-</sup> mice, a strain having one allele mutated in the gene *Trp53*, loss of the WT allele was found at a high rate in MM induced by asbestos fibers (90).

In large-scale analyses alterations of *NF2* TSG are frequently found, in about 20 % of MPM (66, 170). Higher percentages were previously reported in smaller series (184-187). *NF2* has pleiotropic functions, being involved in regulation of cell proliferation, apoptosis, and endocytic trafficking and acting upstream of several signaling pathways including the Hippo signaling pathway (188). Mutations in *NF2* consist of both point mutations and deletions (189). In *Nf2<sup>WT</sup>* and *Nf2<sup>+/-</sup>* FVB mice, *Trp53* alterations were infrequent. *Nf2* mutations were detected in mice exposed to asbestos and exposed to ceramic fibers (92, 180). Alteration in the chromosomal region of the *Trp53* locus was infrequent (190). These results suggest that deletions would be more likely a consequence of the mechanism of action of asbestos, while p53 point mutations could be related to “spontaneous” gene alterations in this model.

Alteration of *NF2* is also consistent with a physical mechanism of action of asbestos fibers with mesothelial cells. The encoded protein, merlin is a regulator involved in signaling pathways that control, among other parameters, cell shape, proliferation (involving the hyaluronic acid receptor, CD44, which is important for proliferation of MM cells), survival, and motility (188). Merlin is a component of the adherens junctions and other types of cell-to-cell contacts (191, 192). As cell division is mechanically impaired by the presence of asbestos fibers, mutation of *NF2* could be responsible for enhanced proliferation as well as impaired mitotic control.

Somatic *BAP1* mutations are frequent in MM. A frequency of about 20% was reported in several studies, although higher rates, up to 60% are reported (88). Bueno et al (66) reported a frequency of mutations in 23% in MPM, that was the highest rate of mutations in comparison with the other predominantly mutated genes *NF2*, *TP53* and *SETD2* (66). In another series including MPM tumors and cultured MPM cells, a higher percentage of *BAP1* mutations was found in the subgroup of epithelioid MPM (subgroup C1) in comparison with subgroup including both epithelioid and sarcomatoid MPM (subgroup C2) (169). *BAP1* germline mutations were found in a few cases of sporadic mesotheliomas (193). However, no germline mutations in *BAP1* was found in a cohort of patients in Australia (194). So far, the weight of germline *BAP1* mutations in asbestos-induced MM is not clear.

Up to recently, *BAP1* was the only gene reported as possibly conferring an increased susceptibility to MPM. A recent paper reported a gene sequencing analysis of 85 cancer susceptibility genes on germline DNA of patients with pleural, peritoneal, and tunica vaginalis MM (195). Twelve percent of patients with MM carried mutations in genes such as *BRCA2*, *CHEK2*, *CDKN2A* and *ATM*, especially those with peritoneal MM, minimal asbestos exposure, young age, and a second cancer diagnosis(195).

So far, no recurrent mutations have been reported in oncogenes. However, a ‘hot spot’ of mutations in the *TERT* gene core promoter has been reported in 15% of MPM MPM (196). *TERT* promoter mutations were significantly more frequent in MPM with sarcomatoid histologic subtype (196).

### **Regulatory Pathways in MM Cells**

Constitutive activation of several signaling pathways has been demonstrated in MPM by the occurrence of mutations and/or deregulated expression of specific regulators in comparison with normal mesothelial cells. These studies have been carried out in primary tumor samples but also in malignant mesothelial cell cultures developed from tissue samples. Pathway deregulation in MM has been shown by gene sequencing and gene expression profiling (197, 198). Alterations were recurrently reported in several pathways: hippo, MAPK, PI3K/AKT/mTOR hedgehog, Wnt signaling pathways, cell cycle, P53/DNA repair and apoptosis and ubiquitin/proteasome system due to the frequent alteration of the deubiquitinase, BAP1.

## The Hippo Pathway

The Hippo pathway is of special interest regarding the high frequency of mutations detected in merlin encoded by the *NF2* gene. As mentioned above, merlin negatively regulates cell proliferation and other cell functions (199, 200). Its activity is affected by interaction between extracellular signals and membrane proteins, and activated merlin transduces signals suppressing the transcriptional activity of YAP coactivator for TEAD and other transcription factors (168, 201). YAP and LATS1/2, regulator kinases of the hippo pathway, may mediate proper organization of cytokinesis machinery and mitosis progression (199). *NF2* co-inactivation with *LATS2* led to loss of cell contact inhibition in human MM cells (202). *LATS2* gene was found to be deleted in three out of six MM cell lines and in one out of 25 tumors by DNA sequencing analyses (203). A more recent study reported *LATS2* mutations in 11% (7/61) MPM cells (202). Merlin exists in two forms, active unphosphorylated or inactive phosphorylated. This later form is found in MPM cells possibly accounting for another mechanism for the deregulation of the hippo pathway in these cells (204).

In an integrated analysis of genomics data hippo pathway was identified as altered in all histological type of MPM due to gene alterations in several members of the pathway (66).

### Cell Cycle

The alteration of CDK inhibitor genes located at the *CDKN2* (*CDKN2A* and *CDKN2B*) locus, as mentioned above, contributes to uncontrolled cell proliferation. Cell cycle control can be affected in MM cells not only by the loss of other negative regulators but also by the overexpression of cyclin-dependent kinases (CDKs), cyclins (CCNs), and regulators of the mitotic checkpoints. These alterations have been shown by gene profiling analyses using microarrays (205-207). Overexpressed genes were involved in the regulation of all phases of the cell cycle and cell replication and control of cell cycle progression (205).

Several genes involved in the control of entry in mitosis and mitosis progression were also detected. Overexpression of aurora kinases (AURK) has been reported in several studies (206, 208). In a recent study, higher expression of aurora kinase A (AURKA) mRNA expression was reported in a subset of MM with poor prognosis (170). Stathmin, a gene involved in the regulation of the microtubule dynamics by inhibiting the formation of microtubules and/or promoting their depolymerization, was strongly overexpressed in MPM, resulting in protein overexpression, possibly by an epigenetic regulation (209-211).

These results can account for the complex, even chaotic, chromosomal alterations mentioned above, as the result of a defective control of cell cycle progression through different phases of the cell cycle, including dysregulation of mitosis.

### P53/DNA repair and apoptosis

Mutations in *TP53* and *BAP1* play a role in MM pathogenesis. *TP53* has multifunctional tumor-suppressor response, including the DNA damage response (DDR) function and regulation of senescence and apoptosis (212). Additionally, *BAP1* encodes a multifunctional ubiquitin C-terminal hydrolase, which is also involved in DNA repair and stress response (213, 214). Epigenetic mechanism was identified as a mechanism involved in gene silencing in DDR responses (215). A nanostring analysis reported that mRNA expression of 12 target genes involved in different DDR pathways was significantly associated with expression levels of miRNAs in a series of 24 epithelioid MPM (216).

Otherwise, specific regulators can contribute to MM resistance to apoptosis, such as low expression of proapoptotic proteins (Bax, Bak, Bad, Bid, or Bim) and high levels or activity of antiapoptotic proteins (Bcl-2, Bcl-xL, and Mcl-1) regulating mitochondrial function (217-220). Approaches to control MM proliferation have focused on the resistance of MM cells to

apoptosis (221, 222). Integrated analysis of the genomics data identified alteration of P53 signaling pathways (66).

From several studies, P53 appears to be stabilized in MM, suggesting basal overexpression and/or another type of dysregulation. The P53 protein is constitutively expressed, not only in MM cells in culture, but also in immunohistological sections of primary tumors (223-226). Candidates for P53 activation could be up-regulation of IGF-1/AKT/mTOR pathway and altered energy metabolism, which have been identified as additional functions of P53, as recently reviewed (227). Energy metabolism of MM cells is characterized as aerobic glycolysis (the Warburg effect), and the P53 protein could be induced to shut down this pathway (227, 228).

#### **MAPK and PI3K/AKT/mTOR Signaling Pathway**

The MAPK and PI3K/AKT/mTOR signaling pathway control various cellular processes, cell proliferation and differentiation, cell migration, survival, apoptosis, response to stress and mitogens, and is deregulated in solid cancers (229). In normal cells, these pathways are triggered by the activating phosphorylation of tyrosine kinase receptors (RTKs), followed by a protein kinase cascade. Downstream networks from RTKs can be activated by RTK mutation or sustained signaling through autocrine or paracrine mechanisms.

MPM cells express both vascular endothelial growth factor (VEGF) and the VEGF receptors (fms-related tyrosine kinases, FLT1 and FLT4, and fetal liver kinase, KDR/FLK1) (230-233). VEGF expression was enhanced in a large proportion of MPM in comparison with nonneoplastic specimens (234). An autocrine role for VEGF in cell proliferation has been suggested (232, 235).

MM cell growth may also be linked to autocrine or paracrine stimulation growth factors such as PDGF (236-242)

Epidermal growth factor receptor (EGFR) is overexpressed in 44–97 % of MM as found by immunohistochemical studies, but no mutation was detected in contrast with others types of cancer (243)

Human MM cells express insulin growth factor (IGF) and insulin growth factor receptors (IGFR), and the activation of IGFR activates downstream signaling (244, 245). IGF-I appears to function as an autocrine growth factor in human mesothelial cells (246). IGFBPs also regulate IGF-dependent growth (245, 247, 248).

Hepatocyte growth factor receptor (MET) is a proto-oncogene and the receptor for the ligand hepatocyte growth factor/scattering factor (HGF/SF). Both MET and HGF/SF proteins are expressed in some MPM suggesting the establishment of an autocrine loop of the (249). *In vitro* HGF/SF increases spreading, motility and/or invasiveness of mesothelial cell lines and inhibition of MET reduced cell proliferation (250-252). The activation status of MET and other RTKs, EGFR family, PDGF-A and PDGFR-B has been investigated in 20 MPM cell lines and 23 primary specimens of MPM, and the effect of MET-specific inhibitors was investigated on cell lines (253). The results showed that inhibition of a single RTK was not sufficient to obtain a tumor suppressor effect but that inhibition of multiple RTK was required (253).

The MAPK signaling pathway is constitutively activated in MM as demonstrated by the phosphorylation and activation of downstream proteins of the MAPK cascade, ERKs, Jun amino-terminal kinases/stress-activated kinases (JNKs/SAPKs) and p38 MAPK, and inhibition of cell proliferation and induction of apoptosis by inhibitors of the pathway (254-256). RTK activation can be achieved by a variety of growth factors, such as EGF family, PDGF, FGF, and HGF/SF, and cytokines such as TGF- $\beta$ , TNF, and IL1. The relative levels of tyrosine phosphorylation of 42 distinct RTKs were determined in MM cell lines established from surgical specimens. A coordinated activation of several RTKs – EGFR, ERBB3, AXL, and MET –

was reported (257). No recurrent mutations were identified in members of MAPK signaling pathway in MM.

Activation of RTKs also induces activation of other downstream signaling cascades including PI3K-AK pathway. PI3K/AKT/mTor is activated in MM (258). Phosphorylation of AKT protein, the active form of the protein, and activation of the Akt pathway have been demonstrated in MM cells (198, 219, 259). In MM cells, PTEN, a TSG and negative regulator of the PI3K-AKT pathway, homozygous deletion has been reported in a very small subset of MPM cell lines (260, 261). Integrated analysis of the genomics data identified mTOR pathway as deregulated in MPM (66). Upregulation of PI3K and mTOR signaling pathways were associated to poor prognosis (170).

### **Other signaling pathways**

Other signal transduction pathways Wnt, Hedgehog and Notch are activated in MM cells. These pathways are important in embryonic development, and also as regulators of cancer stem cells (CSC), a side population which is resistant to chemotherapy and radiotherapy (262, 263).

The Wnt signaling pathway regulates cell proliferation and cell polarity, its activation prevents beta-catenin inactivation, a coactivator of transcription, allowing the expression of a variety of genes exerting pleiotropic effects (264). However, cell growth inhibition and apoptosis of MPM cells was observed according to a beta-catenin-independent inhibition of Wnt signaling (265, 266). In MPM, the Wnt pathway could be altered as a result of promoter hypermethylation of regulatory genes (265, 267, 268). Gene expression profiling of MM cell lines, primary MPM tumors and normal pleural tissue demonstrated that numerous Wnt and Wnt-related genes were upregulated and that some Wnt antagonists were downregulated (269). These results suggest that deregulation of the Wnt signaling pathway is involved in mesothelial carcinogenesis. Hedgehog signaling pathway is inactive in normal mesothelium, it can be reactivated in some MM and targeted to reduce the stemness-related cell population (270-272). Mutations in genes of these pathway have been suggested in MM (273). The deregulation of Notch signaling pathways has been reported in MM, with expression levels of Notch1 and Notch2 being elevated and reduced, respectively in human MM cell lines (274). These proteins act as positive and negative modulators, respectively, of PI3K/Akt/mTOR signaling pathway.

### **Epigenetic pathways**

More recently, alterations in epigenetic pathways, DNA methylation, histone modification, nucleosome remodeling and RNA-mediated targeting (noncoding RNAs) have been reported in MPM. These pathways are important as they are connected to cancer (275). Modifications of DNA methyl transferases, chromatin remodelers and differential expression of noncoding RNAs in comparison with normal mesothelium are found in MM. DNA methylation was associated to silencing TSGs (276). Mutations in genes *SMARCA4*, *ARID1A* and *ARID2* involved in the chromatin remodeling SWI/SNF complexes have been found in a low percentage of MM, and in the histone methyl transferases *SETD2* and *KMT2D* (66, 276). Promoter methylation was associated with alterations of gene expression and an upregulation of several DNA methyltransferases in MM (276, 277). High-throughput integrated analysis of the genomics data identified histone methylation, RNA helicases pathways are altered in MPM (66).

### **Immune checkpoints**

Immune checkpoints are modified in cancer cells. In normal tissue, they permit the maintenance of a self-tolerance function. In cancer cells, the expression of immune-checkpoint proteins is modified, allowing tumor evasion, and blockade of the immune

checkpoints is a developing field in anticancer immunotherapy (278). Among them inhibitory T cell receptors, CTL4 and PD1, or ligand PDL1 are presently targeted using specific antibodies to enhance immune recognition (279). Studies have investigated the level of expression of checkpoints proteins in MM, and an heterogeneity between tumors of their expressions and also of the immune cell content has been reported (66, 170, 280). Further studies should improve the knowledge of immune microenvironment of tumor cell and improve targeted immunotherapy (281).

### **Human MM Molecular Heterogeneity**

MM heterogeneity appears to be one cause of the limited efficiency of treatments (282). Histological diversity reflects the various morphological patterns of MM defined through detailed classifications of the tumors (283). Immunohistochemical markers are useful for differential diagnosis of MPM and molecular markers, such as BAP1 protein expression and deletion of *CDKN2A* locus are currently used (284). CGH arrays and gene mutations analyses of MM have added a level of complexity in MM heterogeneity. DNA sequencing have revealed numerous copy number alteration and gene mutations (166). Moreover, within tumors, mutations are not detected in every mesothelioma cell in the tumor, possibly linked to polyclonal evolution (282, 285).

Recently comprehensive genomic analyses allowed classifying MM in different subtypes through transcriptome analysis alone or coupled to other analysis such as sequencing (66, 169, 170, 202, 208, 286). One transcriptomic study reported a clusterisation of MPM in 2 subtypes loosely correlated with tumor histology, consistent with a molecular diversity partly related to morphological pattern (286). In another study, gene expression profiles of epithelioid versus sarcomatoid MM were analyzed, and led to the identification of genes related to lower survival expressed in sarcomatoid MM, such as aurora kinases A and B and functionally related genes involved in mitosis and cell cycle control (208). The authors developed a prognostic classifier based on their microarray data, but found a limited predictive value (208). However, the identification of diagnostic markers is of potential interest for better patients management (208). Another transcriptomic analysis defined two robust molecular MPM subtypes, C1 and C2, only partly related to histologic types but closely related to prognosis (169). Interestingly, epithelioid MPM were found in both groups, with a worse survival prognosis in the C2 subtype. These MM groups also exhibited differential rate of mutations, with more frequent *BAP1* alterations in C1 subtype. Pathways analysis revealed that EMT was differentially regulated between MPM subtypes, C2 subtype being characterized by a mesenchymal phenotype (169). A subtype of C2, the C2<sup>LN</sup> subtype, characterized by the double inactivation of *NF2* and *LATS2* TSG, was identified by coupling transcriptomic and genetic analyses (202). Another publication has identified four distinct molecular subtypes: sarcomatoid, epithelioid, biphasic-epithelioid (biphasic-E) and biphasic-sarcomatoid (biphasic-S) using RNA-seq data (66). Exome analysis in the same tumor samples confirmed already identified and less commonly known mutated genes, *BAP1*, *NF2*, *TP53*, *SETD2*, *DDX3X*, *ULK2*, *RYR2*, *CFAP45*, *SETDB1* and *DDX51*, and alterations in Hippo, mTOR, histone methylation, RNA helicase and P53 signaling pathways without establishing a link with the 4 molecular subtypes (66). Investigation of the immune microenvironment found highest rates of T cells and M2 macrophages in the sarcomatoid group (66). Finally, one publication reported a comprehensive integrated genomic study providing histology-independent determinants of poor prognosis (170). Four clusters, namely iCluster 1 to 4, were characterized. The authors also defined a genomic subtype with *TP53* and *SETDB1* mutations and extensive loss of heterozygosity, and a strong expression of the immune-checkpoint gene *VISTA* in icluster 1 related to epithelioid MPM (170). Gene methylation seems associated to

prognosis as the methylation level is different between clusters, and higher in better prognosis clusters (170).

The mechanisms of mesothelioma heterogeneity have been recently discussed emphasizing the different levels of MM heterogeneity (287). The recent publications on molecular characterization of MPM and the definition of distinct groups with specific molecular biomarkers linked to prognosis is of paramount interest to refine the diagnosis, to guide the therapeutic option and to develop targeted therapies. In the future, it may be expected that integration of metabolic, epigenetic and genomic data will succeed in proposing therapy adapted to the patients tumor.

## **Conclusions**

Recent studies brought some light on the mechanism of MM carcinogenesis, and some questions remain to be addressed. Carcinogenesis progresses through multi-dependent steps, from fibers inhalation to neoplastic transformation of mesothelial and tumor growth. Asbestos remains the major risk factor for MM and past exposure can explain most of the MM, demonstrating a strong link between asbestos activity and mesothelial cell responsiveness. Lung, larynx, ovary, possibly stomach colon and rectum cancers are other cancers linked to asbestos exposure, but asbestos is not the unique cause for these cancers. Fibers can reach these organs via clearance, translocation and ingestion mechanisms, after inhalation. The relationship with past-asbestos exposure addresses the question of the specific sensitivity of mesothelial cells. The recent investigations carried out with CNT demonstrated a pleural translocation. Further studies would account for a more precise mechanism of particle translocation.

The *BAP1* gene was discovered and suggested as predisposing to MM in a context of asbestos exposure. In human, this gene is mutated in *BAP1* tumor predisposition syndrome (BAP1-TPDS), which increases the risk of a variety of malignant and benign tumors. In MM, *BAP1* mutation may be not a predisposing factor, as other cancers are associated in BAP1-TPDS families, but *BAP1* mutation is more likely a sensitivity factor in subjects exposed to asbestos, asking the question of the role of *BAP1* in mesothelial cell physiology. The results obtained with GEM are consistent with this hypothesis as no MM is found in unexposed *Bap1<sup>+/-</sup>* mice, in contrast with asbestos-exposed *Bap1<sup>+/-</sup>* mice. A recent study suggest the association of other germinal gene mutations associated to MM formation (195).

Carcinogenesis is defined by several capabilities that cells acquire during the neoplastic process (288, 289). Asbestos can induce genotoxicity, an early step in mesothelial cell transformation, due to DNA oxidation generated by oxidative stress and inflammation, and chromosome aberrations generated by mitosis impairment. It seems that there is no evidence that inflammation is a prerequisite for generating DNA oxidation. Chromosome alterations are also reported in human cells exposed to CNT. Further studies carried out with HARNs should improve our knowledge of the mechanism of fiber-induced genotoxicity.

Studies of human MM cells and tissue samples have identified cellular and molecular changes in comparison with normal cells. MM is characterized by numerous copy number alterations including frequent deletions, gene fusions and point mutations in a limited number of genes, most being TSG. In MM, genes are inactivated by mutation or by methylation. Apart from activating mutations in *TERT* promoter, no other recurrent oncogenic activation has been reported. Inactivated genes in MM are involved in the regulation of several pathways, cell cycle, hippo, P53/DNA repair and MAPK and PI3K/AKT/mTOR regulatory pathways. Moreover, epigenetic changes in DNA methylation, histone modification, nucleosome remodeling and miRNA-mediated targeting were more recently reported to occur in

mesothelioma cells. Ongoing researches will improve our knowledge on the molecular ways followed by mesothelial cells during neoplastic transformation.

Several recent clinico-biological studies have performed a molecular classification of MM, based on transcriptomic and multi-omic studies. The results have highlighted the molecular heterogeneity of MM, where tumors can be classified in different subtypes with different gene mutations, level of epithelial-mesenchymal transition, deregulated pathways, immunological microenvironment, and linked to survival outcome. These studies demonstrate that MM are heterogeneous tumors, not only clinically and morphologically but also on a molecular basis. The results are encouraging to go forward and define biomarkers to develop efficient precision medicine.

### **References**

1. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. *Br J Ind Med.* 1960;17:260-71.
2. Albin M, Magnani C, Krstev S, Rapiti E, Shefer I. Asbestos and cancer: An overview of current trends in Europe. *Environ Health Perspect.* 1999;107 Suppl 2:289-98.
3. Kishimoto T, Ozaki S, Kato K, Nishi H, Genba K. Malignant pleural mesothelioma in parts of Japan in relationship to asbestos exposure. *Ind Health.* 2004;42(4):435-9.
4. Goldberg M, Imbernon E, Rolland P, Gilg Soit Ilg A, Saves M, de Quillacq A, et al. The French National Mesothelioma Surveillance Program. *Occup Environ Med.* 2006;63(6):390-5.
5. Park EK, Hannaford-Turner KM, Hyland RA, Johnson AR, Yates DH. Asbestos-related occupational lung diseases in NSW, Australia and potential exposure of the general population. *Ind Health.* 2008;46(6):535-40.
6. Attanoos RL, Churg A, Galateau-Salle F, Gibbs AR, Roggli VL. Malignant Mesothelioma and Its Non-Asbestos Causes. *Arch Pathol Lab Med.* 2018;142(6):753-60.
7. Grosse Y, Loomis D, Guyton KZ, Lauby-Secretan B, El Ghissassi F, Bouvard V, et al. Carcinogenicity of fluoro-edenite, silicon carbide fibres and whiskers, and carbon nanotubes. *Lancet Oncol.* 2014;15(13):1427-8.
8. Kane AB, Hurt RH, Gao H. The asbestos-carbon nanotube analogy: An update. *Toxicol Appl Pharmacol.* 2018.
9. Donaldson K, Poland CA, Murphy FA, Macfarlane M, Chernova T, Schinwald A. Pulmonary toxicity of carbon nanotubes and asbestos - Similarities and differences. *Adv Drug Deliv Rev.* 2013;65(15):2078-86.
10. Jaurand MC, Renier A, Daubriac J. Mesothelioma: Do asbestos and carbon nanotubes pose the same health risk? *Part Fibre Toxicol.* 2009;6:16.
11. Kuempel ED, Jaurand MC, Moller P, Morimoto Y, Kobayashi N, Pinkerton KE, et al. Evaluating the mechanistic evidence and key data gaps in assessing the potential carcinogenicity of carbon nanotubes and nanofibers in humans. *Crit Rev Toxicol.* 2017;47(1):1-58.
12. Kane A, Jean D, Knuutila S, Jaurand MC. Malignant Mesothelioma: Mechanism of Carcinogenesis. In: S. Anttila PB, editor. *Occupational Cancers.* London: Springer-Verlag; 2014. p. 299319.
13. Lippmann M, Yeates DB, Albert RE. Deposition, retention and clearance of inhaled particles. *Br J Industr Med.* 1980;37:337-62.
14. IARC. Man-made vitreous fibres2002.
15. Nielsen GD, Koponen IK. Insulation fiber deposition in the airways of men and rats. A review of experimental and computational studies. *Regul Toxicol Pharmacol.* 2018;94:252-70.

16. Asgharian B, Owen TP, Kuempel ED, Jarabek AM. Dosimetry of inhaled elongate mineral particles in the respiratory tract: The impact of shape factor. *Toxicol Appl Pharmacol.* 2018.
17. Oberdorster G. Evaluation and use of animal models to assess mechanisms of fibre carcinogenicity. *IARC Sci Publ.* 1996(140):107-25.
18. Miserocchi GA, Sancini GA, Mantegazza F, Chiappino G. Translocation pathways for inhaled asbestos fibers. *Environ Health.* 2008;7(1):4.
19. Donaldson K, Murphy FA, Duffin R, Poland CA. Asbestos, carbon nanotubes and the pleural mesothelium: a review and the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. *Part Fibre Toxicol.* 2010;7(1):5.
20. Wang NS. Anatomy of the pleura. *Clin Chest Med.* 1998;19(2):229-40.
21. Wang NS. The preformed stomas connecting the pleural cavity and the lymphatics in the parietal pleura. *Am Rev Respir Dis.* 1975;111(1):12-20.
22. Hammar SP. The pathology of benign and malignant pleural disease. *Chest Surg Clin N Am.* 1994;4(3):405-30.
23. Fleury Feith J, Jaurand MC. [Pleural lymphatics and pleural diseases related to fibres]. *Revue de pneumologie clinique.* 2013;69(6):358-62.
24. Mercer RR, Scabilloni JF, Hubbs AF, Wang L, Battelli LA, McKinney W, et al. Extrapulmonary transport of MWCNT following inhalation exposure. *Part Fibre Toxicol.* 2013;10(1):38.
25. Porter DW, Hubbs AF, Mercer RR, Wu N, Wolfarth MG, Sriram K, et al. Mouse pulmonary dose- and time course-responses induced by exposure to multi-walled carbon nanotubes. *Toxicology.* 2010;269(2-3):136-47.
26. Oberdorster G, Graham U. Predicting EMP hazard: Lessons from studies with inhaled fibrous and non-fibrous nano- and micro-particles. *Toxicol Appl Pharmacol.* 2018;361:50-61.
27. Sinis SI, Hatzoglou C, Gourgoulianis KI, Zarogiannis SG. Carbon Nanotubes and Other Engineered Nanoparticles Induced Pathophysiology on Mesothelial Cells and Mesothelial Membranes. *Front Physiol.* 2018;9:295.
28. Shvedova AA, Yanamala N, Kisin ER, Tkach AV, Murray AR, Hubbs A, et al. Long-Term Effects of Carbon Containing Engineered Nanomaterials and Asbestos in the Lung: One Year Post Exposure Comparisons. *Am J Physiol Lung Cell Mol Physiol.* 2014;306(2):L170-82. .
29. Holt PF. Transport of inhaled dust to extrapulmonary sites. *J Pathol.* 1981;133(2):123-9.
30. Muller KM, Schmitz I, Konstantinidis K. Black spots of the parietal pleura: morphology and formal pathogenesis. *Respiration.* 2002;69(3):261-7.
31. Mitchev K, Dumortier P, De Vuyst P. 'Black Spots' and hyaline pleural plaques on the parietal pleura of 150 urban necropsy cases. *Am J Surg Pathol.* 2002;26(9):1198-206.
32. Pooley FD. Proceedings: The recognition of various types of asbestos as minerals, and in tissues. *Clin Sci Mol Med.* 1974;47(3):11P-2P.
33. Dodson RF, O'Sullivan MF, Huang J, Holiday DB, Hammar SP. Asbestos in extrapulmonary sites: omentum and mesentery. *Chest.* 2000;117(2):486-93.
34. Boutin C, Dumortier P, Rey F, Viallat JR, Devuyst P. Black spots concentrate oncogenic asbestos fibers in the parietal pleura: thoracoscopic and mineralogic study. *Amer J Respir Crit Care Med.* 1996;153(1):444-9.
35. Pairon JC, Laurent F, Rinaldo M, Clin B, Andujar P, Ameille J, et al. Pleural plaques and the risk of pleural mesothelioma. *J Natl Cancer Inst.* 2013;105(4):293-301.
36. Boffetta P. Epidemiology of peritoneal mesothelioma: a review. *Ann Oncol.* 2007;18(6):985-90.

37. Price B, Ware A. Time trend of mesothelioma incidence in the United States and projection of future cases: An update based on SEER data for 1973 through 2005. *Crit Rev Toxicol.* 2009;39(7):576-88.
38. Straif K, Benbrahim-Tallaa L, Baan R, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--part C: metals, arsenic, dusts, and fibres. *Lancet Oncol.* 2009;10(5):453-4.
39. IARC. Arsenic, metals, fibres, and dusts. A review of human carcinogens 69372 Lyon Cedex 08, France: International Agency for Research on Cancer; 2012.
40. Paris C, Thaon I, Herin F, Clin B, Lacourt A, Luc A, et al. Occupational Asbestos Exposure and Incidence of Colon and Rectal Cancers in French Men: The Asbestos-Related Diseases Cohort (ARDCo-Nut). *Environ Health Perspect.* 2017;125(3):409-15.
41. Marant Micallef C, Shield KD, Vignat J, Baldi I, Charbotel B, Fervers B, et al. Cancers in France in 2015 attributable to occupational exposures. *Int J Hyg Environ Health.* 2019;222(1):22-9.
42. Clin B, Thaon I, Boulanger M, Brochard P, Chamming's S, Gislard A, et al. Cancer of the esophagus and asbestos exposure. *Am J Ind Med.* 2017;60(11):968-75.
43. Mutsaers SE. The mesothelial cell. *Int J Biochem Cell Biol.* 2004;36(1):9-16.
44. Michailova KN, Usunoff KG. Serosal membranes (pleura, pericardium, peritoneum). Normal structure, development and experimental pathology. *Adv Anat Embryol Cell Biol.* 2006;183:i-vii, 1-144, back cover.
45. Batra H, Antony VB. Pleural mesothelial cells in pleural and lung diseases. *Journal of thoracic disease.* 2015;7(6):964-80.
46. Mutsaers SE, Whitaker D, Papadimitriou JM. Mesothelial regeneration is not dependent on subserosal cells. *J Pathol.* 2000;190:86-92.
47. Mutsaers SE. Mesothelial cells: their structure, function and role in serosal repair. *Respirology.* 2002;7(3):171-91.
48. Foley-Comer AJ, Herrick SE, Al-Mishlab T, Prele CM, Laurent GJ, Mutsaers SE. Evidence for incorporation of free-floating mesothelial cells as a mechanism of serosal healing. *J Cell Sci.* 2002;115(Pt 7):1383-9.
49. Kienzle A, Servais AB, Ysasi AB, Gibney BC, Valenzuela CD, Wagner WL, et al. Free-Floating Mesothelial Cells in Pleural Fluid After Lung Surgery. *Front Med (Lausanne).* 2018;5:89.
50. IARC. Some nanomaterials and some fibres. 69372 Lyon Cedex 08, France: International Agency for Research on Cancer; 2017.
51. Wagner JC, Berry G. Mesotheliomas in rats following inoculation with asbestos. *British Journal of Cancer.* 1969;23:567-81.
52. Davis JM. Structural variations between pleural and peritoneal mesotheliomas produced in rats by the injection of crocidolite asbestos. *Ann Anat Pathol (Paris).* 1976;21(2):199-210.
53. Davis JM. The histopathology and ultrastructure of pleural mesotheliomas produced in the rat by injections of crocidolite asbestos. *Br J Exp Pathol.* 1979;60(6):642-52.
54. Fleury-Feith J, Lecomte C, Renier A, Matrat M, Kheuang L, Abramowski V, et al. Hemizygoty of Nf2 is associated with increased susceptibility to asbestos-induced peritoneal tumours. *Oncogene.* 2003;22:3799-805.
55. Adamson IYR, Bakowska J. KGF and HGF are growth factors for mesothelial cells in pleural lavage fluid after intratracheal asbestos. *Exp Lung Res.* 2001;27:605-16.
56. Gelzleichter TR, Bermudez E, Mangum JB, Wong BA, Moss OR, Everitt JI. Pulmonary and pleural responses in Fischer 344 rats following short-term inhalation of a synthetic vitreous fiber .2. Pathobiologic responses. *Fund Appl Toxicol.* 1996;30(1):39-46.
57. Everitt JI, Gelzleichter TR, Bermudez E, Mangum JB, Wong BA, Janszen DB, et al. Comparison of pleural responses of rats and hamsters to subchronic inhalation of refractory ceramic fibers. *Environ Health Perspect.* 1997;105 Suppl 5:1209-13.

58. Xu J, Alexander DB, Futakuchi M, Numano T, Fukamachi K, Suzui M, et al. Size- and shape-dependent pleural translocation, deposition, fibrogenesis, and mesothelial proliferation by multiwalled carbon nanotubes. *Cancer Sci.* 2014;105(7):763-9.
59. Liao D, Wang Q, He J, Alexander DB, Abdelgied M, El-Gazzar AM, et al. Persistent Pleural Lesions and Inflammation by Pulmonary Exposure of Multiwalled Carbon Nanotubes. *Chem Res Toxicol.* 2018;31(10):1025-31.
60. Libbus BL, Craighead JE. Chromosomal translocations with specific breakpoints in asbestos-induced rat mesotheliomas. *Cancer Res.* 1988;48:6455-61.
61. Unfried K, Schürkes C, Abel J. Distinct spectrum of mutations induced by crocidolite asbestos : clue for 8-hydroxydeoxyguanosine-dependent mutagenesis in vivo. *Cancer Res.* 2002;62:99-104.
62. Schurkes C, Brock W, Abel J, Unfried K. Induction of 8-hydroxydeoxyguanosine by man made vitreous fibres and crocidolite asbestos administered intraperitoneally in rats. *Mutat Res.* 2004;553(1-2):59-65.
63. Yamaguchi R, Hirano T, Ootsuyama Y, Asami S, Tsurudome Y, Fukada S, et al. Increased 8-hydroxyguanine in DNA and its repair activity in hamster and rat lung after intratracheal instillation of crocidolite asbestos. *Jpn J Cancer Res.* 1999;90(5):505-9.
64. Ni Z, Liu YQ, Keshava N, Zhou G, Whong WZ, Ong TM. Analysis of K-ras and p53 mutations in mesotheliomas from humans and rats exposed to asbestos. *Mutat Res.* 2000;468:87-92.
65. Unfried K, Kociok N, Roller M, Friemann J, Pott F, Dehnen W. P53 mutations in tumours induced by intraperitoneal injection of crocidolite asbestos and benzo[a]pyrene in rats. *Exp Toxicol Pathol.* 1997;49(3-4):181-7.
66. Bueno R, Stawiski EW, Goldstein LD, Durinck S, De Rienzo A, Modrusan Z, et al. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. *Nat Genet.* 2016;48(4):407-16.
67. Jarvis MC, Ebrahimi D, Temiz NA, Harris RS. Mutation Signatures Including APOBEC in Cancer Cell Lines. *JNCI Cancer Spectr.* 2018;2(1).
68. Liu W, Ernst JD, Broaddus VC. Phagocytosis of crocidolite asbestos induces oxidative stress, DNA damage, and apoptosis in mesothelial cells. *Am J Respir cell Mol Biol.* 2000;23:371-8.
69. Shukla A, Gulumian M, Hei TK, Kamp D, Rahman Q, Mossman BT. Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. *Free Radic Biol Med.* 2003;34(9):1117-29.
70. Jiang L, Nagai H, Ohara H, Hara S, Tachibana M, Hirano S, et al. Characteristics and modifying factors of asbestos-induced oxidative DNA damage. *Cancer Sci.* 2008;99(11):2142-51.
71. Huang SX, Jaurand MC, Kamp DW, Whysner J, Hei TK. Role of mutagenicity in asbestos fiber-induced carcinogenicity and other diseases. *J Toxicol Environ Health B Crit Rev.* 2011;14(1-4):179-245.
72. Moller P, Danielsen PH, Jantzen K, Roursgaard M, Loft S. Oxidatively damaged DNA in animals exposed to particles. *Crit Rev Toxicol.* 2013;43(2):96-118.
73. Sayan M, Mossman BT. The NLRP3 inflammasome in pathogenic particle and fibre-associated lung inflammation and diseases. *Part Fibre Toxicol.* 2016;13(1):51.
74. Arnoldussen YJ, Skaug V, Aleksandersen M, Ropstad E, Anmarkrud KH, Einarsdottir E, et al. Inflammation in the pleural cavity following injection of multi-walled carbon nanotubes is dependent on their characteristics and the presence of IL-1 genes. *Nanotoxicology.* 2018;12(6):522-38.
75. Yanamala N, Kisin ER, Gutkin DW, Shurin MR, Harper M, Shvedova AA. Characterization of pulmonary responses in mice to asbestos/asbestiform fibers using gene expression profiles. *J Toxicol Environ Health A.* 2018;81(4):60-79.

76. Teeguarden JG, Webb-Robertson BJ, Waters KM, Murray AR, Kisin ER, Varnum SM, et al. Comparative proteomics and pulmonary toxicity of instilled single-walled carbon nanotubes, crocidolite asbestos, and ultrafine carbon black in mice. *Toxicol Sci.* 2011;120(1):123-35.
77. Chernova T, Murphy FA, Galavotti S, Sun XM, Powley IR, Grosso S, et al. Long-Fiber Carbon Nanotubes Replicate Asbestos-Induced Mesothelioma with Disruption of the Tumor Suppressor Gene *Cdkn2a* (*Ink4a/Arf*). *Curr Biol.* 2017;27(21):3302-14.e6.
78. Sneddon S, Patch AM, Dick IM, Kazakoff S, Pearson JV, Waddell N, et al. Whole exome sequencing of an asbestos-induced wild-type murine model of malignant mesothelioma. *BMC Cancer.* 2017;17(1):396.
79. Port J, Murphy DJ. Mesothelioma: Identical Routes to Malignancy from Asbestos and Carbon Nanotubes. *Curr Biol.* 2017;27(21):R1173-R6.
80. Huaux F, d'Ursel de Bousies V, Parent MA, Orsi M, Uwambayinema F, Devosse R, et al. Mesothelioma response to carbon nanotubes is associated with an early and selective accumulation of immunosuppressive monocytic cells. *Part Fibre Toxicol.* 2016;13(1):46.
81. Kumagai-Takei N, Maeda M, Chen Y, Matsuzaki H, Lee S, Nishimura Y, et al. Asbestos induces reduction of tumor immunity. *Clin Dev Immunol.* 2011;2011:481439.
82. Maeda M, Nishimura Y, Kumagai N, Hayashi H, Hatayama T, Katoh M, et al. Dysregulation of the immune system caused by silica and asbestos. *J Immunotoxicol.* 2010;7(4):268-78.
83. Jean D, Jaurand MC. Mesotheliomas in Genetically Engineered Mice Unravel Mechanism of Mesothelial Carcinogenesis. *International journal of molecular sciences.* 2018;19(8).
84. Guo Y, Chirieac LR, Bueno R, Pass H, Wu W, Malinowska IA, et al. *Tsc1-Tp53* loss induces mesothelioma in mice, and evidence for this mechanism in human mesothelioma. *Oncogene.* 2014;33(24):3151-60.
85. Jongsma J, van Montfort E, Vooijs M, Zevenhoven J, Krimpenfort P, van der Valk M, et al. A conditional mouse model for malignant mesothelioma. *Cancer Cell.* 2008;13(3):261-71.
86. Kadariya Y, Cheung M, Xu J, Pei J, Sementino E, Menges CW, et al. *Bap1* Is a Bona Fide Tumor Suppressor: Genetic Evidence from Mouse Models Carrying Heterozygous Germline *Bap1* Mutations. *Cancer Res.* 2016;76(9):2836-44.
87. Sementino E, Menges CW, Kadariya Y, Peri S, Xu J, Liu Z, et al. Inactivation of *Tp53* and *Pten* drives rapid development of pleural and peritoneal malignant mesotheliomas. *J Cell Physiol.* 2018.
88. Nasu M, Emi M, Pastorino S, Tanji M, Powers A, Luk H, et al. High Incidence of Somatic *BAP1* alterations in sporadic malignant mesothelioma. *J Thorac Oncol.* 2015;10(4):565-76.
89. Bott M, Brevet M, Taylor BS, Shimizu S, Ito T, Wang L, et al. The nuclear deubiquitinase *BAP1* is commonly inactivated by somatic mutations and *3p21.1* losses in malignant pleural mesothelioma. *Nat Genet.* 2011;43(7):668-72.
90. Vaslet CA, Messier NJ, Kane AB. Accelerated progression of asbestos-induced mesotheliomas in heterozygous *p53* (+/-) mice. *Toxicol Sci.* 2002;68:331-8.
91. Altomare DA, Vaslet CA, Skele KL, De Rienzo A, Devarajan K, Jhanwar SC, et al. A mouse model recapitulating molecular features of human mesothelioma. *Cancer Res.* 2005;65(18):8090-5.
92. Lecomte C, Andujar P, Renier A, Kheuang L, Abramowski V, Mellottee L, et al. Similar tumor suppressor gene alteration profiles in asbestos-induced murine and human mesothelioma. *Cell Cycle.* 2005;4(12):1862-9.
93. Altomare DA, Menges CW, Pei J, Zhang L, Skele-Stump KL, Carbone M, et al. Activated *TNF-alpha/NF-kappaB* signaling via down-regulation of *Fas-associated factor 1* in asbestos-induced mesotheliomas from *Arf* knockout mice. *Proc Natl Acad Sci U S A.* 2009;106(9):3420-5.

94. Marsella JM, Liu BL, Vaslet CA, Kane AB. Susceptibility of p53-deficient mice to induction of mesothelioma by crocidolite asbestos fibers. *Environ Health Perspect.* 1997;105(suppl 5):1069-72.
95. Robinson C, Dick IM, Wise MJ, Holloway A, Diyagama D, Robinson BW, et al. Consistent gene expression profiles in MexTAG transgenic mouse and wild type mouse asbestos-induced mesothelioma. *BMC Cancer.* 2015;15:983.
96. Kane A, Jean D, Jaurand MC. Mechanism of mesothelial carcinogenesis. In: Anttila S, Boffetta P, editors. *Occupational Cancers.* London: Springer-Verlag; 2014.
97. Jaurand MC, Kheuang L, Magne L, Bignon J. Chromosomal changes induced by chrysotile fibres or benzo(3-4)pyrene in rat pleural mesothelial cells. *Mutat Res.* 1986;169:141-8.
98. Achard S, Perderiset M, Jaurand MC. Sister chromatid exchanges in rat pleural mesothelial cells treated with crocidolite, attapulgite or benzo 3-4 pyrene. *Br J Ind Med.* 1987;44:281-3.
99. Wang NS, Jaurand MC, Magne L, Kheuang L, Pinchon MC, Bignon J. The interactions between asbestos fibers and metaphase chromosomes of rat pleural mesothelial cells in culture. A scanning and transmission electron microscopic study. *Am J Pathol.* 1987;126:343-9.
100. Yegles M, Saint-Etienne L, Renier A, Janson X, Jaurand MC. Induction of metaphase and anaphase/telophase abnormalities by asbestos fibers in rat pleural mesothelial cells in vitro. *Amer J Respir Cell Mol Biol.* 1993;9(2):186-91.
101. Yegles M, Janson X, Dong HY, Renier A, Jaurand MC. Role of fibre characteristics on cytotoxicity and induction of anaphase/telophase aberrations in rat pleural mesothelial cells in vitro. Correlations with in vivo animal findings. *Carcinogenesis.* 1995;16(11):2751-8.
102. Levresse V, Renier A, Fleury-Feith J, Levy F, Moritz S, Vivo C, et al. Analysis of cell cycle disruptions in cultures of rat pleural mesothelial cells exposed to asbestos fibres. *Amer J Respir Cell Mol Biol.* 1997;17:660-71.
103. Levresse V, Renier A, Levy F, Broaddus VC, Jaurand MC. DNA breakage in asbestos-treated normal and transformed (TSV40) rat pleural mesothelial cells. *Mutagenesis.* 2000;15(3):239-44.
104. Pietruska JR, Kane AB. SV40 oncoproteins enhance asbestos-induced DNA double-strand breaks and abrogate senescence in murine mesothelial cells. *Cancer Res.* 2007;67(8):3637-45.
105. Renier A, Levy F, Pilliere F, Jaurand MC. Uncheduled DNA synthesis in rat pleural mesothelial cells treated with mineral fibres or benzo[a]pyrene. *Mutat Res.* 1990;241:361-7.
106. Dong HY, Buard A, Renier A, Levy F, Saint-Etienne L, Jaurand MC. Role of oxygen derivatives in the cytotoxicity and DNA damage produced by asbestos on rat pleural mesothelial cells in vitro. *Carcinogenesis.* 1994;15(6):1251-5.
107. Dong HY, Buard A, Levy F, Renier A, Laval F, Jaurand MC. Synthesis of poly(ADP-ribose) in asbestos treated rat pleural mesothelial cells in culture. *Mutat Res.* 1995;331:197-204.
108. Fung H, Kow YW, Van Houten B, Mossman BT. Patterns of 8-hydroxydeoxyguanosine formation in DNA and indications of oxidative stress in rat and human pleural mesothelial cells after exposure to crocidolite asbestos. *Carcinogenesis.* 1997;18(4):825-32.
109. Zanella CL, Posada J, Tritton TR, Mossman BT. Asbestos causes stimulation of the extracellular signal-regulated kinase 1 mitogen-activated protein kinase cascade after phosphorylation of the epidermal growth factor receptor. *Cancer Res.* 1996;56:5334-8.
110. Faux SP, Houghton CE, Hubbard A, Patrick G. Increased expression of epidermal growth factor receptor in rat pleural mesothelial cells correlates with carcinogenicity of mineral fibres. *Carcinogenesis.* 2000;12:2275-80.

111. Kopnin PB, Kravchenko IV, Furalyov VA, Pylev LN, Kopnin BP. Cell type-specific effects of asbestos on intracellular ROS levels, DNA oxidation and G1 cell cycle checkpoint. *Oncogene*. 2004;23(54):8834-40.
112. Broaddus VC. Asbestos, the mesothelial cell and malignancy: a matter of life or death. *Am J Respir Cell Mol Biol*. 1997;17(6):657-9.
113. Acencio MM, Soares B, Marchi E, Silva CS, Teixeira LR, Broaddus VC. Inflammatory Cytokines Contribute to Asbestos-Induced Injury of Mesothelial Cells. *Lung*. 2015;193(5):831-7.
114. Bononi A, Giorgi C, Patergnani S, Larson D, Verbruggen K, Tanji M, et al. BAP1 regulates IP3R3-mediated Ca(2+) flux to mitochondria suppressing cell transformation. *Nature*. 2017;546(7659):549-53.
115. Thompson JK, MacPherson MB, Beuschel SL, Shukla A. Asbestos-Induced Mesothelial to Fibroblastic Transition Is Modulated by the Inflammasome. *Am J Pathol*. 2017;187(3):665-78.
116. Casalone E, Allione A, Viberti C, Pardini B, Guarrera S, Betti M, et al. DNA methylation profiling of asbestos-treated MeT5A cell line reveals novel pathways implicated in asbestos response. *Archives of toxicology*. 2018;92(5):1785-95.
117. Nymark P, Lindholm PM, Korpela MV, Lahti L, Ruosaari S, Kaski S, et al. Gene expression profiles in asbestos-exposed epithelial and mesothelial lung cell lines. *BMC Genomics*. 2007;8:62.
118. Shukla A, Macpherson MB, Hillegass J, Ramos-Nino ME, Alexeeva V, Vacek PM, et al. Alterations in Gene Expression in Human Mesothelial Cells Correlate with Mineral Pathogenicity. *Am J Respir Cell Mol Biol*. 2009;in press.
119. Wang H, Gillis A, Zhao C, Lee E, Wu J, Zhang F, et al. Crocidolite asbestos-induced signal pathway dysregulation in mesothelial cells. *Mutat Res*. 2011;723(2):171-6.
120. Burmeister B, Schwerdtle T, Poser I, Hoffmann E, Hartwig A, Muller WU, et al. Effects of asbestos on initiation of DNA damage, induction of DNA-strand breaks, P53-expression and apoptosis in primary, SV40-transformed and malignant human mesothelial cells. *Mutat Res*. 2004;558(1-2):81-92.
121. Lohcharoenkal W, Wang L, Stueckle TA, Dinu CZ, Castranova V, Liu Y, et al. Chronic exposure to carbon nanotubes induces invasion of human mesothelial cells through matrix metalloproteinase-2. *ACS Nano*. 2013;7(9):7711-23.
122. Boulanger G, Andujar P, Pairon JC, Billon-Galland MA, Dion C, Dumortier P, et al. Quantification of short and long asbestos fibers to assess asbestos exposure: a review of fiber size toxicity. *Environ Health*. 2014;13:59.
123. Coin PG, Roggli VL, Brody AR. Persistence of long, thin chrysotile asbestos fibers in the lungs of rats. *Environ Health Perspect*. 1994;102 Suppl 5:197-9.
124. Greillier L, Astoul P. Mesothelioma and asbestos-related pleural diseases. *Respiration*. 2008;76(1):1-15.
125. Fazzo L, Minelli G, De Santis M, Bruno C, Zona A, Conti S, et al. Epidemiological surveillance of mesothelioma mortality in Italy. *Cancer epidemiology*. 2018;55:184-91.
126. Merchant JA. Human epidemiology: a review of fiber type and characteristics in the development of malignant and nonmalignant disease. *Environ Health Perspect*. 1990;88:287-93.
127. Baris YI, Grandjean P. Prospective study of mesothelioma mortality in Turkish villages with exposure to fibrous zeolite. *J Natl Cancer Inst*. 2006;98(6):414-7.
128. IOM. Asbestos: Selected Cancers. Washington, D.C.: The National Academies Press; 2006.
129. Fubini B. Surface reactivity in the pathogenic response to particulates. *Environ health Perspect*. 1997;105(Suppl 5):1013-20.
130. McDonald JC. Epidemiology of malignant mesothelioma--an outline. *Ann Occup Hyg*. 2010;54(8):851-7.

131. McDonald JC, Harris J, Armstrong B. Mortality in a cohort of vermiculite miners exposed to fibrous amphibole in Libby, Montana. *Occup Environ Med.* 2004;61(4):363-6.
132. IARC. Silica and some silicates. *IARC Monogr Eval Carcinog Risk Chem Hum.* 1987;42:1-239.
133. Harik VM. Geometry of carbon nanotubes and mechanisms of phagocytosis and toxic effects. *Toxicol Lett.* 2017;273:69-85.
134. Jaurand MC. Use of in-vitro genotoxicity and cell transformation assays to evaluate potential carcinogenicity of fibres. In: Kane AB, Boffetta P, Sarracci R, Wilbourn JD, editors. *Mechanisms in Fiber Carcinogenesis.* 1401996. p. 55-72.
135. Hei T, Louie D, Zhao YL. Genotoxicity versus carcinogenicity: Implications from fiber toxicity studies. *Inhal Toxicol.* 2000;12(s3):141-7.
136. MacCorkle RA, Slattery SD, Nash DR, Brinkley BR. Intracellular protein binding to asbestos induces aneuploidy in human lung fibroblasts. *Cell Motil Cytoskeleton.* 2006;63(10):646-57.
137. Kisin ER, Murray AR, Sargent L, Lowry D, Chirila M, Siegrist KJ, et al. Genotoxicity of carbon nanofibers: are they potentially more or less dangerous than carbon nanotubes or asbestos? *Toxicol Appl Pharmacol.* 2011;252(1):1-10.
138. Muller J, Decordier I, Hoet PH, Lombaert N, Thomassen L, Huaux F, et al. Clastogenic and aneuploidic effects of multi-wall carbon nanotubes in epithelial cells. *Carcinogenesis.* 2008;29(2):427-33.
139. Lindberg HK, Falck GC, Suhonen S, Vippola M, Vanhala E, Catalan J, et al. Genotoxicity of nanomaterials: DNA damage and micronuclei induced by carbon nanotubes and graphite nanofibres in human bronchial epithelial cells in vitro. *Toxicol Lett.* 2009;186:166-73.
140. Cammisuli F, Giordani S, Gianoncelli A, Rizzardì C, Radillo L, Zweyer M, et al. Iron-related toxicity of single-walled carbon nanotubes and crocidolite fibres in human mesothelial cells investigated by Synchrotron XRF microscopy. *Scientific reports.* 2018;8(1):706.
141. Liu G, Beri R, Mueller A, Kamp DW. Molecular mechanisms of asbestos-induced lung epithelial cell apoptosis. *Chem Biol Interact.*
142. Fubini B, Mollo L. Role of iron in the reactivity of mineral fibers. *Toxicol Lett.* 1995;82-3:951-60.
143. van Berlo D, Clift MJ, Albrecht C, Schins RP. Carbon nanotubes: an insight into the mechanisms of their potential genotoxicity. *Swiss Med Wkly.* 2012;142:w13698.
144. Bernstein D, Castranova V, Donaldson K, Fubini B, Hadley J, Hesterberg T, et al. Testing of fibrous particles: short-term assays and strategies. of an ILSI Risk Science Institute Working Group *Inhal Toxicol.* 2005;17(10):497-537.
145. Berman DW, Crump KS. A meta-analysis of asbestos-related cancer risk that addresses fiber size and mineral type. *Crit Rev Toxicol.* 2008;38 Suppl 1:49-73.
146. Casali M, Carugno M, Cattaneo A, Consonni D, Mensi C, Genovese U, et al. Asbestos Lung Burden in Necroscopic Samples from the General Population of Milan, Italy. *Ann Occup Hyg.* 2015;59(7):909-21.
147. Merler E, Somigliana A, Girardi P, Barbieri PG. Residual fibre lung burden among patients with pleural mesothelioma who have been occupationally exposed to asbestos. *Occup Environ Med.* 2017;74(3):218-27.
148. Pollastri S, Gualtieri AF, Vigliaturo R, Ignatyev K, Strafella E, Pugnali A, et al. Stability of mineral fibres in contact with human cell cultures. An in situ muXANES, muXRD and XRF iron mapping study. *Chemosphere.* 2016;164:547-57.
149. Song Y, Thiagarajah J, Verkman AS. Sodium and chloride concentrations, pH, and depth of airway surface liquid in distal airways. *J Gen Physiol.* 2003;122(5):511-9.
150. Suzuki Y, Yuen SR, Ashley R. Short, thin asbestos fibers contribute to the development of human malignant mesothelioma: pathological evidence. *Int J Hyg Environ Health.* 2005;208(3):201-10.

151. Dodson RF, Hammar SP. Pleural mesothelioma in a woman whose documented past exposure to asbestos was from smoking asbestos-containing filtered cigarettes: the comparative value of analytical transmission electron microscopic analysis of lung and lymph-node tissue. *Inhal Toxicol.* 2006;18(9):679-84.
152. Nagai H, Toyokuni S. Biopersistent fiber-induced inflammation and carcinogenesis: lessons learned from asbestos toward safety of fibrous nanomaterials. *Arch Biochem Biophys.* 2010;502(1):1-7.
153. Shinohara N, Nakazato T, Ohkawa K, Tamura M, Kobayashi N, Morimoto Y, et al. Long-term retention of pristine multi-walled carbon nanotubes in rat lungs after intratracheal instillation. *J Appl Toxicol.* 2016;36(4):501-9.
154. Lechner JF, Tokiwa T, LaVeck M, Benedict WF, Banks-Schlegel S, Yeager H, Jr., et al. Asbestos-associated chromosomal changes in human mesothelial cells. *Proc Natl Acad Sci U S A.* 1985;82(11):3884-8.
155. Hesterberg TW, Hart GA, Chevalier J, Miiller WC, Hamilton RD, Bauer J, et al. The importance of fiber biopersistence and lung dose in determining the chronic inhalation effects of X607, RCF1, and chrysotile asbestos in rats. *Toxicol Appl Pharmacol.* 1998;153:68-82.
156. Kodama Y, Boreiko CJ, Maness SC, Hesterberg TW. Cytotoxic and cytogenetic effects of asbestos on human bronchial epithelial cells in culture. *Carcinogenesis.* 1993;14(4):691-7.
157. Kamp DW, Israbian VA, Yeldandi AV, Panos RJ, Graceffa P, Weitzman SA. Phytic acid, an iron chelator, attenuates pulmonary inflammation and fibrosis in rats after intratracheal instillation of asbestos. *Toxicol Pathol.* 1995;23(6):689-95.
158. Shukla A, Jung M, Stern M, Fukagawa NK, Taatjes DJ, Sawyer D, et al. Asbestos induces mitochondrial DNA damage and dysfunction linked to the development of apoptosis. *Am J Physiol Lung Cell Mol Physiol.* 2003;285(5):L1018-25.
159. Srivastava RK, Lohani M, Pant AB, Rahman Q. Cyto-genotoxicity of amphibole asbestos fibers in cultured human lung epithelial cell line: role of surface iron. *Toxicol Ind Health.* 2010;26(9):575-82.
160. Hei TK, He ZY, Suzuki K. Effects of antioxidants on fiber mutagenesis. *Carcinogenesis.* 1995;16(7):1573-8.
161. Kamp DW, Weitzman SA. The molecular basis of asbestos induced lung injury. *Thorax.* 1999;54(7):638-52.
162. Valinluck V, Sowers LC. Inflammation-mediated cytosine damage: a mechanistic link between inflammation and the epigenetic alterations in human cancers. *Cancer Res.* 2007;67(12):5583-6.
163. Kasai H, Kawai K. DNA methylation at the C-5 position of cytosine by methyl radicals: a possible role for epigenetic change during carcinogenesis by environmental agents. *Chem Res Toxicol.* 2009;22(6):984-9.
164. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med.* 2010;49(11):1603-16.
165. Heintz NH, Janssen-Heininger YM, Mossman BT. Asbestos, lung cancers, and mesotheliomas: from molecular approaches to targeting tumor survival pathways. *Am J Respir Cell Mol Biol.* 2010;42(2):133-9.
166. Kettunen E, Knuutila S, Sarhadi VK. Malignant Mesothelioma: Molecular Markers. In: Anttila S, Boffetta P, editors. *Occupational Cancers.*
167. Yoshikawa Y, Emi M, Hashimoto-Tamaoki T, Ohmuraya M, Sato A, Tsujimura T, et al. High-density array-CGH with targeted NGS unmask multiple noncontiguous minute deletions on chromosome 3p21 in mesothelioma. *Proc Natl Acad Sci U S A.* 2016;113(47):13432-7.
168. Sekido Y. Genomic abnormalities and signal transduction dysregulation in malignant mesothelioma cells. *Cancer Sci.* 2010;101(1):1-6.

169. de Reynies A, Jaurand MC, Renier A, Couchy G, Hysi I, Elarouci N, et al. Molecular classification of malignant pleural mesothelioma: Identification of a poor prognosis subgroup linked to the epithelial-to-mesenchymal transition. *Clin Cancer Res.* 2014;20(5):1323-34.
170. Hmeljak J, Sanchez-Vega F, Hoadley KA, Shih J, Stewart C, Heiman D, et al. Integrative Molecular Characterization of Malignant Pleural Mesothelioma. *Cancer Discov.* 2018;8(12):1548-65.
171. Andujar P, Wang J, Descatha A, Galateau-Sallé F, Abd-Alsamad A, Billon-Galland MA, et al. p16<sup>INK4A</sup> inactivation mechanisms in non small-cell lung cancer patients occupationally exposed to asbestos. *Lung Cancer.* 2010;67(1):23-30.
172. Christensen BC, Godleski JJ, Marsit CJ, Houseman EA, Lopez-Fagundo CY, Longacker JL, et al. Asbestos exposure predicts cell cycle control gene promoter methylation in pleural mesothelioma. *Carcinogenesis.* 2008;29(8):1555-9.
173. Christensen BC, Houseman EA, Godleski JJ, Marsit CJ, Longacker JL, Roelofs CR, et al. Epigenetic profiles distinguish pleural mesothelioma from normal pleura and predict lung asbestos burden and clinical outcome. *Cancer Res.* 2009;69(1):227-34.
174. Toyooka S, Toyooka KO, Maruyama R, Virmani AK, Girard L, Miyajima K, et al. DNA methylation profiles of lung tumors. *Mol Cancer Ther.* 2001;1(1):61-7.
175. Hirao T, Bueno R, Chen CJ, Gordon GJ, Heilig E, Kelsey KT. Alterations of the p16INK4 locus in human malignant mesothelial tumors. *Carcinogenesis.* 2002;23:1127-30.
176. Wong L, Zhou J, Anderson D, Kratzke RA. Inactivation of p16INK4a expression in malignant mesothelioma by methylation. *Lung Cancer.* 2002;38(2):131-6.
177. Marsit CJ, Houseman EA, Christensen BC, Eddy K, Bueno R, Sugarbaker DJ, et al. Examination of a CpG island methylator phenotype and implications of methylation profiles in solid tumors. *Cancer Res.* 2006;66(21):10621-9.
178. Destro A, Ceresoli GL, Baryshnikova E, Garassino I, Zucali PA, De Vincenzo F, et al. Gene methylation in pleural mesothelioma: correlations with clinico-pathological features and patient's follow-up. *Lung Cancer.* 2008;59(3):369-76.
179. Guled M, Lahti L, Lindholm PM, Salmenkivi K, Bagwan I, Nicholson AG, et al. CDKN2A, NF2, and JUN are dysregulated among other genes by miRNAs in malignant mesothelioma -A miRNA microarray analysis. *Genes Chromosomes Cancer.* 2009;48(7):615-23.
180. Andujar P, Lecomte C, Renier A, Fleury-Feith J, Kheuang L, Daubriac J, et al. Clinico-pathological features and somatic gene alterations in refractory ceramic fibre-induced murine mesothelioma reveal mineral fibre-induced mesothelioma identities. *Carcinogenesis.* 2007;28(7):1599-605.
181. Mor O, Yaron P, Huszar M, Yellin A, Jakobovitz O, Brok-imoni F, et al. Absence of p53 mutations in malignant mesothelioma. *Am J Respir Cell Mol Biol.* 1997;16:9-13.
182. Kitamura F, Araki S, Tanigawa T, Miura H, Akabane H, Iwasaki R. Assessment of mutations of Ha- and Ki-ras oncogenes and the p53 suppressor gene in seven malignant mesothelioma patients exposed to asbestos. PCR-SSCP and sequencing analyses of paraffin-embedded primary tumors *Ind Health.* 1998;36:52-6.
183. Andujar P, Pairon JC, Renier A, Descatha A, Hysi I, Abd-Alsamad I, et al. Differential mutation profiles and similar intronic TP53 polymorphisms in asbestos-related lung cancer and pleural mesothelioma. *Mutagenesis.* 2013;28(3):323-31.
184. Bianchi AB, Mitsunaga S, Cheng J, Klein W, Jhanwar SC, Seizinger B, et al. High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesothelioma. *Proc Natl Acad Sci, USA.* 1995;92:10854-8.
185. Sekido Y, Pass HI, Bader S, Mew DJ, Christmas MF, Gazdar AF. Neurofibromatosis type 2 (NF2) gene is somatically mutated in mesothelioma but not in lung cancer. *Cancer Res.* 1995;55:1227-31.

186. Miyanaga A, Masuda M, Tsuta K, Kawasaki K, Nakamura Y, Sakuma T, et al. Hippo pathway gene mutations in malignant mesothelioma: revealed by RNA and targeted exon sequencing. *J Thorac Oncol.* 2015;10(5):844-51.
187. Sekido Y. Molecular pathogenesis of malignant mesothelioma. *Carcinogenesis.* 2013;34(7):1413-9.
188. Stamenkovic I, Yu Q. Merlin, a "magic" linker between extracellular cues and intracellular signaling pathways that regulate cell motility, proliferation, and survival. *Curr Protein Pept Sci.* 2010;11(6):471-84.
189. Murthy SS, Testa JR. Asbestos, chromosomal deletions, and tumor suppressor gene alterations in human malignant mesothelioma. *J Cell Physiol.* 1999;180:150-7.
190. Jean D, Thomas E, Renier A, de Reynies A, Lecomte C, Andujar P, et al. Syntenic relationships between genomic profiles of fiber-induced murine and human malignant mesothelioma. *Am J Pathol.* 2011;176(2):881-94.
191. Lallemand D, Curto M, Saotome I, Giovannini M, McClatchey AI. NF2 deficiency promotes tumorigenesis and metastasis by destabilizing adherens junctions. *Genes Dev.* 2003;17(9):1090-100.
192. Yi C, Troutman S, Fera D, Stemmer-Rachamimov A, Avila JL, Christian N, et al. A Tight Junction-Associated Merlin-Angiomotin Complex Mediates Merlin's Regulation of Mitogenic Signaling and Tumor Suppressive Functions. *Cancer Cell.* 2010;19(4):527-40.
193. Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, et al. Germline BAP1 mutations predispose to malignant mesothelioma. *Nat Genet.* 2011;43(10):1022-5.
194. Sneddon S, Leon JS, Dick IM, Cadby G, Olsen N, Brims F, et al. Absence of germline mutations in BAP1 in sporadic cases of malignant mesothelioma. *Gene.* 2015;563(1):103-5.
195. Panou V, Vyberg M, Weinreich UM, Meristoudis C, Falkmer UG, Roe OD. The established and future biomarkers of malignant pleural mesothelioma. *Cancer Treat Rev.* 2015;41(6):486-95.
196. Tallet A, Nault JC, Renier A, Hysi I, Galateau-Salle F, Cazes A, et al. Overexpression and promoter mutation of the TERT gene in malignant pleural mesothelioma. *Oncogene.* 2014;33(28):3748-452.
197. Jaurand MC, Jean D. Biomolecular Pathways and Malignant Pleural Mesothelioma. In: Mineo TC, editor. *Malignant Pleural Mesothelioma: Present Status and Future Directions*; Bentham Science Publishers; 2015. p. 173-96.
198. Hylebos M, Van Camp G, van Meerbeeck JP, Op de Beeck K. The Genetic Landscape of Malignant Pleural Mesothelioma: Results from Massively Parallel Sequencing. *J Thorac Oncol.* 2016;11(10):1615-26.
199. Liu XL, Zuo R, Ou WB. The hippo pathway provides novel insights into lung cancer and mesothelioma treatment. *J Cancer Res Clin Oncol.* 2018;144(11):2097-106.
200. Sato T, Sekido Y. NF2/Merlin Inactivation and Potential Therapeutic Targets in Mesothelioma. *International journal of molecular sciences.* 2018;19(4):988.
201. Felley-Bosco E, Stahel R. Hippo/YAP pathway for targeted therapy. *Translational lung cancer research.* 2014;3(2):75-83.
202. Tranchant R, Quetel L, Tallet A, Meiller C, Renier A, de Koning L, et al. Co-occurring Mutations of Tumor Suppressor Genes, LATS2 and NF2, in Malignant Pleural Mesothelioma. *Clin Cancer Res.* 2017;23(12):3191-202.
203. Murakami H, Mizuno T, Taniguchi T, Fujii M, Ishiguro F, Fukui T, et al. LATS2 Is a Tumor Suppressor Gene of Malignant Mesothelioma. *Cancer Res.* 2011;71(3):873-83.
204. Thurneysen C, Opitz I, Kurtz S, Weder W, Stahel RA, Felley-Bosco E. Functional inactivation of NF2/merlin in human mesothelioma. *Lung Cancer.* 2009;64(2):140-7.

205. Romagnoli S, Fasoli E, Vaira V, Falleni M, Pellegrini C, Catania A, et al. Identification of potential therapeutic targets in malignant mesothelioma using cell-cycle gene expression analysis. *Am J Pathol.* 2009;174(3):762-70.
206. Crispi S, Fagliarone C, Biroccio A, D'Angelo C, Galati R, Sacchi A, et al. Antiproliferative effect of Aurora kinase targeting in mesothelioma. *Lung Cancer.* 2010;70(3):271-9.
207. Roe OD, Anderssen E, Sandeck H, Christensen T, Larsson E, Lundgren S. Malignant pleural mesothelioma: genome-wide expression patterns reflecting general resistance mechanisms and a proposal of novel targets. *Lung Cancer.* 2010;67(1):57-68.
208. Lopez-Rios F, Chuai S, Flores R, Shimizu S, Ohno T, Wakahara K, et al. Global gene expression profiling of pleural mesotheliomas: overexpression of aurora kinases and P16/CDKN2A deletion as prognostic factors and critical evaluation of microarray-based prognostic prediction. *Cancer Res.* 2006;66(6):2970-9.
209. Rubin CI, Atweh GF. The role of stathmin in the regulation of the cell cycle. *J Cell Biochem.* 2004;93(2):242-50.
210. Kim JY, Harvard C, You L, Xu Z, Kuchenbecker K, Baehner R, et al. Stathmin is overexpressed in malignant mesothelioma. *Anticancer Res.* 2007;27(1A):39-44.
211. Birnie KA, Yip YY, Ng DC, Kirschner MB, Reid G, Prele CM, et al. Loss of mir-223 and JNK Signalling Contribute to Elevated Stathmin in Malignant Pleural Mesothelioma. *Mol Cancer Res.* 2015;13(7):1106-18. .
212. Aubrey BJ, Strasser A, Kelly GL. Tumor-Suppressor Functions of the TP53 Pathway. *Cold Spring Harb Perspect Med.* 2016;6(5).
213. Carbone M, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G. BAP1 and cancer. *Nat Rev Cancer.* 2013;13(3):153-9.
214. Luchini C, Wood LD, Cheng L, Nottegar A, Stubbs B, Solmi M, et al. Extranodal extension of lymph node metastasis is a marker of poor prognosis in oesophageal cancer: a systematic review with meta-analysis. *J Clin Pathol.* 2016.
215. Knijnenburg TA, Wang L, Zimmermann MT, Chambwe N, Gao GF, Cherniack AD, et al. Genomic and Molecular Landscape of DNA Damage Repair Deficiency across The Cancer Genome Atlas. *Cell Rep.* 2018;23(1):239-54 e6.
216. Mairinger FD, Werner R, Flom E, Schmeller J, Borchert S, Wessolly M, et al. miRNA regulation is important for DNA damage repair and recognition in malignant pleural mesothelioma. *Virchows Arch.* 2017;470(6):627-37.
217. Soini Y, Kinnula V, Kaarteenaho-Wiik R, Kurttila E, Linnainmaa K, Paakko P. Apoptosis and expression of apoptosis regulating proteins bcl-2, mcl-1, bcl-X, and bax in malignant mesothelioma. *Clin Cancer Res.* 1999;5(11):3508-15.
218. O'Kane SL, Pound RJ, Campbell A, Chaudhuri N, Lind MJ, Cawkwell L. Expression of bcl-2 family members in malignant pleural mesothelioma. *Acta Oncol.* 2006;45(4):449-53.
219. Daubriac J, Fleury-Feith J, Kheuang L, Galipon J, Saint-Albin A, Renier A, et al. Malignant pleural mesothelioma cells resist anoikis as quiescent pluricellular aggregates. *Cell Death and Differentiation.* 2009;16 (8):1146-55.
220. Jin L, Amatya VJ, Takeshima Y, Shrestha L, Kushitani K, Inai K. Evaluation of apoptosis and immunohistochemical expression of the apoptosis-related proteins in mesothelioma. *Hiroshima J Med Sci.* 2010;59(2):27-33.
221. Leard LE, Broaddus VC. Mesothelial cell proliferation and apoptosis. *Respirology.* 2004;9(3):292-9.
222. Wilson SM, Barbone D, Yang TM, Jablons DM, Bueno R, Sugarbaker DJ, et al. mTOR mediates survival signals in malignant mesothelioma grown as tumor fragment spheroids. *Am J Respir Cell Mol Biol.* 2008;39(5):576-83.
223. Kafiri G, Thomas DM, Shepherd NA, Krausz T, Lane DP, Hall PA. p53 expression is common in malignant mesothelioma. *Histopathology.* 1992;21(4):331-4.

224. Ramael M, Lemmens G, Eerdekens C, Buysse C, Deblieer I, Jacobs W, et al. Immunoreactivity for p53 protein in malignant mesothelioma and non-neoplastic mesothelium. *J Pathol.* 1992;168:371-5.
225. Mayall FG, Goddard H, Gibbs AR. The Frequency of p53 Immunostaining in Asbestos-Associated Mesotheliomas and Non-Asbestos-Associated Mesotheliomas. *Histopathology.* 1993;22(4):383-6.
226. Attanoos RL, Griffin A, Gibbs AR. The use of immunohistochemistry in distinguishing reactive from neoplastic mesothelium. A novel use for desmin and comparative evaluation with epithelial membrane antigen, p53, platelet-derived growth factor-receptor, P-glycoprotein and Bcl-2. *Histopathology.* 2003;43(3):231-8.
227. Feng Z, Levine AJ. The regulation of energy metabolism and the IGF-1/mTOR pathways by the p53 protein. *Trends Cell Biol.* 2010;20(7):427-34.
228. Singhal S, Wiewrodt R, Malden LD, Amin KM, Matzie K, Friedberg J, et al. Gene expression profiling of malignant mesothelioma. *Clin Cancer Res.* 2003;9(8):3080-97.
229. Lei YY, Wang WJ, Mei JH, Wang CL. Mitogen-activated protein kinase signal transduction in solid tumors. *Asian Pac J Cancer Prev.* 2014;15(20):8539-48.
230. Ohta Y, Shridhar V, Bright RK, Kalemkerian GP, Du W, Carbone M, et al. VEGF and VEGF type C play an important role in angiogenesis and lymphangiogenesis in human malignant mesothelioma tumours. *Br J Cancer.* 1999;81(1):54-61.
231. Konig J, Tolnay E, Wiethage T, Muller K. Co-expression of vascular endothelial growth factor and its receptor flt-1 in malignant pleural mesothelioma. *Respiration.* 2000;67:36-40.
232. Strizzi L, Catalano A, Vianale G, Orecchia S, Casalini A, Tassi G, et al. Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. *J Pathol.* 2001;193:468-75.
233. Filho AL, Baltazar F, Bedrossian C, Michael C, Schmitt FC. Immunohistochemical expression and distribution of VEGFR-3 in malignant mesothelioma. *Diagn Cytopathol.* 2007;35(12):786-91.
234. Lee AY, Raz DJ, He B, Jablons DM. Update on the molecular biology of malignant mesothelioma. *Cancer.* 2007;109(8):1454-61.
235. Masood R, Kundra A, Zhu S, Xia G, Scalia P, Smith DL, et al. Malignant mesothelioma growth inhibition by agents that target the VEGF and VEGF-C autocrine loops. *Int J Cancer.* 2003;104(5):603-10.
236. Jacobson A, Brinck J, Briskin MJ, Spicer AP, Heldin P. Expression of human hyaluronan synthases in response to external stimuli. *Biochem J.* 2000;348:29-35.
237. Heldin P, Asplund T, Ytterberg D, Thelin S, Laurent TC. Characterization of the molecular mechanism involved in the activation of hyaluronan synthetase by platelet-derived growth factor in human mesothelial cells. *Biochem J.* 1992;283 ( Pt 1):165-70.
238. Gerwin BI, Lechner JF, Reddel RR, Roberts AB, Robbins KC, Gabrielson EW, et al. Comparison of production of transforming growth factor-beta and platelet-derived growth factor by normal human mesothelial cells and mesothelioma cell lines. *Cancer Res.* 1987;47(23):6180-4.
239. Versnel MA, Claessonwelsh L, Hammacher A, Bouts MJ, Vanderkwast TH, Eriksson A, et al. Human malignant mesothelioma cell lines express PDGF beta-receptors whereas cultured normal mesothelial cells express predominantly PDGF alpha-receptors. *Oncogene.* 1991;6(11):2005-11.
240. Metheny-Barlow LJ, Flynn B, van Gijssel HE, Marrogi A, Gerwin BI. Paradoxical effects of platelet-derived growth factor-A overexpression in malignant mesothelioma. Antiproliferative effects in vitro and tumorigenic stimulation in vivo. *Am J Respir Cell Mol Biol.* 2001;24(6):694-702.

241. Van der Meeren A, Seddon MB, Betsholtz CA, Lechner JF, Gerwin BI. Tumorigenic conversion of human mesothelial cells as a consequence of platelet-derived growth factor-A chain overexpression. *Amer J Respir Cell Mol Biol.* 1993;8(2):214-21.
242. Honda M, Kanno T, Fujita Y, Gotoh A, Nakano T, Nishizaki T. Mesothelioma cell proliferation through autocrine activation of PDGF-beta receptor. *Cell Physiol Biochem.* 2012;29(5-6):667-74.
243. Agarwal V, Lind MJ, Cawkwell L. Targeted epidermal growth factor receptor therapy in malignant pleural mesothelioma: Where do we stand? *Cancer Treat Rev.* 2011;37(7):533-42.
244. Hoang CD, Zhang X, Scott PD, Guillaume TJ, Maddaus MA, Yee D, et al. Selective activation of insulin receptor substrate-1 and -2 in pleural mesothelioma cells: association with distinct malignant phenotypes. *Cancer Res.* 2004;64(20):7479-85.
245. Whitson BA, Kratzke RA. Molecular pathways in malignant pleural mesothelioma. *Cancer Lett.* 2006;239(2):183-9.
246. Jaurand MC, Fleury-Feith J. Mesothelial cells. In: Light RW, Lee YCG, editors. *Textbook of pleural diseases* Second edition. London: Hodder Arnold; 2008. p. 27-37.
247. Lee TC, Zhang Y, Aston C, Hintz R, Jagirdar J, Perle MA, et al. Normal human mesothelial cells and mesothelioma cell lines express insulin-like growth factor I and associated molecules. *Cancer Res.* 1993;53(12):2858-64.
248. Liu Z, Klominek J. Regulation of matrix metalloprotease activity in malignant mesothelioma cell lines by growth factors. *Thorax.* 2003;58(3):198-203.
249. Thayaparan T, Spicer JF, Maher J. The role of the HGF/Met axis in mesothelioma. *Biochemical Society transactions.* 2016;44(2):363-70.
250. Harvey P, Warn A, Dobbin S, Arakaki N, Daikuhara Y, Jaurand MC, et al. Expression of HGF/SF in mesothelioma cell lines and its effects on cell motility, proliferation and morphology. *Br J Cancer.* 1998;77:1052-9.
251. Mukohara T, Civiello G, Davis IJ, Taffaro ML, Christensen J, Fisher DE, et al. Inhibition of the met receptor in mesothelioma. *Clin Cancer Res.* 2005;11(22):8122-30.
252. Jagadeeswaran R, Ma PC, Seiwert TY, Jagadeeswaran S, Zumba O, Nallasura V, et al. Functional analysis of c-Met/hepatocyte growth factor pathway in malignant pleural mesothelioma. *Cancer Res.* 2006;66(1):352-61.
253. Kawaguchi K, Murakami H, Taniguchi T, Fujii M, Kawata S, Fukui T, et al. Combined inhibition of MET and EGFR suppresses proliferation of malignant mesothelioma cells. *Carcinogenesis.* 2009;30(7):1097-105.
254. Vintman L, Nielsen S, Berner A, Reich R, Davidson B. Mitogen-activated protein kinase expression and activation does not differentiate benign from malignant mesothelial cells. *Cancer.* 2005;103(11):2427-33.
255. de Melo M, Gerbase MW, Curran J, Pache JC. Phosphorylated extracellular signal-regulated kinases are significantly increased in malignant mesothelioma. *J Histochem Cytochem.* 2006;54(8):855-61.
256. Eguchi R, Fujimori Y, Takeda H, Tabata C, Ohta T, Kuribayashi K, et al. Arsenic trioxide induces apoptosis through JNK and ERK in human mesothelioma cells. *J Cell Physiol.* 2011;226(3):762-8.
257. Ou WB, Hubert C, Corson JM, Bueno R, Flynn DL, Sugarbaker DJ, et al. Targeted inhibition of multiple receptor tyrosine kinases in mesothelioma. *Neoplasia.* 2011;13(1):12-22.
258. Zhou S, Liu L, Li H, Eilers G, Kuang Y, Shi S, et al. Multipoint targeting of the PI3K/mTOR pathway in mesothelioma. *Br J Cancer.* 2014;110(10):2479-88.
259. Besson A, Robbins SM, Yong VW. PTEN/MMAC1/TEP1 in signal transduction and tumorigenesis. *Eur J Biochem.* 1999;263(3):605-11.

260. Altomare DA, You H, Xiao GH, Ramos-Nino ME, Skele KL, De Rienzo A, et al. Human and mouse mesotheliomas exhibit elevated AKT/PKB activity, which can be targeted pharmacologically to inhibit tumor cell growth. *Oncogene*. 2005;24(40):6080-9.
261. Suzuki Y, Murakami H, Kawaguchi K, Taniguchi T, Fujii M, Shinjo K, et al. Activation of the PI3K-AKT pathway in human malignant mesothelioma cells. *Molecular Medicine Reports*. 2009;2(2):181-8.
262. Makena MR, Ranjan A, Thirumala V, Reddy AP. Cancer stem cells: Road to therapeutic resistance and strategies to overcome resistance. *Biochim Biophys Acta Mol Basis Dis*. 2018.
263. Takebe N, Miele L, Harris PJ, Jeong W, Bando H, Kahn M, et al. Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update. *Nat Rev Clin Oncol*. 2015;12(8):445-64.
264. Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell*. 2006;127(3):469-80.
265. Lee AY, He B, You L, Xu Z, Mazieres J, Reguart N, et al. Dickkopf-1 antagonizes Wnt signaling independent of beta-catenin in human mesothelioma. *Biochem Biophys Res Commun*. 2004;323(4):1246-50.
266. He B, Lee AY, Dadfarmay S, You L, Xu Z, Reguart N, et al. Secreted frizzled-related protein 4 is silenced by hypermethylation and induces apoptosis in beta-catenin-deficient human mesothelioma cells. *Cancer Res*. 2005;65(3):743-8.
267. Batra S, Shi Y, Kuchenbecker KM, He B, Reguart N, Mikami I, et al. Wnt inhibitory factor-1, a Wnt antagonist, is silenced by promoter hypermethylation in malignant pleural mesothelioma. *Biochem Biophys Res Commun*. 2006;342(4):1228-32.
268. Kohno H, Amatya VJ, Takeshima Y, Kushitani K, Hattori N, Kohno N, et al. Aberrant promoter methylation of WIF-1 and SFRP1, 2, 4 genes in mesothelioma. *Oncol Rep*. 2010;24(2):423-31.
269. Mazieres J, You L, He B, Xu Z, Twogood S, Lee AY, et al. Wnt2 as a new therapeutic target in malignant pleural mesothelioma. *Int J Cancer*. 2005;117(2):326-32.
270. Felley-Bosco E, Opitz I, Meerang M. Hedgehog Signaling in Malignant Pleural Mesothelioma. *Genes*. 2015;6(3):500-11.
271. Kim HA, Kim MC, Kim NY, Kim Y. Inhibition of hedgehog signaling reduces the side population in human malignant mesothelioma cell lines. *Cancer gene therapy*. 2015;22(8):387-95.
272. Mutti L, Peikert T, Robinson BWS, Scherpereel A, Tsao AS, de Perrot M, et al. Scientific Advances and New Frontiers in Mesothelioma Therapeutics. *J Thorac Oncol*. 2018;13(9):1269-83.
273. Lim CB, Prele CM, Cheah HM, Cheng YY, Klebe S, Reid G, et al. Mutational analysis of hedgehog signaling pathway genes in human malignant mesothelioma. *PLoS ONE*. 2013;8(6):e66685.
274. Rossini M, Rizzo P, Bononi I, Clementz A, Ferrari R, Martini F, et al. New Perspectives on Diagnosis and Therapy of Malignant Pleural Mesothelioma. *Frontiers in oncology*. 2018;8:91.
275. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. *Cell*. 2012;150(1):12-27.
276. McLoughlin KC, Kaufman AS, Schrupp DS. Targeting the epigenome in malignant pleural mesothelioma. *Translational lung cancer research*. 2017;6(3):350-65.
277. Vandermeers F, Neelature Sriramareddy S, Costa C, Hubaux R, Cosse JP, Willems L. The role of epigenetics in malignant pleural mesothelioma. *Lung Cancer*. 2013;81(3):311-8.
278. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12(4):252-64.

279. Lievens LA, Sterman DH, Cornelissen R, Aerts JG. Checkpoint Blockade in Lung Cancer and Mesothelioma. *Am J Respir Crit Care Med*. 2017;196(3):274-82.
280. Minnema-Luiting J, Vroman H, Aerts J, Cornelissen R. Heterogeneity in Immune Cell Content in Malignant Pleural Mesothelioma. *International journal of molecular sciences*. 2018;19(4).
281. Guazzelli A, Bakker E, Krstic-Demonacos M, Lisanti MP, Sotgia F, Mutti L. Anti-CTLA-4 therapy for malignant mesothelioma. *Immunotherapy*. 2017;9(3):273-80.
282. Oehl K, Vrugt B, Opitz I, Meerang M. Heterogeneity in Malignant Pleural Mesothelioma. *International journal of molecular sciences*. 2018;19(6).
283. Galateau-Salle F, Churg A, Roggli V, Travis WD. The 2015 World Health Organization Classification of Tumors of the Pleura: Advances since the 2004 Classification. *J Thorac Oncol*. 2016;11(2):142-54.
284. Husain AN, Colby TV, Ordonez NG, Allen TC, Attanoos RL, Beasley MB, et al. Guidelines for Pathologic Diagnosis of Malignant Mesothelioma 2017 Update of the Consensus Statement From the International Mesothelioma Interest Group. *Arch Pathol Lab Med*. 2018;142(1):89-108.
285. Comertpay S, Pastorino S, Tanji M, Mezzapelle R, Strianese O, Napolitano A, et al. Evaluation of clonal origin of malignant mesothelioma. *J Transl Med*. 2014;12:301.
286. Gordon GJ, Rockwell GN, Jensen RV, Rheinwald JG, Glickman JN, Aronson JP, et al. Identification of novel candidate oncogenes and tumor suppressors in malignant pleural mesothelioma using large-scale transcriptional profiling. *Am J Pathol*. 2005;166(6):1827-40.
287. Felley-Bosco E. Special Issue on Mechanisms of Mesothelioma Heterogeneity: Highlights and Open Questions. *International journal of molecular sciences*. 2018;19(11).
288. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100:57-70.
289. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2012;144(5):646-74.