Chapter 2 Asbestos and mesothelioma :What is recent advance in research on asbestos-induced molecular carcinogenesis?

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Abstract. The relationship between asbestos exposure and malignant mesothelioma is established since the middle of the 20th century. From this time, scientific researches have progressed investigating the mechanism of action of asbestos on mesothelial cells, and more intensively during the beginning of the 21th century the analysis of the molecular changes in mesothelioma. Indeed, asbestos fibers were reported to induce chromosomal and genetic damage in mammalian cells. Mesothelioma is characterized by chromosomal alterations, which include numerous chromosome rearrangements, gene mutations and gene deletions. Recent studies have enhanced our knowledge of the molecular landscape of mesothelioma, emphasizing mutations targeting more specifically tumor suppressor genes, differential gene expression and DNA methylation in comparison with normal cells and between mesotheliomas, expression of non coding RNAs and alterations of regulatory pathways. Researches also provided knowledge of susceptibility factors in malignant mesothelioma families and relationships with asbestos exposure. It is time to review on the recent advances on asbestos-induced molecular changes related to mesothelial carcinogenesis.

Introduction

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

Researches on malignant mesothelioma

Molecular characteristics of malignant mesothelioma

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

Gene expression profiles in MPM revealed the differential expression of specific genes in comparison with normal mesothelial cells or lung tissues, or other thoracic cancers and provided a variety of information on the mechanism of mesothelioma carcinogenesis and the prognostic value of the expression level of specific genes [11, 12, 4, 13-15].

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

MPM heterogeneity was further investigated by transcriptome analyses using deconvolution methods [17]. This approach allowed to define a set of genes that define epithelioid-like and/or sarcomatoid-like types of MPM. Then, a MPM tumor can be decomposed as epithelioid-like and sarcomatoid-like components, and can be defined by a E- and S-score, which refers to the proportion of these components. Interestingly, the S-score is strongly associated with prognosis [17]. Besides, this study also revealed that markers of the adaptive immune response were predominant in tumors with a high S-score, whereas markers of the innate immune response are found in tumors with a high E-score, consistent with an impact of the

tumor micro-environment on survival [17]. The interest of associating molecular investigations and histological analysis was later proposed in a review recommending to update the histologic classification of MPM by a more multidisciplinary approach to support clinical practice, research investigation and clinical trials [18]. An influence of the micro-environment on patients' outcome was further suggested using deep learning based on MPM histology slides [19]. Contribution of histone methyl transferases can be illustrated by the overexpression of *EZH2*, a component of the polycomb complex PRC-2, which silent histone H3 by trimethylation [20]. Recent studies highlighted the strong contribution of epigenetic regulation through DNA methylation or miRNA expression deregulation in MPM. Integration of miRNome and methylome data revealed the contribution of epigenetic regulation in the epithelioid-like and sarcomatoid-like components of the tumors [21, 17]. Some genes such as *WT1* and *PI3KR1*, or *RUNX1* and *PBRM1* were hypermethylated and underexpressed in tumors with a high E-score or S-score, respectively [17]. Next generation sequencing analyses linked alterations of histone methylation pathway to inactivation of histone lysine methyltransferases, mainly *SETD2* and *SETDB1* [4].

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

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

Proteomic approaches were also used to characterize MM secretome and exosome. MM secretome was analyzed in six cell lines by iTRAQ® mass spectrometry, and compared to non-malignant cell lines. Results showed differential expression of proteins involved in metabolic energetic pathways, up-regulation of proteins involved in cancer invasion and metastasis, and down-regulation of proteins involved in cell adhesion [26]. Protein content of MM-derived exosomes was investigated in the four MPM cell lines studied in the previously quoted paper. A majority of proteins detected is expressed in various types of cancer, but specific proteins were identified in MM, either shared with all MM of differential between

the MPM [27]. The proteomic findings correlated with gene expression reported in transcriptomic studies of MPM and identified biomarkers known to be expressed from immunohistochemical studies, as well as immunomodulatory components and tumor-derived antigens [27].

State of signaling pathways in malignant mesothelioma

Several signaling pathways are deregulated in human MM, leading to an un-maintained mesothelial cell homeostasis. Pathways analyses from transcriptomic data have revealed alterations in cell proliferation control, apoptosis, differentiation, cell migration and survival [28, 29]. In cancer, both the MAPKs and PI3K/AKT/mTOR pathways are often affected by activating oncogenic mutations in genes involved in these signaling pathways, but these mutations are rare in MM [30]. In MM, these pathways are activated as assessed with the use of specific inhibitors that reduce cell growth or cell viability, and their activation may result from overexpression of specific growth factors or receptors such as EGFR and MET [30, 29]. Pathway analyses carried out in recent comprehensive integrative genomic studies highlighted P53 and mTOR pathways as deregulated in MPM [6, 17, 4]. Other pathways were identified as differentially activated between MPM tumors, depending on the E/S-scores (angiogenesis, EMT, immune checkpoints and metabolic pathways) [17].

One prominent feature in MM is the deregulation of Hippo, an evolutionarily conserved pathway involved in development and control of organ size. When turned on, this pathway negatively controls cell proliferation, partly maintaining cell-cell contacts. Protein players of the pathway are merlin (*NF2*), LATS1 and LATS2 that silence YAP and TAZ by phosphorylation, and consequently avoid the transcription of downstream genes such as *CTGF*, *CYR61* or *c-MYC* [31]. In MPM, several members (*NF2*, *LATS2*, *LATS1*, *SAV1*...) of Hippo pathway are inactivated due to gene mutations and/or deletions [5, 32]. This pathway crosstalks with other pathways, Hedgehog, Wnt and P53. This last cross is of particular interest regarding the different rate of mutations of *NF2* and *TP53* in MM, with a possible repercussion of alteration of one pathway on the other. A recent review sheds light in the interactions between Hippo and P53 pathways, which show both mutated member genes in MPM [33]. YAP and P53 can bind to the *TP53* and *YAP* promoters, respectively. Moreover, LATS1/2 binds to MDM2, a negative regulator of P53, and YAP1 can bind to mutant P53 and members of the P53 family [33]. Finally, these two pathways may coordinately maintain genomic stability in response to stress by the modulation of cell senescence, apoptosis and growth.

Gene susceptibility factors

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

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

Although germline mutations in *BAP1* are susceptibility factors in the induction of MM in individuals exposed to asbestos, they do not seem to lead to MM in the absence of exposure. This hypothesis is supported by experimental studies using heterozygous *Bap1*^{+/-} mutant mice not treated with asbestos showing no or a low rate of spontaneous mesotheliomas, despite a high incidence of other types of malignant tumors, and an increased incidence *Bap1*^{+/-} asbestos-exposed mice in comparison with their *Bap1*^{+/+} counterparts [45, 46]. Moreover, homozygous conditional knockout mice *Bap1*^{-/-} generated by the injection of Adeno-*Cre* in the

pleural cavity also developed a low rate of pleural mesothelioma (1/32 mice) [47, 48].

Asbestos fibers and mesothelial carcinogenesis

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

Global mechanism of action of mineral fibers

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

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

Molecular features of MPM possibly related to the mechanism of action of mineral fibers

Genetic damage in MPM

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

DNA double strand breaks (DSB) are other forms of DNA damage that can be caused by different sorts of clastogenic agents, by mechanical stress on chromosomes or in case of replication stress, and also promoted by abnormal mitosis [65, 66]. Several experimental works carried out with different types of cultured cells, including mesothelial cells, have shown that asbestos may interfere with mitosis [67-69]. Abnormal mitoses are revealed by various observations including the occurrence of aneuploidy, chromosome and chromatin damages, defects in spindle formation, lagging chromosomes, centrosome amplifications, multipolar mitoses and alterations of cytokinesis [70-72, 51, 73, 74, 36]. Cell cycle investigations have shown an accumulation of asbestos-treated cells in the G2/M phases of the cell cycle, consistent with a protracted mitosis [75-77]. It is known that mitosis impairment may promote chromosome missegregation, rearrangements and aneuploidy, and delayed mitosis may promote DNA breakage, as shown with agents interacting with microtubule dynamics and other different conditions [66]. Therefore, the impact of asbestos on mitosis, which is due to the fiber internalization and the interaction with cell, is also an important effect to consider in the mechanisms of asbestos-induced carcinogenesis.

Repair processes are very important to resolve DNA damages. They include homologous and non-homologous recombination that may result in error-prone

repair [78]. They may play a role in the genesis of MPM. In one hand, asbestos induces DNA breakage, as shown by the genotoxicity data in experimental assays. On the other hand, several publications reported pathogenic variants in DNA repair systems including recombination repair genes [39, 42].

Cell and molecular heterogeneity in MPM

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

Molecular heterogeneity of MPM is attested both by mutations and deregulation of signaling pathways. Molecular heterogeneity, in terms of mutations, is likely linked to the polyclonal and sub-clonal evolution of tumor cells, as shown by the intra-tumor heterogeneity [82-84]. Hippo pathway inactivation is a characteristic of some MPM. A role of Hippo pathway is possibly linked to the structure of the pleura and to the mechanism of action of asbestos fibers. First, normal mesothelial cells form a monolayer at the serosal surface and are joined by junctions, which assure cell-cell and cell-basal membrane contacts [85, 86]. Hippo pathway activity is regulated by mechano-transduction and cell-cell adhesion, and controls tight junctions [87, 31]. Its inactivation may abolish control of claudins, which are expressed in tight junctions, and differentially expressed in epithelioid compared to nonepithelioid MPM, and in MPM compared to healthy tissue [88, 89, 4, 90, 17]. Second, asbestos fibers provoke numerical chromosome changes and alteration of mitosis, especially abolishment of cytokinesis, leading to aneuploid cells including tetraploid cells. Interestingly, Hippo pathway regulates the proliferation of tetraploid cells and blocks their proliferation. Asbestos fibers avoid cell abscission, and tetraploid and near-tetraploid cells are observed in asbestos-treated mesothelial cells and in MPM [91, 92]. Therefore, knockout of proliferation control may facilitate chromosome instability, and the appearance of hypo-tetraploid or hyperdiploid cells, and lead to neoplastic evolution. It may be paradoxical that *NF2* seems more frequently mutated in non-exposed patients than in exposed patients, but *NF2* mutations in asbestos-exposed cells would lead to catastrophic mitosis [32]. Conversely *BAP1*, the most frequently mutated gene in MPM, might

prevent chromosome instability, by the regulation of γ -tubulin ubiquitination in *BAP1* wild-type cells [93, 94].

Conclusions

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

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

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

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

relationships between mechanisms of action of asbestos and the molecular mechanism of mesothelial carcinogenesis.

1. Musti M, Kettunen E, Dragonieri S, Lindholm P, Cavone D, Serio G et al. Cytogenetic and molecular genetic changes in malignant mesothelioma. *Cancer Genet Cytogenet.* 2006;170(1):9-15.
2. Toyooka S, Kishimoto T, Date H. Advances in the molecular biology of malignant mesothelioma. *Acta Med Okayama.* 2008;62(1):1-7.
3. 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. *Nature genetics.* 2011;43(7):668-72. doi:10.1038/ng.855.
4. 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. *Nature genetics.* 2016;48(4):407-16. doi:10.1038/ng.3520.
5. Tranchant R, Quétel 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. doi:10.1158/1078-0432.ccr-16-1971.
6. Hmeljak J, Sanchez-Vega F, Hoadley KA, Shih J, Stewart C, Heiman D et al. Integrative Molecular Characterization of Malignant Pleural Mesothelioma. *Cancer discovery.* 2018;8(12):1548-65. doi:10.1158/2159-8290.cd-18-0804.
7. Guo G, Chmielecki J, Goparaju C, Heguy A, Dolgalev I, Carbone M et al. Whole exome sequencing reveals frequent genetic alterations in BAP1, NF2, CDKN2A and CUL1 in malignant pleural mesothelioma. *Cancer research.* 2015;75(2):264-9. doi:10.1158/0008-5472.can-14-1008.
8. Carbone M, Yang H. Mesothelioma: recent highlights. *Ann Transl Med.* 2017;5(11):238. doi:10.21037/atm.2017.04.29.
9. Maki-Nevala S, Sarhadi VK, Knuuttila A, Scheinin I, Ellonen P, Lagstrom S et al. Driver Gene and Novel Mutations in Asbestos-Exposed Lung Adenocarcinoma and Malignant Mesothelioma Detected by Exome Sequencing. *Lung.* 2016;194(1):125-35. doi:10.1007/s00408-015-9814-7.
10. Ahadi MS, Gill AJ. SMARCA4 Loss Is Very Rare in Thoracic Mesothelioma. *The American journal of surgical pathology.* 2019;43(8):1154-5. doi:10.1097/pas.0000000000001262.
11. Andujar P, Lacourt A, Brochard P, Pairon JC, Jaurand MC, Jean D. Five years update on relationships between malignant pleural mesothelioma and exposure to asbestos and other elongated mineral particles. *Journal of toxicology and environmental health Part B, Critical reviews.* 2016;19(5-6):151-72. doi:10.1080/10937404.2016.1193361.

12. Bueno R, De Rienzo A, Dong L, Gordon GJ, Hercus CF, Richards WG et al. Second generation sequencing of the mesothelioma tumor genome. *PloS one*. 2010;5(5):e10612. doi:10.1371/journal.pone.0010612.
13. Gordon GJ. Transcriptional profiling of mesothelioma using microarrays. *Lung cancer (Amsterdam, Netherlands)*. 2005;49 Suppl 1:S99-S103.
14. Dong L, Jensen RV, De Rienzo A, Gordon GJ, Xu Y, Sugarbaker DJ et al. Differentially expressed alternatively spliced genes in malignant pleural mesothelioma identified using massively parallel transcriptome sequencing. *BMC medical genetics*. 2009;10:149. doi:10.1186/1471-2350-10-149.
15. Kane AB, Jean D, Knuutila S, Jaurand MC. Malignant Mesothelioma: Mechanism of Carcinogenesis. In: S. Anttila PB, editor. *Occupational Cancers*. Springer-Verlag; In Press.
16. 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. doi:10.1158/1078-0432.CCR-13-2429.
17. Blum Y, Meiller C, Quétel L, Elarouci N, Ayadi M, Tashtanbaeva D et al. Dissecting heterogeneity in malignant pleural mesothelioma through histomolecular gradients for clinical applications. *Nature Communications*. 2019;10(1):1333. doi:doi: 10.1038/s41467-019-09307-6.
18. Nicholson AG, Sauter JL, Nowak AK, Kindler HL, Gill RR, Remy-Jardin M et al. EURACAN/IASLC Proposals for Updating the Histologic Classification of Pleural Mesothelioma: Towards a More Multidisciplinary Approach. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer*. 2020;15(1):29-49. doi:10.1016/j.jtho.2019.08.2506.
19. Courtiol P, Maussion C, Moarii M, Pronier E, Pilcer S, Sefta M et al. Deep learning-based classification of mesothelioma improves prediction of patient outcome. *Nature medicine*. 2019;25(10):1519-25. doi:10.1038/s41591-019-0583-3.
20. McLoughlin KC, Kaufman AS, Schrump DS. Targeting the epigenome in malignant pleural mesothelioma. *Transl Lung Cancer Res*. 2017;6(3):350-65. doi:10.21037/tlcr.2017.06.06.
21. Blum Y, Jaurand MC, Reynies d, Jean D. Unraveling the cellular heterogeneity of malignant pleural mesothelioma through a deconvolution approach. *Molecular & Cellular Oncology*. 2019;DOI: 10.1080/23723556.2019.1610322. doi:DOI: 10.1080/23723556.2019.1610322.
22. Singh AS, Heery R, Gray SG. In Silico and In Vitro Analyses of LncRNAs as Potential Regulators in the Transition from the Epithelioid to Sarcomatoid Histotype of Malignant Pleural Mesothelioma (MPM). *International journal of molecular sciences*. 2018;19(5). doi:10.3390/ijms19051297.
23. Giusti L, Ciregia F, Bonotti A, Da Valle Y, Donadio E, Boldrini C et al. Comparative proteomic analysis of malignant pleural mesothelioma: Focusing on the biphasic subtype. *EuPA open proteomics*. 2016;10:42-9. doi:10.1016/j.euprot.2016.01.006.

24. Grosserueschkamp F, Bracht T, Diehl HC, Kuepper C, Ahrens M, Kallenbach-Thieltges A et al. Spatial and molecular resolution of diffuse malignant mesothelioma heterogeneity by integrating label-free FTIR imaging, laser capture microdissection and proteomics. *Scientific reports*. 2017;7:44829. doi:10.1038/srep44829.
25. 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. doi:10.1016/j.ctrv.2015.05.001.
26. Creaney J, Dick IM, Leon JS, Robinson BW. A Proteomic Analysis of the Malignant Mesothelioma Secretome Using iTRAQ. *Cancer genomics & proteomics*. 2017;14(2):103-17. doi:10.21873/cgp.20023.
27. Greening DW, Ji H, Chen M, Robinson BW, Dick IM, Creaney J et al. Secreted primary human malignant mesothelioma exosome signature reflects oncogenic cargo. *Scientific reports*. 2016;6:32643. doi:10.1038/srep32643.
28. Chapel DB, Churg A, Santoni-Rugiu E, Tsujimura T, Hiroshima K, Husain AN. Molecular pathways and diagnosis in malignant mesothelioma: A review of the 14th International Conference of the International Mesothelioma Interest Group. *Lung cancer (Amsterdam, Netherlands)*. 2019;127:69-75. doi:10.1016/j.lungcan.2018.11.032.
29. 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; 2016. p. 169-92.
30. Sekido Y. Molecular pathogenesis of malignant mesothelioma. *Carcinogenesis*. 2013;34(7):1413-9. doi:10.1093/carcin/bgt166.
31. Meng Z, Moroishi T, Guan KL. Mechanisms of Hippo pathway regulation. *Genes & development*. 2016;30(1):1-17. doi:10.1101/gad.274027.115.
32. Quetel L, Meiller C, Assie JB, Blum Y, Imbaud S, Montagen F et al. Genetic alterations of malignant mesothelioma: association to tumor heterogeneity and overall survival. *Molecular Oncology*.
33. Raj N, Bam R. Reciprocal Crosstalk Between YAP1/Hippo Pathway and the p53 Family Proteins: Mechanisms and Outcomes in Cancer. *Frontiers in cell and developmental biology*. 2019;7:159. doi:10.3389/fcell.2019.00159.
34. Ugolini D, Neri M, Ceppi M, Cesario A, Dianzani I, Filiberti R et al. Genetic susceptibility to malignant mesothelioma and exposure to asbestos: the influence of the familial factor. *Mutation research*. 2008;658(3):162-71. doi:10.1016/j.mrrev.2007.08.001.
35. Melaiu O, Gemignani F, Landi S. The genetic susceptibility in the development of malignant pleural mesothelioma. *Journal of thoracic disease*. 2018;10(Suppl 2):S246-s52. doi:10.21037/jtd.2017.10.41.
36. Neri M, Ugolini D, Dianzani I, Gemignani F, Landi S, Cesario A et al. Genetic susceptibility to malignant pleural mesothelioma and other asbestos-associated diseases. *Mutation research*. 2008;659(1-2):126-36. doi:10.1016/j.mrrev.2008.02.002.

37. Cadby G, Mukherjee S, Musk AW, Reid A, Garlepp M, Dick I et al. A genome-wide association study for malignant mesothelioma risk. *Lung cancer* (Amsterdam, Netherlands). 2013;82(1):1-8. doi:10.1016/j.lungcan.2013.04.018.
38. Matullo G, Guarrera S, Betti M, Fiorito G, Ferrante D, Voglino F et al. Genetic variants associated with increased risk of malignant pleural mesothelioma: a genome-wide association study. *PloS one*. 2013;8(4):e61253. doi:10.1371/journal.pone.0061253.
39. Betti M, Aspesi A, Sculco M, Matullo G, Magnani C, Dianzani I. Genetic predisposition for malignant mesothelioma: A concise review. *Mutation research*. 2019;781:1-10. doi:10.1016/j.mrrev.2019.03.001.
40. Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E et al. Germline BAP1 mutations predispose to malignant mesothelioma. *Nature genetics*. 2011;43(10):1022-5. doi:10.1038/ng.912.
41. Panou V, Gadiraju M, Wolin A, Weipert CM, Skarda E, Husain AN et al. Frequency of Germline Mutations in Cancer Susceptibility Genes in Malignant Mesothelioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2018;36(28):2863-71. doi:10.1200/jco.2018.78.5204.
42. Bertelsen B, Tuxen IV, Yde CW, Gabrielaite M, Torp MH, Kinalis S et al. High frequency of pathogenic germline variants within homologous recombination repair in patients with advanced cancer. *NPJ genomic medicine*. 2019;4:13. doi:10.1038/s41525-019-0087-6.
43. Guo R, DuBoff M, Jayakumaran G, Kris MG, Ladanyi M, Robson ME et al. Brief Report: Novel Germline Mutations in DNA Damage Repair in Patients with Malignant Pleural Mesotheliomas. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer*. 2019. doi:10.1016/j.jtho.2019.12.111.
44. Pastorino S, Yoshikawa Y, Pass HI, Emi M, Nasu M, Pagano I et al. A Subset of Mesotheliomas With Improved Survival Occurring in Carriers of BAP1 and Other Germline Mutations. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2018;Jco2018790352. doi:10.1200/jco.2018.79.0352.
45. Xu J, Kadariya Y, Cheung M, Pei J, Talarchek J, Sementino E et al. Germline mutation of Bap1 accelerates development of asbestos-induced malignant mesothelioma. *Cancer research*. 2014;74(16):4388-97. doi:10.1158/0008-5472.can-14-1328.
46. 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 research*. 2016;76(9):2836-44. doi:10.1158/0008-5472.can-15-3371.
47. Kukuyan AM, Sementino E, Kadariya Y, Menges CW, Cheung M, Tan Y et al. Inactivation of Bap1 Cooperates with Losses of Nf2 and Cdkn2a to Drive the Development of Pleural Malignant Mesothelioma in Conditional Mouse Models. *Cancer research*. 2019;79(16):4113-23. doi:10.1158/0008-5472.can-18-4093.

48. Napolitano A, Pellegrini L, Dey A, Larson D, Tanji M, Flores EG et al. Minimal asbestos exposure in germline BAP1 heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma. *Oncogene*. 2016;35(15):1996-2002. doi:10.1038/onc.2015.243.
49. Attanoos RL, Churg A, Galateau-Salle F, Gibbs AR, Roggli VL. Malignant Mesothelioma and Its Non-Asbestos Causes. *Archives of pathology & laboratory medicine*. 2018;142(6):753-60. doi:10.5858/arpa.2017-0365-RA.
50. 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. *The Lancet Oncology*. 2014;15(13):1427-8. doi:10.1016/s1470-2045(14)71109-x.
51. Toyokuni S. Mechanisms of asbestos-induced carcinogenesis. *Nagoya journal of medical science*. 2009;71(1-2):1-10.
52. Huang SX, Jaurand MC, Kamp DW, Whysner J, Hei TK. Role of mutagenicity in asbestos fiber-induced carcinogenicity and other diseases. *Journal of toxicology and environmental health Part B, Critical reviews*. 2011;14(1-4):179-245. doi:10.1080/10937404.2011.556051.
53. Moller P, Danielsen PH, Jantzen K, Roursgaard M, Loft S. Oxidatively damaged DNA in animals exposed to particles. *Critical reviews in toxicology*. 2013;43(2):96-118. doi:10.3109/10408444.2012.756456.
54. Solbes E, Harper RW. Biological responses to asbestos inhalation and pathogenesis of asbestos-related benign and malignant disease. *Journal of investigative medicine : the official publication of the American Federation for Clinical Research*. 2018;66(4):721-7. doi:10.1136/jim-2017-000628.
55. Ospina D, Villegas VE, Rodriguez-Leguizamon G, Rondon-Lagos M. Analyzing biological and molecular characteristics and genomic damage induced by exposure to asbestos. *Cancer management and research*. 2019;11:4997-5012. doi:10.2147/cmar.s205723.
56. 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.
57. Miserocchi G, Sancini G, Mantegazza F, Chiappino G. Translocation pathways for inhaled asbestos fibers. *Environmental health : a global access science source*. 2008;7:4. doi:10.1186/1476-069x-7-4.
58. Broaddus VC, Everitt JI, Black B, Kane AB. Non-neoplastic and neoplastic pleural endpoints following fiber exposure. *Journal of toxicology and environmental health Part B, Critical reviews*. 2011;14(1-4):153-78. doi:10.1080/10937404.2011.556049.
59. Asgharian B, Owen TP, Kuempel ED, Jarabek AM. Dosimetry of inhaled elongate mineral particles in the respiratory tract: The impact of shape factor. *Toxicology and applied pharmacology*. 2018;361:27-35. doi:10.1016/j.taap.2018.05.001.
60. Oberdörster G. Lung Dosimetry: Pulmonary Clearance of Inhaled Particles. *Aerosol Science and Technology*. 1993;18:279-89

61. Batra H, Antony VB. Pleural mesothelial cells in pleural and lung diseases. *Journal of thoracic disease*. 2015;7(6):964-80. doi:10.3978/j.issn.2072-1439.2015.02.19.
62. Mutsaers SE, Prele CM, Brody AR, Idell S. Pathogenesis of pleural fibrosis. *Respirology (Carlton, Vic)*. 2004;9(4):428-40. doi:10.1111/j.1440-1843.2004.00633.x.
63. Tubbs A, Nussenzweig A. Endogenous DNA Damage as a Source of Genomic Instability in Cancer. *Cell*. 2017;168(4):644-56. doi:10.1016/j.cell.2017.01.002.
64. Kawanishi S, Ohnishi S, Ma N, Hiraku Y, Murata M. Crosstalk between DNA Damage and Inflammation in the Multiple Steps of Carcinogenesis. *International journal of molecular sciences*. 2017;18(8). doi:10.3390/ijms18081808.
65. Mehta A, Haber JE. Sources of DNA double-strand breaks and models of recombinational DNA repair. *Cold Spring Harbor perspectives in biology*. 2014;6(9):a016428. doi:10.1101/cshperspect.a016428.
66. Ganem NJ, Pellman D. Linking abnormal mitosis to the acquisition of DNA damage. *The Journal of cell biology*. 2012;199(6):871-81. doi:10.1083/jcb.201210040.
67. Jaurand MC. Mechanisms of fiber-induced genotoxicity. *Environ Health Perspect*. 1997;105(Suppl 5):1073-84.
68. Jean D, Jaurand MC. Causes and pathophysiology of malignant pleural mesothelioma. *Lung Cancer Management*. 2015;4(5):219-29.
69. Felley-Bosco E, MacFarlane M. Asbestos: Modern Insights for Toxicology in the Era of Engineered Nanomaterials. *Chemical research in toxicology*. 2018;31(10):994-1008. doi:10.1021/acs.chemrestox.8b00146.
70. 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.
71. 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.
72. Jensen CG, Watson M. Inhibition of cytokinesis by asbestos and synthetic fibres. *Cell Biol Intl*. 1999;23(12):829-40.
73. Cortez BA, Rezende-Teixeira P, Redick S, Doxsey S, Machado-Santelli GM. Multipolar mitosis and aneuploidy after chrysotile treatment: a consequence of abscission failure and cytokinesis regression. *Oncotarget*. 2016;7(8):8979-92. doi:10.18632/oncotarget.6924.
74. 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.
75. 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.

76. Cortez Bde A, Quassollo G, Caceres A, Machado-Santelli GM. The fate of chrysotile-induced multipolar mitosis and aneuploid population in cultured lung cancer cells. *PloS one*. 2011;6(4):e18600. doi:10.1371/journal.pone.0018600.
77. Liu W, Ernst JD, Broaddus VC. Phagocytosis of crocidolite asbestos induces oxidative stress, DNA damage, and apoptosis in mesothelial cells. *American journal of respiratory cell and molecular biology*. 2000;23(3):371-8. doi:10.1165/ajrcmb.23.3.4094.
78. Rodgers K, McVey M. Error-Prone Repair of DNA Double-Strand Breaks. *Journal of cellular physiology*. 2016;231(1):15-24. doi:10.1002/jcp.25053.
79. 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. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer*. 2016;11(2):142-54. doi:10.1016/j.jtho.2015.11.005.
80. 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.
81. Wang NS. Anatomy of the pleura. *Clin Chest Med*. 1998;19(2):229-40.
82. Oehl K, Vrugt B, Opitz I, Meerang M. Heterogeneity in Malignant Pleural Mesothelioma. *International journal of molecular sciences*. 2018;19(6). doi:10.3390/ijms19061603.
83. Oey H, Daniels M, Relan V, Chee TM, Davidson MR, Yang IA et al. Whole-genome sequencing of human malignant mesothelioma tumours and cell lines. *Carcinogenesis*. 2019. doi:10.1093/carcin/bgz066.
84. 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. doi:10.1186/s12967-014-0301-3.
85. Markov AG, Amasheh S. Tight junction physiology of pleural mesothelium. *Frontiers in physiology*. 2014;5:221. doi:10.3389/fphys.2014.00221.
86. Plouffe SW, Hong AW, Guan KL. Disease implications of the Hippo/YAP pathway. *Trends in molecular medicine*. 2015;21(4):212-22. doi:10.1016/j.molmed.2015.01.003.
87. Halder G, Dupont S, Piccolo S. Transduction of mechanical and cytoskeletal cues by YAP and TAZ. *Nature reviews Molecular cell biology*. 2012;13(9):591-600. doi:10.1038/nrm3416.
88. Soini Y. Claudins in lung diseases. *Respiratory research*. 2011;12:70. doi:10.1186/1465-9921-12-70.
89. Soini Y, Kinnula V, Kahlos K, Paakko P. Claudins in differential diagnosis between mesothelioma and metastatic adenocarcinoma of the pleura. *Journal of clinical pathology*. 2006;59(3):250-4. doi:10.1136/jcp.2005.028589.
90. Rouka E, Vavougios GD, Solenov EI, Gourgoulialis KI, Hatzoglou C, Zarogiannis SG. Transcriptomic Analysis of the Claudin Interactome in Malignant Pleural Mesothelioma: Evaluation of the Effect of Disease Phenotype, Asbestos

- Exposure, and CDKN2A Deletion Status. *Frontiers in physiology*. 2017;8:156. doi:10.3389/fphys.2017.00156.
91. Hagemeijer A, Versnel MA, Van Drunen E, Moret M, Bouts MJ, Van der Kwast TH et al. Cytogenetic analysis of malignant mesothelioma. *Cancer Genet Cytogenet*. 1990;47:1-28.
  92. Pyrhonen S, Tiainen M, Rautonen J, Tammilehto L, Laasonen A, Mattson K et al. Comparison of DNA and karyotype ploidy in malignant mesothelioma. *Cancer Genet Cytogenet*. 1992;60(1):8-13.
  93. Peng J, Ma J, Li W, Mo R, Zhang P, Gao K et al. Stabilization of MCRS1 by BAP1 prevents chromosome instability in renal cell carcinoma. *Cancer letters*. 2015;369(1):167-74. doi:10.1016/j.canlet.2015.08.013.
  94. Zarrizi R, Menard JA, Belting M, Massoumi R. Deubiquitination of gamma-tubulin by BAP1 prevents chromosome instability in breast cancer cells. *Cancer research*. 2014;74(22):6499-508. doi:10.1158/0008-5472.can-14-0221.
  95. Karaman R, Halder G. Cell Junctions in Hippo Signaling. *Cold Spring Harbor perspectives in biology*. 2018;10(5). doi:10.1101/cshperspect.a028753.
  96. Misra JR, Irvine KD. The Hippo Signaling Network and Its Biological Functions. *Annual review of genetics*. 2018;52:65-87. doi:10.1146/annurev-genet-120417-031621.