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Abbreviation

*BAP1*: BRCA1 associated protein-1

*CDKN2A*: cyclin-dependent kinase inhibitor 2A

*CDKN2B*: cyclin-dependent kinase inhibitor 2B

*EGFR*: epithelial growth factor receptor

*EPP*: Extrapleural pneumonectomy

*GM mice*: genetically modified mice

*GM-CSF*: granulocyte macrophage-colony stimulating factor

*HDACi*: histone deacetylase inhibitors

*IFN*: interferon

*IL*: interleukin

*LATS2*: large tumor suppressor, homolog 2

*LFT*: localized fibrous tumors

*MM*: Malignant mesothelioma

*MPM*: Malignant mesothelioma

*ADCC*: antibody-dependent cellular cytotoxicity

*NF2*: neurofibromin 2

*P/D*: pleurectomy/decortication

*PDGFR*: platelet-derived growth factor receptor

*PFS*: progression-free survival

*SV40*: simian virus 40

*TK*: tyrosine kinase

*TP53*: tumor protein 53

*TSG*: tumor suppressor genes

*VEGF*: vascular endothelial growth factor

*VEGFR*: vascular endothelial growth factor receptor

*VPA*: valproic acid

*VT*: videothoracoscopy
Synopsis

Epidemiology

Malignant mesothelioma (MM) is mainly attributable to environmental or occupational asbestos exposure, but other causes or cofactors could account for the occurrence of MM. Despite the ban of asbestos use in several countries, MM still arises due to its long latency period, typically longer than 30 years. The incidence and mortality trends of malignant mesothelioma in various countries are strongly influenced by asbestos consumption patterns in the past. In the other countries there is substantial concern that the increased use of asbestos may result in an increase in the number of cases of MM for many decades. Nowadays, MM remains an environmental and occupational concern for a large population.

Clinical Aspects

The pleura is the most frequent location of MM. Spontaneous survival of patients is less than 6 to 10 months. However, differences in spontaneous behavior of MPM are observed. The clinical presentation usually includes dyspnea and chest pain due to a massive pleural effusion. After removal of this effusion, chest computed tomography and positron emission tomography are helpful to evaluate the clinical stage. Cytologic analysis on the pleural fluid and serum biomarkers are currently used for the diagnosis, and monitoring of MPM. The most definitive approach to obtain a formal diagnosis is videothoracoscopy (VT) allowing adequate tissue sampling. The surgical and medical treatment depends on the patient’s performance status, the characteristics of the tumor and the stage of the disease using IMIG staging system. The role of any specific therapy should be evaluated in the context of a multimodality program including palliative therapy.

Morphological Mesothelioma Classification

The diagnosis of MM is difficult because of the histological heterogeneity of MM. MM mimics other malignancies located to the pleura, and may resemble benign pleural lesions or metastatic lesions. Its distinction from other neoplasms and reactive processes requires adequate tissue sampling. Histological classification is based on morphological features, and on immunohistochemical reactivity using both negative and positive markers. There are four main subtypes: epithelioid, sarcomatoid, desmoplastic and biphasic mesotheliomas. Several unusual morphological variants have also been identified. Distinguishing benign and malignant mesothelioma can be a difficult challenge for both clinicians and pathologists. Before making a definitive diagnosis of MM, the pathologist should consider the clinical and radiological presentation together with immunohistochemistry. In the near future molecular data will probably be useful for MM diagnosis and classification.
Molecular Classification
The biological diversity of MPM evidenced in morphological studies is also demonstrated by molecular analyses. MPM exhibit large chromosomal imbalance, a heterogeneous mutation spectrum, epigenetic changes and alterations of gene expression and signaling pathways. This heterogeneity may account for the differences in spontaneous behavior of MPM, and the lack of efficient treatment. The establishment of a molecular classification of MPM would have paramount interest for diagnostic, prognostic and therapeutic issues. Integrative approaches taking account data of large-scale molecular studies such as tumor genome sequencing or high-throughput genomic, epigenetic or transcriptomic analyses will improve the MPM classification according to tumor-specific criteria.

Mesothelioma Therapy
To date, MM remains a cancer without effective curative treatment. Depending on the clinical context, therapeutic strategies include chemotherapy, radiotherapy, surgery or multimodal treatments. Radical surgery is limited to a small number of patients. New strategies, immunotherapy (cytokine treatments, antibody-based cytotoxicity, or vaccination) and gene therapy, alone or combined with conventional anti-cancer therapies are under investigation. Some results may be promising. Results of molecular studies in MM led to the design of novel molecular targeted agents, used alone or in combination with chemotherapy. So far, results remain limited. A better knowledge of the molecular characteristics, and classification of the tumors should improve targeted pharmacological strategies for treatment of MPM.

Animal and In vitro Models
Pre-clinical investigations are needed to study the mechanism of neoplastic transformation of mesothelial cells, and to test the efficacy of anti-cancer drugs and therapeutic strategies. For that purpose, several so-called “models” of mesothelioma have been developed. Asbestos-induced MM have been generated by intra-pleural or intra-peritoneal injection of asbestos fibers in rodents, and “spontaneous” MM in mice genetically engineered in genes, known to be altered in human MM, without asbestos exposure. MM models can be derived from the transplantation of human MM cells in immunosuppressed mice. Cell systems are relevant tools to assess the response to drugs. Two-dimensional and 3D (cell aggregates, spheroids) are currently used. The advantage of the cell assays concerns their potential to study well-characterized human cells at the genomic level representative of the MM molecular diversity. Present developments in our knowledge on MM features should lead developing new powerful models.
Introduction

Malignant mesothelioma (MM) is an aggressive tumor that commonly affects the mesothelial surfaces of the pleural and peritoneal cavities, and occasionally, the testicular tunica vaginalis and the pericardium. MM is mainly due to asbestos past exposure. However, in about 20% of MM cases no asbestos exposure can be found, addressing the question of the role of other risk factors. The latency period between date of first exposure to asbestos and onset of disease is typically longer than 30 years and the median survival time after diagnosis is 9–12 months. So far, no curative treatment is available supporting the development of basic and applied biological researches to improve MM outcome.

Epidemiology of Malignant Mesothelioma

Etiological factors

Asbestos

MM is mainly attributable to environmental or occupational asbestos exposure. Asbestos is the commercial name given to a family of natural silicate mineral fibers including two varieties: fibers called serpentines (chrysolite, so-called white asbestos), and amphiboles (crocidolite, blue asbestos; amosite, brown asbestos), anthophyllite, tremolite, and actinolite). Evidence in humans supports the carcinogenicity of all forms of asbestos. Because of their interesting physical and chemical properties (high tensile strength, high heat resistance and resistance to most chemicals and acids), asbestos fibers have been commonly used in a variety of building construction materials for insulation and as a fire-retardant. Because of the fibers’ strength and heat-resistant properties, asbestos has been used for a wide range of manufactured goods, mostly in building materials, friction products, heat-resistant fabrics, packaging, gaskets, and coatings.

The link between MM and asbestos exposure was first established by Wagner among asbestos-mine-workers in South-African’s Cape Province in the 1950s. Malignant pleural mesothelioma is mainly due to asbestos past exposure at work, with an attributable fraction of 80% or more in men and less than 40% in women. Over the last decades, a shift has been observed in the exposure history of MM cases, from primary asbestos workers (handling raw asbestos material) to end-users often exposed when installing asbestos products or handling asbestos materials that are still in place. Elevated risks were found in plumbers, sheet-metal workers, welders, metal molders, coremakers and cabinetmakers and in the industries of shipbuilding, construction, manufacturing of metal products, chemicals, and railroad and aircraft equipment. An English case-control study estimates
lifetime risks for male Britons born in the 1940s with ≥10 years of exposure before the age of 30 years between 0.6 and 5.9% depending on occupation.

When asbestos-containing materials are damaged or disturbed by human activities such as repair or demolition, asbestos fibers can be inhaled and reach the deep lung and the pleura. The most important physicochemical properties of asbestos fibers related to pathogenicity are fiber dimensions, surface chemistry and reactivity and biopersistence. Chrysotile is less biopersistent in the lungs than amphiboles. Chrysotile, amosite and crocidolite have all been widely used for industrial purposes. Fiber shape and length-to-width ratio are important physical attributes that determine how deeply into the lung the fibers are inhaled and whether they then have the capacity to penetrate the lung and to reach the pleural space. The most dangerous asbestos fibers are long and thin. Asbestos fibers may pierce the mitotic spindle of cells and thereby disrupt mitosis, resulting in aneuploidy and other forms of chromosomal damage and induce also the generation of reactive oxygen species that cause DNA damage.

MM is also linked to environmental exposure to asbestos, either in areas of the world where asbestos (generally tremolite) exists as a geological component of the soil (Turkey, Corsica, Cyprus and New Caledonia) or to neighborhood exposures in people living close to asbestos mines or factories. Domestic cases are described in households of asbestos workers, mainly because of asbestos exposure via clothes used at work washed at home.

**Others causes**

Exposure to other types of fibers has been linked to MM occurrence. Erionite, and talc or vermiculite contaminated with asbestos (Libby, Montana, United States) are all also strongly associated with the risk of development of MM. Erionite, a fibrous zeolite mineral is an endemic natural mineral fiber in Turkey; it is also present as road surfaces in North Dakota, and in other parts in USA. Fluoro-edenite, a silicate similar in morphology and composition to the actinolite-tremolite series of minerals was suggested as the cause of MM.

MM has been attributed to the effect of ionizing radiations, especially in patients exposed to thorotrast used as a radiographic contrast material in the 1930s to 1950s. A potential role for the simian SV40 virus has been suggested and discussed, as a cofactor of asbestos.

Several reports have emphasized familial clustering of MM cases suggesting a genetic predisposition. One cluster was thought to show a possible autosomal dominant pattern in subjects studied in Cappadocia, Turkey. More recently, germline mutations in BRCA1-associated-protein-1 (BAP1) were found to predispose to MM. In these studies, there was no significant correlation with asbestos exposure. In one study, patients did not seem to
have been exposed to asbestos, but chrysotile asbestos and traces of tremolite and chrysotile asbestos were detected in homes in which all family members were affected, in agreement with an environmental exposure.

Incidence and Global Mortality

MM incidence varies markedly from one country to another although some of that variance may be due to differences in reporting since the sources of information are very different from one country to another. In some countries specific mesothelioma registries cover all or a part of the national territory. In many countries, MM incidence is estimated on the basis of mortality data. The highest annual crude incidence rates (about 25 to 30 cases per million) are observed in Australia, Great Britain and Belgium, followed by The Netherlands, Italy and Norway (15 to 24 cases per million), and Denmark, Germany Sweden, France and Finland (10 to 14 cases per million). In the United States and in Canada it is estimated to 9 cases per million. MM incidence is much higher in men than women with a male-to-female ratio about 4:1.

Industrialized countries have much higher rates of MM than non-industrialized countries, reflecting the past production and use of asbestos in industry. The developed world has seen an epidemic of MM since World War II because of the demand for asbestos of all varieties that accompanied industrialization. This epidemic did not take place immediately because of the long latency period, typically longer than 30 years, although latency periods of around 15 years have been described.

According to the World Health Organization mortality database, the crude and age-adjusted mortality rates for all mesothelioma deaths were 6.2 and 4.9 per million respectively, and the mean age at death was 70 years between 1994 and 2002 in a total of 83 countries. The gender-specific age-adjusted mortality rate for males was 9.0 per million compared with 1.9 per million for females.

Incidence and Mortality Predictions

The future occurrence of MM can be predicted from the pattern of asbestos use around the world. While asbestos consumption leveled off during the 1960s and 1970s in the United States, Australia, United Kingdom and Nordic countries, this did not happen in Italy and France until the early 1980s. Different estimates of peak mesothelioma mortality have been predicted in many countries: 700 cases per year in 2010 in Australia, approximately 2000 deaths per year in the year 2016 in Great-Britain, with a rapid decline thereafter to 900 cases per year of pleural mesothelioma around the year 2028 in the Netherlands. Recently, the incidence of MM in the United States and in some European countries (especially in France and Sweden) should have started to decline before or around the year 2000. Recent predictions in some western countries were lower than previously published. There is
substantial concern that the increased use of asbestos in developing countries may result in an increase in the number of cases of MM for many decades to come unless strong occupational health controls are performed.

Conclusions

MM is mainly attributable to environmental or occupational asbestos exposure with a long latency period, typically longer than 30 years. The incidence and mortality trends of MM in various countries are strongly influenced by asbestos consumption patterns in the past. The developed world has seen an epidemic of MM since World War II. Based on the asbestos consumption, an increase of MM cases can be predicted in the future in developing countries.

Clinical Aspects of Malignant Pleural Mesothelioma

Malignant pleural mesothelioma (MPM) is the most frequent location of MM. Its aggressiveness and the lack of curative treatment make MPM a tumor with poor prognosis, spontaneous survival being of less than 6 to 10 months. However, differences in spontaneous behavior of MPM according to the histological type are well recognized, epithelioid MPM being associated with significant better survival.

Clinical presentation and natural History

Dyspnea, cough or diffuse chest wall pains are the usual presenting symptoms observed in 90% of patients. These symptoms are mainly due to pleural effusion usually more abundant in epithelioid MPM compared to sarcomatoid MPM. In early-stage of MPM, symptoms normally disappear after pleural drainage. Intense and localized chest pain, not reversible by drainage, generally occurs in thoracic wall invasion suggesting neural invasion (intercostal, sympathetic chain or brachial plexus). In tumors extended to apical pleura, Pancoast-Tobias or Horner’s syndrome generally occurs, severely enough to require opioids. Vena cava syndrome may occur on right MPM with mediastinal invasion and dysphagia generally due to esophagus compression by pleural mass lesions. Pericardial effusion leads to arrhythmias, cardiac failure or even tamponade. Digestive disorders or obstructive bowel symptoms suggest peritoneal carcinomatosis by transdiaphragmatic tumor spread. Several para-neoplastic syndromes have been described, but their frequency remains unknown. These are non-specific, common to a number of malignancies, and include coagulation disorders, hypercalcemia, hypoglycemia, autoimmune hemolytic anemia etc.

Initially, the tumor may be limited to some areas on lower parietal, diaphragmatic or mediastinal pleura. Accumulation of pleural fluid compresses the underlying lung and impairs diaphragmatic movements. Because
of tumor cell clusters floating in the effusion, tumor seeding may gradually occur in all the parts of the pleural surface. Local extension of tumor generally occurs towards the chest wall (extrapleural fat, muscles, ribs), the mediastinal structures (fat, phrenic nerve, vena cava, aorta, brachial plexus) and the three adjacent cavities (peritoneal, pericardic and contralateral pleural). The paths of peritoneal invasion are mainly the phrenic tendinous center and the posterior thoraco-abdominal holes. The direct pericardial invasion rapidly worsens the prognosis because of cardiac constriction by increasing pericardial thickening and fluid accumulation. The pattern of lymphatic spread is different from that observed in lung cancer. The lymph nodes involved in MPM are located near the thoracic wall (internal mammary lymph nodes, sympathetic thoracic chain or near the costodiaphragmatic sinus). MPM mainly remains a loco-regional disease with mortality usually due to cardiothoracic or digestive complications. Distant metastases, while significantly more frequent in sarcomatoid MPM, are rarely observed overall clinically and are extremely rare as a presenting manifestation. Nevertheless, in a post-mortem series on MPM, extra-thoracic dissemination of mesothelioma has been reported. The most frequent sites of metastasis are the liver, adrenal gland, kidney, contralateral lung and rarely brain. Respiratory failure appears to be the major cause of mortality due to local bulky tumor increased. Typically, metastases, either diagnosed or not, do not seem to be the cause of death raising the possibility of physiological and metabolic causes of death.

**Diagnosis**

Chest CT is helpful in increasing the clinical suspicion of a malignant pleural process by revealing pleural thickening with extension to mediastinal pleura and fissure (Figure 1). However no radiological examination can definitively establish the diagnosis, which requires histological analysis. The first step in all patients in whom MPM is suspected is a cytologic analysis done on the exudative pleural fluid despite its very low sensibility. While it is not generally recommended to make a diagnosis of MPM on cytology alone, a cell block with appropriate immunohistochemistry may be sufficient in some cases. Molecular analysis of cytology fluids using a FISH assay has also been reported to be helpful. In older or very sick patients, a simple transcutaneous biopsy with cytologic analysis can make a diagnosis but is only 30% sensitive. A risk of wall tumor seeding on intervention tracts (between pleura and skin) exists following transthoracic procedures done for diagnostic purposes. To avoid this drawback, prophylactic irradiation of VT port or skin puncture is commonly done. However no consensus has been reached to support this practice.
The most definitive approach to obtain a formal diagnosis is videothoracoscopy (VT). In experienced hands VT allows to take large and targeted pleural biopsies to perform a complete histological study giving a positive diagnosis in 90% to 98% of cases. Moreover, VT allows a direct examination of the pleural cavity to evaluate the stage of the disease and to perform talc pleurodesis, in order to avoid a recurrence of major pleural effusion. Recent researches have evaluated serum biomarkers (osteopontin and soluble mesothelin, and more recently fibulin-3) to diagnose, detect and monitor disease. Mesothelin is currently the most studied serum biomarker of MPM. From a recent meta-analysis study, a negative blood test for mesothelin does not appear to be a useful to exclude mesothelioma in symptomatic or high-risk (asbestos-exposed) individuals while a positive blood test for mesothelin should prompt further investigations.

Management of Patients

Most patients with MPM, whether treated or untreated, die of complications of the local disease. Since the mid-1990s a more aggressive surgical and medical treatment has been proposed to treat MPM, depending on the general status of the patients, the histological subtype and the stage of the disease using IMIG staging system. Older patients (over 70) were referred to standard chemotherapy if no major co-morbidities were detected. A curative approach utilizing surgery, radiotherapy and chemotherapy, was proposed in rare selected cases (younger patients with good general status, at early stage of the disease and mostly with MPM of epithelioid type) in some trials. Only stage I, II and rare stage III, without mediastinal lymph node invasion, may be referred to resection, but accurate preoperative staging is often impossible even by using more invasive procedures as mediastinoscopy and laparoscopy. Two different surgical approaches have been used. While extrapleural pneumonectomy (EPP) with adjuvant chemotherapy and chest wall irradiation (trimodality therapy) showed very interesting initial results (better survival in Stage-I patients with epithelioid histological type and negative resection margins after EPP) more recent studies have not confirmed the results. Despite this aggressive treatment, with 90-day mortality of 3.3% to 10.8% and 40% of morbidity, tumor recurrence occurred near the thoracic cavity but also in the peritoneum (26%), contralateral pleural (17%) and distant organs. To improve local tumor control, adding intraoperative chemotherapy inside the cavity of EPP has been evaluated, but the early results show an increased morbidity, and the impact on survival is pending. The second surgical procedure is the pleurectomy/decortication (P/D) that preserves the lung. Although it is necessarily an incomplete tumor removal, its advantages may be an adequate cytoreduction procedure allowing symptoms control, with lower morbidity and mortality rates, and at least similar survival rates. Finally, the place of surgery remains uncertain moving towards palliative therapy included in a multimodality program.
Morphological Mesothelioma Classification

The histological heterogeneity of MM creates a variety of complex challenges to pathologists in making a reliable diagnosis. Firstly, MM mimics other malignancies located to the pleura, the most frequent being metastatic pleural tumors originating from other sites especially lung or breast carcinoma. Secondly, MM has varied and deceptive appearance in a high percentage of cases, and may resemble benign pleural lesions and vice versa. Figure 2 shows different morphological and immunohistochemical features of MM.

Histological Diagnosis and Classification

MM can be subdivided into four major histological subtypes: epithelioid, sarcomatoid, desmoplastic and biphasic types. Within these subtypes there is a myriad of patterns, which are important to be aware of in terms of histopathological diagnosis, but they are not significant clinically. Epithelioid MM consists of tubules, trabecules, papillae, micropapillae or sheets of cuboidal or polygonal epithelioid mesothelial cells. EMM must be distinguished from reactive mesothelial proliferations as well as metastatic carcinoma. This is the most common histologic variant. The most important criteria for malignancy is the presence of invasion, especially into visceral pleural adipose tissue as discussed in detail below. It is this requirement that makes a deep tissue biopsy imperative, since otherwise relatively bland mesothelial proliferations can be invasive and hence malignant.

Well-differentiated papillary mesothelioma is another tumor of mesothelial origin that spreads on the surface without invasion. It should be well discriminated from the papillary epithelioid type of mesothelioma because this former lesion is usually associated with prolonged survival. This is a distinct tumor usually found in the peritoneal cavity in women without asbestos exposure.

Sarcomatoid mesotheliomas consist of a pure spindled pattern resembling a true sarcoma e.g. fibrosarcoma or malignant fibrous histiocytoma. In a small percentage of cases, areas resembling osteosarcoma and/or chondrosarcoma, leiomyosarcoma and rhabdomyosarcoma may be present. In some cases, these areas are so prominent that calcified densities can be seen within the tumor radiographically. Sarcomatoid MM must be distinguished from organizing fibrous pleuritis, localized fibrous tumors of the serous membranes (LFT), sarcomatoid carcinomas, primary and metastatic sarcomas involving the pleura, and desmoid tumors. Histologic features and cytokeratin staining can help to exclude LFT, desmoid tumors, and metastatic sarcomas. The differentiation between sarcomatoid carcinoma and sarcomatoid mesothelioma is highly dependant of the clinical presentation.
Biphasic mesotheliomas consist of a combined epithelioid and sarcomatoid pattern with the same immunohistochemical pattern seen in the individual components as previously described. It is arbitrarily recommended that there should be at least 10% of each component to diagnose biphasic mesothelioma. It is not uncommon for some biphasic MM to have a desmoplastic component of less than fifty per cent, which can appear extremely benign and can be mistaken with reactive pleural fibroblastic reactive tissue.

Desmoplastic mesotheliomas are a sarcomatoid mesothelioma with a predominance (>50 percent) of dense collagenous stroma and haphazardly arranged slit-like spaces made up of cells with slightly atypical nuclei. The expression of pancytokeratin is focally and haphazardly distributed. This form is poorly vascularized and the vessels are haphazardly distributed, compared to highly vascularized organizing pleuritis with vessels disposed perpendicular to the surface. The diagnosis is especially difficult with reactive processes and should be made very circumspectly on a closed needle biopsy.

**Immunohistochemistry and mesothelioma diagnosis.** The most important point in mesothelioma histologic diversity is that there is sufficient overlap with other tumors and reactive conditions that immunohistochemistry is mandatory to make a diagnosis. Immunohistochemistry also allows recognition of deeply invasive tumor that is otherwise not histologically apparent particularly in desmoplastic mesothelioma. Diagnosis of epithelioid MPM requires the use of two positive mesothelial markers (nuclear markers such as anti-calretinin and anti-WT1 or the membrane marker anti-EMA, or anti-CK5/6, antiD2-40 (podoplanin), anti-mesothelin, etc…) and two negative markers that stain carcinoma but not mesothelioma. While the full discussion about the use of antibodies in pleural tumor diagnosis is beyond the scope of this article, the general concept is that these latter should include both antibodies that stain epithelial tumors in general (CEA, Ber-EP4/ MOC31) as well as antibodies that will stain specific subsets of epithelial tumors (TTF-1, lung adenocarcinoma, ER/PR, breast cancer).

For sarcomatoid and desmoplastic mesothelioma, it is necessary to use two broad-spectrum anti-cytokeratin antibodies (negative immunostaining with a single antibody does not exclude the diagnosis), and two markers with negative predictive value which stain other sarcomas (such as anti-CD34, anti-BCL2, anti-desmin, anti-S100) to confirm the diagnosis. Sarcomatoid mesothelial tumors generally don’t stain for the mesothelioma markers seen in epithelioid tumors, thus diagnosis is commonly one of exclusion and requiring close clinical correlation. With regard to atypical mesothelial hyperplasia (mesothelial proliferations of undetermined malignancy), there are currently no commercially available immunohistochemical markers that will identify the benign or malignant nature of the cells. As the distinction is made on depth and pattern of invasion, it speaks to
the importance of an adequate biopsy. Precursor lesions to mesothelioma are not well defined, either histologically or molecularly.

Molecular Classification of Malignant Mesothelioma

As discussed above, MPM classification is presently based on histology according to three main subtypes: epithelioid, sarcomatoid, and biphasic. However, large-scale molecular studies such as high-throughput genomic, epigenetic or transcriptomic analyses have demonstrated marked tumor heterogeneity among patients independently of the histological subtype. Biological diversity of MPM is also underlined by the difficulty to define a single specific biomarker. Indeed, MPM displays different sensitivities to different anti-cancer drugs in preclinical assays and in clinical trials. Although multiple patterns of each histological subtypes have been described, leading to a more complete histological classification, the establishment of a molecular classification of MPM would have diagnostic, prognostic and therapeutic interests.

Classification Mesothelioma Based on Molecular Data

Growing evidence leads to consider a given tumor type as a heterogeneous group characterized by distinct molecular alterations. Molecular classification is now available for several tumors such as hepatocellular carcinoma or breast cancer. However, only few studies have proposed a classification based on molecular data, and linked to clinical characteristic in MPM. A first classification has been based on transcriptomic profile, defining two subclasses by unsupervised hierarchical clustering, using the 1405 genes with the most variable expression across all samples. The two potential subclasses of mesothelioma were correlated loosely with tumor histology. Previously, a transcriptomic classification of twelve MPM cell lines also showed to also separate MPM based on their histological subtypes. A second classification has been based on methylation profile where MPM were divided into two groups, a high methylation group and a low methylation group, using 445 genes commonly methylated in more than one-third of MPM cases. Survival of patients with a low methylation level was significantly longer than in those with high methylation. A third classification has been also based on methylation profile and seven subclasses were identified by unsupervised hierarchical clustering using the 750 most variable autosomal CpG loci, and a β mixture model. MPM subgroups were characterized by different clinical outcomes and methylation class membership was also significantly associated with lung tissue asbestos body burden. Future studies aiming to establish a molecular classification of MPM would need to take into account the different molecular aspects of MPM diversity that include chromosomal abnormalities, gene
mutations, epigenetic alterations, gene expression changes and more generally signal pathway deregulation. So far, few integrative approaches have been performed in MPM.

**Molecular Alterations in Malignant Mesothelioma**

MPM are characterized by numerous chromosomal abnormalities involving alterations in both chromosome number and structure. Comparison between recent studies with high-throughput analyses, such as comparative genomic hybridization array or single nucleotide polymorphism array, allowed localizing recurrent regions of chromosomal alterations. Some of these recurrent regions have been linked to short-term (less than 12 months) disease recurrence after surgery, such as deletion in 9p21 or to asbestos exposure such as loss in 14q11.2–q21, but no attempt was performed to classify MPM based on these genomic data.

A limited number of tumor suppressor genes have been described to be recurrently mutated in MPM while no recurrent oncogene has been identified. Mutations in *BAP1* (BRCA1 associated protein-1), *CDKN2A* (cyclin-dependent kinase inhibitor 2A), *CDKN2B* (cyclin-dependent kinase inhibitor 2B) and *NF2* (neurofibromin 2) have been reported in a high percentage of MPM, and *TP53* (tumor protein 53) has been found mutated at a lower rate in comparison with other human cancers. Recently, frequent genetic alterations in *LATS2* (large tumor suppressor, homolog 2) were also observed, but not yet confirmed. So far, publications of large mutational analyses did not discuss any link between mutations and MM classification. In our laboratory gene mutation sequencing of a series of MPM in culture from our MesoCellbank (about seventy cases) did not permit discrimination of MM subclasses based on gene mutations. Recently, the first studies describing large genome or transcriptome sequencing of MPM have been published. The low number of tumor samples sequenced prevents drawing definite conclusions. Ongoing whole genome or exome sequencing projects may allow identifying new recurrent genetic alterations in MPM, and will be useful to define molecular MPM subclasses.

Earlier epigenetic studies exploring DNA methylation of transcriptional promoters of key genes pointed out mesothelioma heterogeneity. High throughput methylation analyses confirmed this heterogeneity and two classifications have been proposed as mentioned above. MiRNA expression pattern could also be used to classify tumors. However, so far, large scale miRNA studies have mainly focused on the differences between MPM and normal pleura or others tumor types. Down-regulation of miR-17 and miR-30c in sarcomatoid MPM, and upregulation of miR-29c in epithelioid MPM have been significantly associated with better patient’s survival, indicating that miRNAs expression should be taken into account to establish MPM subclasses.

Several transcriptomic studies were performed on MPM mainly looking for diagnostic or prognostic biomarkers. Several studies separated MPM in several clusters using unsupervised hierarchical clustering. These studies were
performed either in cell lines or in tumor samples. However, they did not report any difference in clinical characteristics or metabolic and signaling pathways between the different clusters. Deregulation of numerous signaling pathways related to differentiation, survival, proliferation, apoptosis, cell cycle control, metabolism, migration, and invasion has been shown in MPM.

Mesothelioma Therapy

To date, the therapeutic strategies for mesothelioma are limited. The standard first-line chemotherapy (cisplatin-pemetrexed) allows an increase of about 3 months in median survival. Radiotherapy, surgery or multimodal treatments currently being developed have not made substantial improvements. Radical surgery is limited to a small number of patients. Nevertheless, molecular studies in mesothelioma have emphasized the role of regulatory molecules in mesothelioma pathogenesis, offering a number of proteins and signaling pathways that could be targeted to abolish mesothelioma growth or induce cell death.

Targeted Therapies

Thyrosine kinase inhibitors

Epithelial growth factor receptor (EGFR): EGFR plays a role in tumor cell proliferation, differentiation, migration, adhesion, and survival. It is overexpressed in more than half of MPM. However, clinical trials testing single-agent EGFR tyrosine kinase (TK) inhibitors (gefitinib or erlotinib) have failed to show any response in MPM patients. Potential explanations include rare EGFR mutations in mesothelioma, the controversial prognostic value of EGFR over-expression in MPM, and significant cross-talks between the EGFR pathway and other receptor signaling pathways such as c-MET receptor and IGF-1R pathways.

Vascular Endothelial Growth Factor (VEGF/VEGFR). VEGF and VEGFR are overexpressed in mesothelioma. High circulating levels of VEGF found in MPM patients positively correlate with microvascular density and the stage of the tumor, and are associated with a poor prognosis. Finally, MPM cell growth is inhibited by anti-VEGF antibodies. Several phase II trials have been performed. Combination of erlotinib and bevacizumab did not improve the median progression-free survival (PFS) or median overall survival. Similarly, a cisplatin-gemcitabine and bevacizumab combination showed no improvement compared to the same chemotherapy alone. However, patients with low plasma VEGF level prior to treatment had a longer PFS. Cisplatin, pemetrexed, and bevacizumab failed to improve PFS rate in comparison with historical controls treated with cisplatin and pemetrexed, in a small number of patients with advanced malignant mesothelioma. Results from a larger
ongoing randomized phase II/III trial (“MAPS”) comparing cisplatin and pemetrexed with or without bevacizumab as first-line treatment in inoperable MPM patients may permit more confident conclusions.

**Other tyrosine kinase inhibitors.** Valatanib (VEGFR, PDGFR, and c-Kit TK inhibitor), cediranib (pan-VEGFR and PDGFR TKI) inhibitor, semaxanib (inhibitor of VEGF-1R, PDGFR and c-Kit inhibitor) provided limited or negative results in phase II trials. Similar results were found with Imatinib, a selective inhibitor of c-kit, PDGFRs and of mutated bcr/abl tyrosine kinase in phase II studies in refractory or chemonaive MPM patients. Furthermore, trials assessed imatinib combined with cisplatin and Pemetrexed or gemcitabine with negative results. As dasatinib (BCR/ABL and Src family tyrosine kinase inhibitor) exhibited cytotoxic effects in mesothelioma cell lines, two phase II trials tested dasatanib as neoadjuvant treatment in operable MPM patients, or as monotherapy in inoperable patients, had also negative results.

**Other Targeted Agents**

**Histone Deacetylase Inhibitors (HDACi).** Inhibition of histone deacetylases results in acetylation of histone proteins and in expression of genes potentially associated with cell cycle arrest, apoptosis and tumour suppression. HDACi also lead to acetylation of non-histone proteins leading to other anti-cancer effects such as inhibition of angiogenesis, motility and invasion of tumour cells. Many specific or pan HDACi have been tested in MPM, including suberoylanilide hydroxamic acid (SAHA/vorinostat), panobinostat or valproic acid (VPA), alone or in combination with chemotherapy. Vorinostat failed to show any overall survival improvement in a phase 2/3 trial and Belinostat did not show better response. In vitro data have suggested that VPA had a pro-apoptotic effect in MPM, which was synergized with chemotherapy, including doxorubicin. A phase II study using VPA combined with doxorubicin after at least one chemotherapy (platinium-pemetrexed) has shown encouraging results.

**Proteasome inhibitor.** The Bortezomib inhibitor exhibited cytotoxic effect in preclinical models of mesothelioma. Limited results were found in phase II trials.

**Immunotherapy**

Although MPM is not recognized as a typically immune therapy responsive cancer as is for example melanoma, several studies relate the existence of both humoral and cellular immune responses associated with mesothelioma. First, antibody responses have been demonstrated in some patients, whose prognosis seemed favorable. Second, lymphocytic infiltration of the tumors has been observed in MPM, and a good correlation has been noted between prognosis and the presence of the lymphocytes. Other evidences concern the spontaneous regression associated with infiltration by lymphocytes, and recent other investigations demonstrating a
correlation between the lymphocyte infiltration and better prognosis. Together these studies highlight the interest of immunological strategies to treat MPM.

Immunotherapy consisting in cytokine or interleukin treatments was comparable in terms of therapeutic efficacy, to those currently reported for chemotherapy treatments. In fact, even though some treatments that activate the immune system using cytokines appear to be well tolerated, especially those acting in the intrapleural cavity, a high degree of toxicity was observed. Thus, these therapeutic approaches have been now abandoned.

Antibody based immunotherapy was also investigated. Mesothelin is expressed at the cell surface of mesothelial cells but is overexpressed by several cancers, including epithelioid MM. Mesothelin antibodies (humanized monoclonal antibody, MORAb-009 and a recombinant immunotoxin, SS1P) was evaluated to treat mesothelioma. Stable disease, but no response, was obtained in phase I trials testing SS1P and MORAb-009 in mesothelioma patients. Anti-EGFR monoclonal antibodies (cetuximab) have potent anti-MPM activity both in vitro and in vivo, notably through the immunologic mechanism of antibody-dependent cellular cytotoxicity (ADCC).

A therapeutic strategy was proposed in the early 2000’s to treat MPM by vaccination, based on the injection of activated autologous dendritic cells loaded with tumor antigens. This tumor immunotherapy combined with chemotherapy provided encouraging results in a Phase I clinical study. Other approaches under investigations are dead cancer cells expressing danger signals or autologous tumor cells, with recombinant granulocyte macrophage colony stimulating factor (GM-CSF).

Gene therapy

Mesothelioma is a compartmentalized tumor that remained in the pleural cavity until late in its course and is easily accessible for in vivo gene delivery. Despite these advantages, few clinical studies based on genetic therapy have been carried out for the treatment of mesothelioma. Intrapleural instillation of replication-deficient, recombinant adenovirus was used to deliver into the tumor cells the herpes simplex virus thymidine kinase suicide gene, which induced cell death when combined with ganciclovir, an antiviral drug. Preclinical data then early phase clinical trial validated this approach. The introduction of viral vectors into the intrapleural cavity allowing the production of immune-activating cytokines (IL-2, IFN-gamma, IFN-beta) at the site of tumor development (intra-pleural injection), has been performed in MPM. Recently a new approach involving virus vaccines was initiated. Indeed, the measles vaccine is actually in a clinical trial (Mayo clinic), related to the previous pre-clinical data obtained in vitro.

Conclusions
Targeted therapies, alone or in combination with standard treatments chemotherapy regimens, exhibited limited value so far in clinical trials for MPM. However a multidisciplinary approach and the compulsory development of new tools predicting the response to treatment (biomarkers, imaging...) may guide the use of such targeted therapies in the future, more likely in combination with standard treatments (chemotherapy, surgery...), improving the management and outcome of MPM patients. The recent international iMig meeting held in Boston in September 2012, clearly stated that immunotherapeutical strategies for the treatment of mesothelioma should previously consider immunomodulatory cell populations present in the tumor of the patients.

Animal and In vitro Models of Mesothelioma

Pre-clinical investigations allow the study the of mechanism of neoplastic transformation of mesothelial cells, and to test the efficacy of anti-cancer drugs and therapeutic strategies that may bring proof of concept. Several “models” of mesothelioma have been developed in rodents to obtain mesothelioma samples and neoplastic cells in culture. Moreover, human MPM tumor samples have also been used in this context. A flow-chart summarizes the different procedures (Figure 3). Schematically, there are three main routes to obtain mesotheliomas: exposure of rodents to asbestos fibers, xenografts of mesothelioma cells, and animal gene engineering, focusing on genes altered in human mesothelioma, without asbestos exposure (“spontaneous” mesotheliomas).

Asbestos-induced mesotheliomas in rodents

Mesothelioma can be obtained in conventional rodents, rats or mice, or genetically modified mice to asbestos fibers, after injection of asbestos fibers in either the pleural or the peritoneal cavity, and in the peritoneal cavity in mice. (Figure 3A). This model is difficult to exploit for drug testing, because of the long delay (several months) to obtain mesotheliomas. However, the asbestos-induced mesotheliomas can be minced into small fragments, and mesothelioma cells can be grown in culture and further used. Similarly, cells from pleural effusions or ascites can be cultured (Figure 3A). These cultures can be transplanted into relevant animals using subcutaneous or intra-cavitary injections. Rodent cells may be transplanted into syngenic animals if appropriate, or in immunodeficient animals when the cells originate from outbred sources. In the literature, a few cell lines are currently used, especially from mice. In rats, one cell line, IL-45, derived from a chrysotile-induced peritoneal mesothelioma in Fisher 344 rat. This cell line has been used to screen the cell response to growth-inhibitory agents, or for mesothelioma imaging. Several cell lines, have been obtained from mice. AC29 is a murine MM cell line developed from asbestos-induced malignant MM in CBA/CAH (H-2k) mice, and AB1 and
AB12 are derived from asbestos-induced MM in Balb/c mice. The morphology of the asbestos-induced tumors has been studied, demonstrating similarities with human mesothelioma. These models have already been used to evaluate the efficacy of chemical anti-cancer agents, as well as the mesothelioma cells response to immunotherapies.

“Spontaneous” mesotheliomas in genetically engineered mice

More recently, genetically modified mice (GM mice) have been exposed to asbestos using similar methods to investigate mesothelioma pathogenesis associated with asbestos exposure, and the mesothelioma cells have been used for mechanistic studies (gene alterations, allelic imbalance, changes in gene expression) (Figure 3B). So far, according to our knowledge, they have not been used for preclinical purposes. These studies have been performed with several types of mice carrying a heterozygous mutation in tumor suppressor genes. *Trp53*+/– mice carried a heterozygous mutation in *Trp53*, the gene orthologous to the human gene *TP53*. These p53-deficient mice show accelerated development and progression of asbestos-induced peritoneal malignant mesothelioma. As the rate of *TP53* mutations is not high in human mesothelioma, other studies have focused on *NF2*, a gene inactivated at a frequency of about 60% in human mesothelioma. Heterozygous *Nf2*+/– mice developed mesotheliomas at a much higher frequency than unmodified mice, but the delay between exposure and mesothelioma occurrence was not substantially modified. Histological studies have found that the sarcomatoid type of mesothelioma was the most frequent.

MexTAg mice, is another type of mice engineered by insertion of a coding sequence of SV40 TAg cloned downstream of the mesothelin promoter. Then, mesothelial cells express the monkey virus (SV40 large T antigen), a protein inhibiting both p53 and pRb. This construct was based on the hypothesis that the simian SV40 virus has a role in the development of mesothelioma. While no spontaneous mesotheliomas were seen, targeted expression of the TAg transgene causes mesothelioma to develop more rapidly after asbestos exposure in MexTAg wild-type mice compared to wild-type mice.

Several other GM mice have been employed with the aim to generate mesotheliomas in the pleural cavity without exposure to asbestos fibers. The so-called “spontaneous” mesotheliomas have been obtained from mice according to a strategy inactivating several tumor suppressor genes. Three murine genes orthologous of genes frequently inactivated in human mesothelioma, *Nf2, Cdkn2a/p16* and *Cdkn2a/Arf* have been selected, as well as *Trp53* that shows a lower mutation rate in mesothelioma but plays an important role in carcinogenesis generally. Generation of heterozygous or homozygous gene deletion was made using recombination systems based on Cre/loxP strategy. Gene inactivation was achieved by injection of adenoviral-mediated delivery of Cre
recombinase (AdCre) in the pleural cavity. However, in mice, the growth of tumors in the pleural cavity is difficult to follow because of the lack of symptoms allowing detection of tumor occurrence. To counteract these drawbacks, these mice were crossed to LucRep mice carrying a conditional reporter luciferase transgene which is expressed as the targeted genes are inactivated. With these engineered crossed mice, it is possible to follow tumor growth and growth inhibition by bioluminescence, after intra-peritoneal injection of luciferin.

Spontaneous MM in the absence of asbestos exposure has been generated in both heterozygous and homozygous double mutants for Nf2 and Trp53, Nf2 and Ink4a/Arf, and for triple mutants Nf2, Trp53 and Ink4a/Arf and mice. MM developed rapidly and at a high incidence. The highest rate of tumor incidence (100%) was found in triple homozygous mutants Nf2<sup>-/-</sup>;Trp53<sup>-/-</sup>;Ink4a/Arf<sup>-/-</sup>. The lowest rate (34%) was observed in Nf2<sup>+/−</sup>;Ink4a/Arf<sup>-/-</sup> mice with a median survival of 30 weeks. Murine MM closely mimicked the human disease characterized by peritoneal ascites, a long latency between fiber injection and MM development, and histological subtypes, epithelioid, sarcomatoid and biphasic.

**Mesothelioma Models Derived from Human Cells**

Studies with human cells can be performed with mesothelioma cells collected from pleural effusions or ascites, or from finely minced tumor samples, and expanded in culture conditions (Figure 3C). Several types of cultures can be used. In the classical 2D method mesothelioma cells grow attached to the culture support. In the 3D method mesothelioma cells are prevented from attaching to the culture support (polyHEMA-coated support) or are grown in agar (semi-solid medium); they form micropapillary-like structures of epithelial cells and spheroidal aggregates or spheroids. This procedure is interesting as these models reconstitute morulas and clusters of mesothelioma cells seen in pleural and peritoneal liquids, and the tumor microenvironment when made from tumor samples.

**Conclusions**

The question of the choice pre-clinical models is critical. This has been recently debated at the 2012 iMig meeting. Among solutions none is fully satisfactory. Either human cells are used, but they are transplanted in immunosuppressed mice, escaping the immunological survey, or immunocompetent mice are used, but murine mesothelioma cell have to be transplanted. Spontaneous mesotheliomas are of interest. Nevertheless, a great number of different engineered mice would be necessary to represent the genetic diversity of mesothelioma. In this context, the cellular models remain of great interest regarding the heterogeneity that is seen among types of mesotheliomas and even within an individual cancer. With cell systems, the response to drugs can be studied with well characterized human cells, knowing their morphology and that of the original tumor, the molecular
status of critical genes or more wide genetic data, and some physiological and metabolic changes. This knowledge will permit to better understand the link between the drug and cell response, and to help performing a targeted treatment more adapted to a given type of tumor cells. To have more powerful systems, it is needed first to increase our knowledge of the heterogeneity of the molecular and physiological characteristics of mesothelioma cells, and second to develop more sophisticated in-vitro systems allowing for instance dynamic studies and reconstruction of the cell microenvironment.
Legend to Figures

Figure 1:
(A) CT image showing a thickening of the parietal and mediastinal pleura. (B) Tumor extending into the fissure.

Figure 2
(A) Epithelioid mesothelioma invading the visceral pleura (low power view).
(B) Immunohistochemical analysis showing strong nuclear staining with calretinin antibodies.
(C) High power view of an epithelioid mesothelioma with micropapillary architecture. Calretinin staining.
(D) Diffuse membranous staining with EMA antibodies of an epithelioid mesothelioma with micropapillary architecture.
(E) Sarcomatoid mesothelioma showing a fibroblastic type architecture. Few mitosis are present.
(F) Immunohistochemical analysis of a sarcomatoid mesothelioma showing a diffuse staining with anti-AE1/AE3 antibodies.
(G) Low power view of a parietal pleura showing diffuse transmural spindle cells proliferation invading the adipose tissue.
(H) High power view highlighting the diffuse cytokeratin expression of the spindle cells present in the adipose tissue. AE1/AE3 antibodies.
(I) Biphasic mesothelioma showing a mixture of epithelioid and spindle cells.
(J) Atypical mesothelial hyperplasia lining the serosal cavities, made of large epithelioid cells loosely connected between themselves and showing atypical nuclei.

Figure 3:
Flow-chart summarizing the different procedures to obtain mesothelioma cells and mesothelioma cultures for pre-clinical studies. (A) Cells from serosal effusions can be cultured and transplanted into relevant animals using subcutaneous or intra-cavitary injections. (B) Mesothelioma can be obtained after injection of asbestos fibers in the pleural or the peritoneal cavity of conventional rodents, rats or mice, or genetically modified mice. Mesothelioma cells from serosal effusions can be grown in culture, or from finely minced tumor samples. (C) Human mesothelioma cells can be collected from pleural effusions, or from finely minced tumor samples, and expanded in culture. Both classical 2D cultures and 3D cultures of mesothelioma cells can be made. In 3D cultures, mesothelioma cells form micropapillary-like structures of epithelial cells and spheroidal aggregates or spheroids.
REVIEWS


EPIDEMIOLOGY


CLINICAL ASPECTS

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**MORPHOLOGICAL CLASSIFICATION**


**MOLECULAR CLASSIFICATION**


MESOTHELIOMA THERAPY


**ANIMAL AND IN VITRO MODELS**


A

Asbestos

Rat (I.Pl; I.Pe)  Mice (I.Pe)

Serosal effusions

Cultured cells

Syngenic rat/mice

Mesothelioma

B

Asbestos or no asbestos

GM Mice

Pleural fluid

Cultured cells

Immuno-suppressed mice

Subcutaneous or intra-cavitary inoculation

Mesothelioma

C

Human

Pleural fluid

Mesothelioma

Cultured cells

2D - 3D cultures

Immuo-suppressed mice

Subcutaneous or intra-cavitary inoculation

Mesothelioma