The Molecular Basis of Malignant Pleural Mesothelioma

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KEYWORDS

• Mesothelioma • Molecular • Genetic • Gene expression • Epigenetic

KEY POINTS

- Malignant pleural mesothelioma (MPM) is highly heterogeneous at the molecular level, leading to challenges in diagnosis, prognosis, and treatment.
- MPM is associated with asbestos exposure in greater than 80% of cases. Mechanisms of asbestos-associated tumorigenesis include reactive oxygen species, chronic inflammation, direct cytotoxicity, and cytokine and growth factor dysregulation.
- Epigenetic hallmarks of MPM include widespread chromosomal loss and aberrant gene methylation, although these patterns are complex and variable.
- MPM is characterized by the presence of fewer protein-altering somatic point-mutations compared with other cancers. Key mutated genes include tumor suppressors *BAP1*, *NF2*, *CDKN2A/B*, *TP53*, and *SETD2.*
- Integrated multi-omic analyses identify up to 4 distinct clusters of MPM, with 2 extreme epithelioid-like and mesenchymal-like groups separated by molecular gradient along the epithelial-to-mesenchymal transition spectrum.

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a rare but aggressive cancer associated with asbestos exposure in greater than 80% of cases.[1] It is almost uniformly lethal, and although decreasing use of asbestos has led to a plateau of incidence in Western countries, a long latency period after exposure combined with continued global asbestos use makes MPM an ongoing area of concern.[2] MPM is classified into 3 histologic subtypes: epithelioid, sarcomatoid, and biphasic. Epithelioid histology confers the most favorable prognosis and sarcomatoid the least, and a greater proportion of epithelial differentiation in biphasic tumors correlates with longer survival.[3,4]

At the molecular level, MPM is a highly heterogeneous disease both between patients and within individual tumors.[5–8] Intratumor heterogeneity can be further conceptualized as a combination of longitudinal (change over time) and spatial (among samples of the same tumor) heterogeneity.[7] The broad molecular variation seen in MPM and its microenvironment poses a significant challenge in diagnosis, prognostication, and treatment of this devastating disease. Although advances in molecular oncology have led to effective novel therapeutics for several solid organ cancers, first-line medical therapy for MPM in the form of cytotoxic combination cisplatin/pemetrexed-based chemotherapy has remained unchanged for decades.[9,10] The application of surgery and hemithoracic radiation in multimodal approaches

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prolongs survival only in a subset of patients, and the ability to accurately predict patient response to any form of treatment is limited.[9,11]

Next-generation sequencing technology and novel computational techniques combined with international collaborative efforts have resulted in greater understanding of the molecular basis of MPM. This article describes current molecular mechanisms behind MPM tumorigenesis, reviews the epigenetic, genetic, and transcriptomic hallmarks of MPM, and discusses the implications of advances in MPM molecular biology in clinical practice.

CURRENT EVIDENCE AND RESEARCH

Tumorigenesis

MPM arises from malignant transformation of the mesothelial cell monolayer on the surface of the parietal pleura. Approximately 70% to 90% of cases are associated with exposure to asbestos fibers.[12,13] Most research into asbestos as a cause of MPM relies on self-report of exposure, and quantitative data on the relation of asbestos exposure to mesothelioma risk are rare.[14]

Asbestos, unlike chemical carcinogens, exerts its effects over a long period, which is consistent with the 10- to 40-year latency period between estimated exposure and MPM diagnosis.[15] There is debate over the specific mechanisms through which asbestos causes mesothelioma.[6] Implicated pathways include generation of reactive oxygen species (ROS), direct cytotoxicity, kinase-mediated signaling, chronic inflammation, and cytokine and growth factor dysregulation.[6,13,16] A small subset of MPM tumors exhibit a distinctive widespread loss of heterozygosity, which may be consistent with spindle damage induced directly by asbestos fibers as well.[2] It is also likely that these mechanisms overlap and that no single pathway can be identified as a sole sufficient cause of malignant transformation.

Reactive oxygen species

Asbestos fibers generate ROS both directly and indirectly (eg, through immune-mediated inflammation). These asbestos fibers in turn lead to epigenetic and somatic genetic changes in mesothelial cells.[17] At the epigenetic level, increased ROS causes altered DNA methylation.[17] In vitro treatment of Met5A cells with asbestos, for example, resulted in significant methylation changes in CpG islands located in the promoter regions of genes involved in migration/cell adhesion. However, no correlation between changes in methylation and expression of these genes was observed except for a significant inverse correlation with *DKK1*, whose protein is an antagonist of the Wnt/β-catenin signaling pathway.[18] Interestingly, in the absence of asbestos, in vitro treatment of MPM cell lines with exemestane, an aromatase inhibitor that generates ROS, had an antiproliferative effect.[19]

Downstream effects of environmental stress: mutagenesis and failure of DNA repair

MPM has a relatively low rate of somatic mutation compared with other solid cancers.[5] However, whether through ROS or other molecular mechanisms, asbestos fibers are clearly mutagenic at the chromosomal and gene levels in both in vitro and in vivo models, leading to tumorigenesis.[16,20] These damage patterns are consistent with known frequent alterations in DNA repair genes.[5] In contrast, asbestos-induced pleural thickening and plaques are produced by changes in gene regulation secondary to inflammation and ROS without mutagenesis,[16,21,22] suggesting that mutagenesis is a key step in development of asbestos-induced MPM. However, whether there is a threshold of asbestos exposure below which cancer does not develop remains controversial and likely depends on patient-specific genetic factors.[16,23]

Although chronic inflammation is implicated in MPM development, the role of inflammation-related genes in development of MPM following asbestos exposure is still controversial. Crovella and colleagues[23] investigated the role of 93 genetic variants in 12 genes encoding inflammasome and iron metabolism proteins in relation to the number of asbestos bodies (ABs), considered a hallmark of asbestos exposure, in 81 patients who died of MPM. Although there was no association between the number of ABs and most of the selected genes, the frequency of the single nucleotide polymorphism rs12150220 A/T (17p13.2) in the NLRP1 gene correlated with a significantly lower number of ABs, suggesting that NLRP1 inflammasome may contribute in the development of lung ABs. A subsequent analysis by the same group found no association between polymorphisms in *NLRP1* or *NLRP3* and susceptibility to MPM in asbestos-exposed individuals.[24]

Nonasbestos causes of malignant pleural mesothelioma

Despite the myriad of pathways through which asbestos can cause MPM, the risk of developing MPM among high-risk individuals with industrial asbestos exposure is only ~5%.[25] Other factors have been associated with mesothelioma: nonasbestos mineral fibers (eg, erionite, fluoroedenite, carbon nanotubes), therapeutic radiation, chronic

pleural inflammation, and (in rare cases) germline genetic mutations.[12] Apart from germline mutations, a definitive molecular signature to differentiate these causes has not yet been developed.

Epigenetics

Chromosomal losses

Aneuploidy, particularly chromosomal loss, is an epigenetic hallmark of MPM.[7,26–28] However, the copy number alteration (CNA) profiles of individual tumors are complex.[29] In one analysis of CNAs in 53 primary MPM tumor samples, 77% demonstrated a predominance of losses and 23% a predominance of gains.[30] The most common losses are in 1p, 3p14-p21, whole chromosome 4, 6q, 9p, and 22q.[7,26,27] None of these losses individually predominate in MPM. The frequencies of common losses include 9p21 (34%), 22q (32%), 4q31-32 (29%), 4p12-13 (25%), and 3p21 (16%).[28] These regions contain some of the most commonly mutated genes in MPM, including *BAP1* (3p), *CDKN2A* (9p), and *NF2* (22q).[7]

In addition to large chromosomal losses, focal losses have been described. For example, in the Guo and colleagues[27] series, deletion of 9p21 containing *CDKN2A/2B* was identified. In The Cancer Genome Atlas (TCGA) analysis,[2] focal copy number deletions were found to affect canonical MPM tumor suppressor genes, including *CDKN2A* (>50% of samples) and *NF2* (>70%). Deletions of *CDKN2A* often involve the adjacent gene *MTAP*, which has been linked to increased sensitivity to pharmacologic inhibition.[2] Loss of *CDKN2A* was also associated with shorter overall survival.[2]

The TCGA analysis also identified a rare MPM subtype in a small number of tumors exhibiting genomic near-haploidization, absent alteration in *BAP1*, *PBRM1*, or *SETD2*, and universal inactivation of *SETDB1*. Women were overrepresented in this subtype (4 Females:1 Male), whereas histologic subtype showed no difference from the MPM cohort at large.[2]

Chromosomal gains

Although less common than losses, gains in some MPM chromosomal regions have been described. In one comparative genomic hybridization (CGH) analysis of 26 MPM tumors, 7 (27%) were found to have recurrent gains in 17q, involving known cancer-related genes, such as *MAP3K3*, *SMARCD2*, *ERN1*, and *PRKCA*.[31] Krismann and colleagues[28] examined 90 MPM cases using CGH and DNA cytometry, finding common gains in 8q22-23 (18%), 1q23/1q32 (16%), 7p14-15 (14%), and 15q22-25 (14%). An analysis of 41 epithelioid MPM revealed relative gains in the regions encompassing *KDM5A* (*12p13*), *DVL1* (1p36), and *MYC* (8q24) compared with peritoneal mesothelioma samples.[32]

Chromosomal alterations by histologic subtype

In the Krismann cohort,[28] aneuploidy was significantly less frequently in sarcomatoid samples (75%) and significantly more frequently in epithelioid samples (88%), although absolute differences were small. Imbalances were detected by CGH in 84% of all samples with an average of 6.2 defects per sample. Losses of chromosomal regions were twice as frequent as gains, consistent with observations in other studies.[30] Epithelioid MPM had distinct recurrent losses at several locations, including 3p21 (33% vs 16% in the whole cohort) and 17p12-pter (26%); sarcomatoid MPM had distinct recurrent losses at 7q31-qter (21%) and 15q (18%). Biphasic tumors demonstrated a CGH pattern consistent with a combination of the other 2 subtypes.[28]

Changes in gene regulation

Dysregulation of epigenetic control of tumor suppressor genes is also present in MPM, particularly hypermethylation.[33] In a high-throughput global screening analysis for aberrant DNA hypermethylation in 50 MPM specimens, an average of 6.3% of genes was found to be hypermethylated in MPM compared with 8.8% in lung adenocarcinoma.[34] Methylation patterns were distinct between the two tumors based on hierarchical cluster analysis, and three of the hypermethylated genes (*TMEM30 B*, *KAZALD1*, and *MAPK13*) were unique to MPM, suggesting a potential role for these genes as diagnostic markers.[34] Interestingly, four patients included in this study showed low levels of gene methylation and longer survival, suggesting that methylation may affect the progression of this disease. In addition, the number of methylated genes increased significantly in stages III and IV disease compared with stages I and II.[34] An analysis published in the same year by Christensen and colleagues[35] identified distinct epigenetic profiles between normal pleura and MPM. These data suggest a unique epigenetic landscape in MPM compared with other forms of thoracic disease.[33–35]

Several key genes mutated in MPM are involved in epigenetic regulation. For example, in the Bueno cohort,[5] 8% of tumors exhibited mutations in *SETD2*, which encodes a histone methyltransferase, often leading to loss of function. Mutations in the *SETDB1* and *SETD5* histone methyltransferase genes were also identified. The downstream effects of these and other mutations in genes involved in epigenetic programming have not yet

been fully elucidated. However, for example, *ITGA7* is a known tumor suppressor gene that may be epigenetically regulated, and decreased expression of ITGA7 has been associated with decreased overall survival in MPM.[36] Tsou and colleagues[33] evaluated 52 MPM samples using the MethylLight technique for 28 methylation markers and found significant changes in methylation in the *ESR1* (increased) and *APC* (decreased) loci, which are known to be involved in tumorigenesis. Similarly, tumors without DNA losses affecting *DNMT1*, a methyltransferase, exhibited higher average methylation indicating a significant change in the epigenetic landscape.[37]

Small noncoding microRNAs (miRNAs) also participate in posttranscriptional regulation of gene expression; irreversible alterations in miRNA expression are associated with cancer development.[17] miR-126 in particular is known to play a crucial role in MPM pathogenesis, where it fails to act as an oncosuppressor by inhibition of the PI3K/AKT pathway. Treatment with exogenous miR-126 under these circumstances results in tumor suppression in vitro.[17] Over the last decade, the biological activity of many other miRNAs has been associated with MPM, including in the roles of tumor suppressor (miR-16-5p and miR-193a-3p) and cellular function (miR-182-5p, miR-183-5p, miR-24-3p) (reviewed in Reid and colleagues,[38] 2020).

DNA Mutation Signatures

Somatic mutations

As previously described, MPM has a relatively low rate of protein-altering somatic point mutations compared with other solid cancers.[5] In a cohort of 74 MPM tumors, whole-exome sequencing confirmed an overall rate of less than two nonsynonymous mutations per megabase in all but one sample[2] and demonstrated that MPM had a lower rate of protein-altering mutations than many other cancers except thyroid carcinoma and acute myeloid leukemia.[5]

In the Bueno series, targeted (n = 103) and whole-exome (n = 99) sequencing of paired MPM tumors revealed an average of 24 ± 11 protein-coding alterations per sample with no significant differences between molecular subtypes.[5] Quetel and colleagues[39] demonstrated in 49 MPM primary cultures and in 35 frozen tumor specimens that mutations in MPM exhibit an enrichment in C > T transitions. A recent review summarizing massively parallel sequencing studies has observed that genetic variations tend to cluster in the TP53/DNA repair pathway and the PI3K/AKT pathway.[29] Recent high-throughput analyses have identified recurrent mutations in several genes, which underlie key features of MPM molecular biology.

Somatic mutations: BAP1 and other tumor suppressors

The main recurrent genetic alterations in MPM have been identified in tumor suppressor genes. The most frequently mutated gene in most series is *BRCA1-Associated Protein 1* (*BAP1*), which is located in 3p21 (a region frequently lost in MPM) and altered in up to 60% of tumors.[2,5,26,30,40,41] *BAP1* encodes a deubiquitinating enzyme involved in DNA repair, cell cycle, cellular differentiation, and DNA damage response.[42–44] BAP1 also promotes apoptosis in wild-type cells through deubiquitylation and stabilization of the IP3R3 channel.[45] Loss of nuclear BAP1 expression by immunohistochemistry (IHC) is currently used as a diagnostic marker in MPM. However, although loss of nuclear BAP1 staining can sometimes distinguish reactive versus neoplastic stroma particularly in biphasic tumors, BAP1 staining even within MPM is known to be heterogeneous.[46,47] There is also evidence that *BAP1*-mutant malignancies may be sensitive to epigenetically based therapies.[48] However, patient survival does not correlate with presence of *BAP1* mutation itself.[2] In addition, MPM patients with germline *BAP1* mutations have fewer chromosomal alterations than others.[2,49]

Beyond *BAP1*, frequently mutated tumor suppressor genes in MPM include *CDKN2A*, *CDKN2B*, *NF2*, and *TP53*.[2,5,26,50] Seven additional significantly mutated genes, *SETD2*, *ULK2*, *CFAP45*, *SETDB1*, *RYR2*, *DDX51*, and *DDX3X*, were identified in the Bueno cohort.[5] Mutations in *TP53* were absent in epithelioid tumors. In this cohort, patients carrying *TP53* mutation showed lower overall survival compared with patients with wild-type *TP53* ($P = .0167$). Another analysis of 49 MPM primary cell lines and 35 frozen tumors for 22 genes confirmed the high frequency of *BAP1*, *NF2*, *CDKN2A/B*, *TP53*, and *SETD2* mutations in MPM.[39]

Another gene frequently mutated in MPM is *LATS2*, a member of the *Hippo* signaling pathway.[39] An analysis found alteration in *LATS2* in 11% of 61 MPM primary cell lines.[51] Mutations in *NF2* gene, another member of the Hippo pathway, were found to co-occur with *LATS2* mutations in 8% of the cases. Although other studies did not report a high rate of *LATS2* mutation, large deletions of chromosome 13, where *LATS2* resides, may indicate potential loss of this gene and possible underestimation of the prevalence of *LATS* alterations.[51]

Germline mutations

Germline mutations have been identified in up to 7-12% of patients with MPM.[52–54] Pleural site in general is less frequently associated with germline mutations than other primary mesothelioma sites.[52] Few studies have shown that germline mutation frequency increases with decreasing age at diagnosis.[52,54] In addition, patients with germline mutations are less likely to report asbestos exposure, more likely to report a second cancer diagnosis, and more likely to have epithelioid histology.[52,54]

Pathogenic germline variants in MPM are often involved in DNA damage repair and chromatin remodeling pathways, and *BAP1* is the most frequently identified germline mutation.[52,54,55] Germline mutations in *BAP1* are known to predispose families to mesothelioma.[56] *BAP1* is also known to be frequently inactivated in cancers, such as uveal melanoma, clear cell renal cancer, and cholangiocarcinoma.[57] Taken together, loss-of-function germline mutations in *BAP1* constitute what is termed the familial BAP1 syndrome, including MPM, uveal melanoma, cutaneous melanoma, and other dermatologic tumors, as well as renal cell carcinoma and meningioma.[58,59] MPM patients with germline *BAP1* mutations almost always exhibit a second somatic *BAP1* mutation leading to likely complete loss of function.[53] Germline *BAP1* mutation is associated with less aggressive disease than sporadic MPM.[60]

Hassan and colleagues[53] investigated the impact of inherited loss-of-function mutations on survival in mesothelioma following platinum-based chemotherapy. In a cohort of 385 MPM patients, they found significantly longer overall survival following platinum-based chemotherapy in patients with any germline mutation, including *BAP1*, compared with patients without germline mutations (7.9 years vs 2.4 years, $P = .0012$). The benefit was comparable across all the genes under investigation. Interestingly, the effect of genotype was significant for pleural, but not peritoneal mesothelioma. In addition, there was no difference in tumor histology or reported asbestos exposure between the germline mutant and control patients. Overall, these results suggest that MPM patients with germline mutations in DNA repair and other tumor suppressor genes may benefit from platinum chemotherapy.[53] There is also evidence that the presence of germline mutations may predict sensitivity to PARP inhibition.[53,61]

Application of gene mutations to diagnosis

MPM subtypes may be difficult to distinguish from benign pleural proliferation and from other tumors, such as adenocarcinoma (for epithelioid MPM) and sarcoma (for sarcomatoid MPM).[62] No single IHC stain is diagnostic, and agreement among expert pathologists classifying histologically biphasic MPM is moderate at best.[63] Homozygous deletion of *CDKN2A* by fluorescent in situ hybridization (FISH) can be useful in distinguishing benign florid stromal reaction from sarcomatoid components of biphasic MPM tumors.[64] Because chromosomal losses of *CDKN2A* often involve the adjacent gene *MTAP*, and IHC for MTAP correlates well with *CDKN2A* FISH, it has been suggested that IHC for MTAP may be clinically useful in diagnosis of MPM.[2,63]

Characterization of Gene Expression

Recent advances in gene expression profiling have allowed for the simultaneous analysis of thousands of genes. Gene expression data have been applied across major cancer types to identify novel subtypes, predict outcomes, and define heterogeneity and the need for personalized treatments.[65]

Some of the first molecular MPM classifications were generated in the early 2000s primarily using microarrays.[66–69] Microarray data have also been used to identify candidate tumor-associated genes. For example, an analysis of miRNA dysregulation implicated *CDKN2A*, *NF2*, *JUN*, *HGF*, and *PDGF2A* as frequently affected in mesothelioma.[70] A subsequent meta-analysis of several sets of microarray data defined a list of potential novel biomarkers for MPM, including *PTGS2*, *BIRC5*, *ASS1*, *JUNB*, *MCM2*, *AURKA*, *FGF2*, *MKI67*, *CAV1*, *SFRP1*, *CCNB1*, *CDK4*, and *MSLN*.[71]

Several efforts have been made over the years to classify MPM tumors according to molecular characteristics. Gordon and colleagues[72] used expression arrays to analyze 40 MPM tumors as well as normal pleura, normal lung, and MPM cell lines. Unsupervised hierarchical clustering revealed two distinct groups of tumor samples that correlated loosely with tumor histology. Suraokar and colleagues[73] used microarray and pathway analysis to define three molecular subgroups of MPM, which correlated only partially with histologic subtypes.

Another analysis was published by de Reynies and colleagues[74] in 2014. This group investigated microarray profiles of 67 MPM cell lines and generated 2 MPM subclasses (termed C1 and C2) partially related to histologic type and closely related to prognosis. These clusters were characterized by the differential expression of epithelial-to-mesenchymal (EMT) genes with C1 expressing

an epithelial and C2 a mesenchymal phenotype. C1 was characterized by more frequent *BAP1* and *CDKN2A* mutations, whereas C2 contained all the sarcomatoid/desmoplastic samples among other subtypes of MPM. The investigators created a predictor tool to discriminate samples between C1 and C2 using the expression levels of three genes: *PPL*, *UPK3B*, and *TFPI*. This tool was then used to validate the C1/C2 classification in 108 MPM tumor specimens with epithelioid and biphasic samples in both C1 and C2, and sarcomatoid samples only in C2.[74]

In 2016, 211 MPM transcriptomes were characterized using unsupervised consensus clustering, and 4 distinct molecular subtypes of MPM were identified: epithelioid, biphasic-E, biphasic-S, and sarcomatoid.[5] These subtypes were associated to a degree with the spectrum from epithelioid-to-sarcomatoid histology. The 62% of histologically epithelioid samples classified into the biphasic-E, biphasic-S, or sarcomatoid clusters showed significantly lower overall survival than those in the epithelioid cluster, indicating that epithelioid MPM can be distinguished into multiple different molecular groups. Differential expression analysis revealed that gene expression in the four clusters was related to the EMT process, consistent with previous findings.[74] Furthermore, a simple ratio of two genes, *CLDN15* and *VIM*, was able to significantly differentiate the samples in the four clusters. Four (*SETD2*, *TP53*, *NF2*, and *ULK2*) of the most significantly mutated genes showed mutation rates significantly different between cluster E and clusters BE, BS, and S.[75] Pathways implicated in this integrated analysis included histone methylation (consistent with previous findings, eg, Goto and colleagues,[34] 2009), Hippo, mTOR, RNA helicase, and p53 signaling.

In 2018, TCGA performed integrated analysis of 74 MPM tumors, including epigenetic, exomic, and transcriptomic profiles.[2] Integrative clustering performed using two separate algorithms (iCluster[76] and PARADIGM[77]) identified four distinct subtypes of MPM in each. These subtypes were highly concordant, particularly with respect to the more extreme clusters 1 and 4. These two clusters correlated significantly with survival even when controlling for histologic subtype and deletion of *CDKN2A*.[78] Cluster 1 was enriched for epithelioid histology, whereas cluster 4 was enriched for sarcomatoid tumors similarly to the Bueno cohort.[5] Genes associated with EMT transition were again differentially expressed between clusters. In addition, each cluster was characterized by a distinct immune profile. In particular, cluster 1 expressed the checkpoint inhibitor gene *VISTA* at high levels.

In an effort to deconvolute the signatures of epithelioid and sarcomatoid-like cell populations within bulk MPM samples, Blum and colleagues[8] performed a meta-analysis using several publicly available datasets.[5,72,74,79,80] Initially, they used transcriptome data to classify 63 MPM samples into four distinct clusters (C1A, C1B, C2A, and C2B). Next, they compared the expression profile of each cluster with the previously published expression-based cluster data. They identified two highly correlated molecular groups among all datasets corresponding with the most extreme epithelioid and sarcomatoid subtypes. The intermediary tumors, however, did not form distinguishable clusters, and therefore, the investigators suggest they reflect a continuum, or gradient, between epithelioid and sarcomatoid tumors. A panel of 150 common genes was used to generate 2 different scores, termed *E*-score and *S*-score, to determine the relative epithelioid-like and sarcomatoid-like molecular components present in individual tumors. Increased expression of *UPK3B*, *MSLN*, and *CLDN15* was correlated with *E*-score and *LOXL2* and *VIM* with the *S*-score. Pathway analysis revealed correlation of the *S*-score with EMT, TP53 signaling, cell cycle, angiogenesis, and immune checkpoints. The increasing sarcomatoid component identified by *S*-score was associated with worse outcomes in each series individually as well as in aggregate.[8]

Clinical applications of gene expression

MPM can be challenging to diagnose. Pleural plaques are not diagnostic for mesothelioma, and as previously described, the different MPM subtypes may be difficult to distinguish from other thoracic tumors on a histologic basis alone.[62] In addition, efforts to develop molecular predictors of clinical outcomes in MPM date back to the early 2000s, corresponding with the rapid proliferation of novel and cost-effective sequencing technologies, but few are regularly used in practice.[69,74,81–83]

The gene ratio-based method, developed by the authors' laboratory, is able to overcome the difficulty of validating large gene signatures and offers improved clinical applicability.[75,84] Developed by comparing expression profiles between patients with different clinicopathologic parameters, these tests can then predict tumor characteristics or clinical outcomes based on a small number of genes.[66,75] With respect to diagnosis, Gordon and colleagues[84] used 181 tissue samples to develop a 6-gene 3-ratio test to differentiate MPM from adenocarcinoma with 99% accuracy. De Rienzo and colleagues[85] used microarray data for 113 assorted MPM, non-MPM malignant, and benign samples to develop a sequential

combination of binary gene-expression ratio tests in frozen tissues to discern MPM from other thoracic cancers, as well as to distinguish epithelioid from sarcomatoid MPM. Bruno and colleagues[86] used NanoString technology to develop and validate a diagnostic tool using 117 genes, of which 25 and 18 were upregulated and downregulated in MPM, respectively, as compared with benign mesothelial hyperplasia. Designed to work with small quantities of RNA, this test could be performed on formalin-fixed paraffin-embedded (FFPE) specimens.[86]

Similar strategies have been applied to prognosis. For example, a 4-gene 3-ratio (TM4SF1/PKM2, TM4SF1/ARHGDIA, COBLL1/ARHGDIA) test was developed to predict treatment-related outcome independent of histology based on real-time polymerase chain reaction expression data.[83,84] Although originally based on fresh-frozen tissue specimens, this score was later validated using FFPE tissue under Clinical Laboratory Improvement Amendments–approved guidelines in an independent multicenter cohort of MPM specimens.[85] It proved able to provide orthogonal risk information preoperatively, and, postoperatively, predict overall survival when combined with histopathologic information.

In addition to gene ratio tests, expression-based molecular subtype[74] and FAK protein expression[87] have been shown to correlate with sensitivity to the targeted agents verteporfin and defactinib, respectively. However, these and other targeted agents have not succeeded in clinical trials, and there are no current guidelines recommending their use.[20,29] Immunotherapy, although promising in several other cancer types, currently lacks biomarkers to predict efficacy in MPM because programmed death-ligand 1 (PD-L1) expression by IHC does not associate with treatment response.[88,89]

The immune microenvironment

Immunotherapy has expanded treatment options for tumors, such as melanoma and non–small cell lung cancer. Defining the immune microenvironment in MPM is an area of active investigation. In an early study, Burt and colleagues[90] demonstrated a significantly higher number of monocytes and tumor-infiltrating macrophages in nonepithelioid tumors. This study also found a significant association between higher monocyte counts and shorter survival. The checkpoint ligand PD-L1 is expressed in almost 40% of MPM tumors by RNA-seq, with significantly higher expression in sarcomatoid tumors.[5] Expression of CTLA-4, another checkpoint molecule, was found in varying levels in 56% of MPM tumor samples by IHC, and higher in the epithelioid subtype.[91] In contrast, serum levels of soluble CTLA-4 were higher in patients with sarcomatoid disease as measured by enzyme-linked immunosorbent assay.[91]

Expression of immune mediators can drive tumor biology. An analysis of 87 advanced-stage (III or IV) MPM tumors combining IHC for PD-L1 and NanoString analysis for 805 genes revealed PD-L1 expression in 16% of samples with significantly higher PD-L1 expression in sarcomatoid and biphasic samples.[92] Using hierarchical clustering by gene expression, these investigators identified 3 subgroups of MPM: one with moderate T-cell effector gene expression but high B-cell gene expression (*CD19*, *CD20*); one with high PD-L1 expression and high T effector/T regulatory cell gene expression (including *GZMA*/*GZMB*, *CXCL9*, *EOMES*, *FOXP3*, *ICOS*, *CTLA4*); and one "immunologically ignorant" group with low expression of immune compartment-related genes but high stroma-related gene expression, including *CTGF*, *DKK3*, *FN1*, *FAP*, *MMP2*, and several genes encoding collagen subunits.[92] Taken together, these results suggest heterogeneity in the interaction between MPM and the immune microenvironment that warrants further exploration.

SUMMARY AND FUTURE DIRECTIONS

MPM is a rare and aggressive cancer caused by asbestos exposure in most cases. It is characterized by heterogeneity not only at the histologic but also at the molecular level. Its hallmarks include widespread chromosomal loss, mutations in tumor suppressor genes, such as *BAP1*, *CDKN2A/2B*, *NF2*, and *TP53*, and diverse transcriptomic phenotypes leading to several distinct molecular clusters. These clusters are defined at the extremes by epithelial and mesenchymal characteristics, with a histopathologic gradient stratifying the tumors in between. Multiple groups are working to develop predictive scores to classify individual tumors into these different subtypes, which have prognostic significance and may help guide choice of therapy.

Indeed, despite substantial advances in understanding the molecular biology of MPM, to date there have been relatively few changes in standard clinical practice based on these findings. MPM continues to present a diagnostic challenge and is often advanced at the time of detection. Histology remains the primary tool of prognostication in terms of overall survival and selection of therapeutic approach. Blunt, cytotoxic chemotherapy remains first-line systemic treatment for a nuanced, recalcitrant, and biologically complex disease.

Ongoing work in MPM molecular oncology will focus on deconvoluting the biological pathways involved in MPM tumorigenesis, growth, interaction with the tumor microenvironment, and response to therapy. Single-cell and single-nucleus transcriptomics have led to meaningful discoveries in several other cancers and offer the opportunity to define the contributions of individual tumor and immune/stromal cells to bulk tumor signatures. These techniques also provide a means to dissect intratumor heterogeneity and evaluate whether there are significant differences between malignant cells within an individual tumor, and how these might affect clinical outcomes.

Building on ever-expanding large datasets, deep learning and other advanced computational techniques are being used to integrate clinical, histopathologic, and molecular data to refine diagnostic approaches and identify new prognostic biomarkers (Courtiol and colleagues, 2019). New fields of study, such as proteomics and metabolomics, have yet to be incorporated into many of these analyses but show promise and merit further exploration (Sato and colleagues, 2018; Tomasetti and colleagues, 2019). Finally, the development of unique molecular signatures for individual tumors will help guide treatment selection and identify approaches to meaningfully improve patient survival on an individualized basis.

Clinics Care Points

- More than 80% of MPM is caused by asbestos exposure.
- MPM in patients with germline mutations is less aggressive and more chemotherapy-responsive than sporadic MPM, but these patients have a higher incidence of multiple other cancers.
- Gene ratio tests can be useful in distinguishing MPM from other thoracic disease processes, as well as for predicting response to treatment and overall survival.
- Transcriptomic and integrated multi-omic analyses can stratify MPM into distinct molecular clusters, which associate to a degree with histology and have independent implications for outcomes.
- Biomarkers to identify candidates for targeted therapy or immunotherapy in MPM are currently lacking.

DISCLOSURE

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