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Review

Molecular pathways and diagnosis in malignant mesothelioma: A review of the 14th International Conference of the International Mesothelioma Interest Group

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ABSTRACT

The pathologist plays a central role in the diagnosis and management of malignant mesothelioma, including definitive tissue-based diagnosis in conjunction with clinical and radiographic data; diverse ancillary studies of diagnostic, prognostic, and predictive importance; and research efforts to better define the pathobiology of mesothelioma and develop novel clinical applications. The pivotal role of pathology in care of mesothelioma patients was on display at the recent meeting of the International Mesothelioma Interest Group (iMig) in Ottawa, Canada. This review summarizes the key findings of the "Molecular Pathways and Diagnosis in Malignant Mesothelioma" plenary session, including a large multi-institutional validation of a composite nuclear grading system for pleural mesothelioma, including incorporation of tumor necrosis as an additional independent prognostic factor; the correlation between nuclear grading in small biopsies and paired resection specimens in pleural mesothelioma; a multi-institutional study of important clinical and pathologic prognostic factors in peritoneal mesothelioma; the diagnostic role of HEG1 immunohistochemistry as a highly sensitive and specific marker of mesothelial lineage; the prevalence and diagnostic significance of MET protein overexpression in mesothelioma, as well as the correlation between MET protein overexpression and *MET* gene amplification; and the prognostic role of EZH2 protein overexpression in mesothelioma, together with data indicating an important pathogenic role for EZH2 in mesothelioma tumorigenesis. Special consideration is given to the convergence of diagnostic, prognostic, and predictive tools and their role in guiding highly personalized patient-centered management, and to the translation of novel research findings to practical techniques for routine pathologic practice.

1. Introduction

The International Mesothelioma Interest Group (iMig) (www.imig.org) holds a biennial meeting, at which clinical and research experts in the fields of medical oncology, surgical oncology, radiation oncology, radiology, and pathology convene to discuss advances in the field and promote consensus on rigorously derived, evidence-based standards of care for the diagnosis and management of malignant mesothelioma (MM). Pathologists have long played a central role in the diagnosis of MM, providing tissue-based diagnoses in conjunction with critical clinical and radiographic information. Increasingly, pathologists are asked not only to render a diagnosis of MM, but also to evaluate a wide array of histopathologic and immunophenotypic parameters with known prognostic or predictive importance. And with the increasing relevance of molecular-based testing for diagnosis, risk stratification, and guidance of patient management, pathologists are in a position not only to select tissue for molecular-based studies, but in many instances subspecialized teams of pathologists are also to develop, validate, perform, and interpret a wide battery of molecular tests. These manifold roles of pathologists were all on display at the 2018 iMig meeting in Ottawa, Canada. This report summarizes the advances in the pathology of MM that were presented and discussed in the "Molecular

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Pathways and Diagnosis in Malignant Mesothelioma" session of that meeting.

The iMig conference provides two invaluable opportunities to pathologists with expertise in MM: first, a forum in which to convene with other expert MM pathologists from around the world, to discuss matters of current diagnostic uncertainty, novel diagnostic and pathobiological insights, and future research directions; and second, the unique and important opportunity to convene with clinical colleagues across disciplines, to more precisely and productively define the role of the pathologist in the multidisciplinary team that cares for each MM patient. Indeed, numerous important contributions to the MM literature have been made through collaborative efforts that originated through iMig, including work among pathologists [1–3] and interdisciplinary projects [4–6].

The quintessential role of the pathologist as master of morphology-based diagnosis is as relevant today as ever. Certainly, fears that ancillary immunohistochemical, cytogenetic, or molecular studies would somehow obviate the essential role of tissue morphology in diagnosis and characterization of MM have not been realized. Histopathologic assessment of tumor tissue remains a rapid, cost-effective, and multifaceted tool for the pathologist's armamentarium. And far from being a settled field of study, MM histopathology remains an active, dynamic area of research, driven at least in part by the interplay between morphologic evaluation, novel pathobiological insights, and ancillary testing [7]. The correlation between cytomorphology, architecture, and prognosis in MM has become increasingly well defined in recent years, with publications detailing composite tumor grading systems that predict patient outcome [8,9]. Work to better define the role of MM grading in biopsy tissue is ongoing.

Compared to its counterpart in the pleura, peritoneal MM is rare, accounting for 10–15% of new MM cases. Given the rarity of peritoneal MM and the intrinsic challenges of managing relatively indolent yet inexorable intraabdominal disease, cases of peritoneal MM are widely referred to a small handful of tertiary centers with particular expertise. These factors have complicated confident pathologic characterization of peritoneal MM, with most studies to date limited to single institutions, examining one or few pathologic parameters.

While histomorphology takes a leading role in the diagnosis and characterization of MM, ancillary studies have become indispensable in all aspects of MM diagnosis, classification, and management. Immunohistochemistry (IHC) is a cost-effective technique with short turnaround time, and is conducive to in situ assessment of protein expression and localization. Because of its low cost and relative technical simplicity, many molecular findings are ultimately translated to more widely available IHC tests [10,11]. Among the most fundamental but most critical roles of IHC in MM is the verification of mesothelial lineage, particularly in high-grade epithelioid or sarcomatoid MM in which the morphologic differential diagnosis with high-grade or sarcomatoid carcinoma is challenging. New mesothelial immunomarkers are needed, given the limited sensitivity and specificity of the markers currently in clinical use.

The link between IHC assessment of protein expression and FISH studies of gene copy number variation is central to multiple recent and ongoing studies of MM, including investigations of MTAP IHC as a surrogate marker for *CDKN2A* FISH [11], and investigations of *MET* expression and amplification in MM. Comparison of IHC and molecular techniques has also been important in understanding the role of EZH2 expression in MM tumorigenesis, including a potential link between EZH2 and the widespread diagnostic marker BAP1.

Here we report new findings and subjects of ongoing research in the pathology of MM, with the aim of moving these findings toward clinical applications, spurring additional investigations, and fostering new collaborations, both among pathologists and across disciplinary lines.

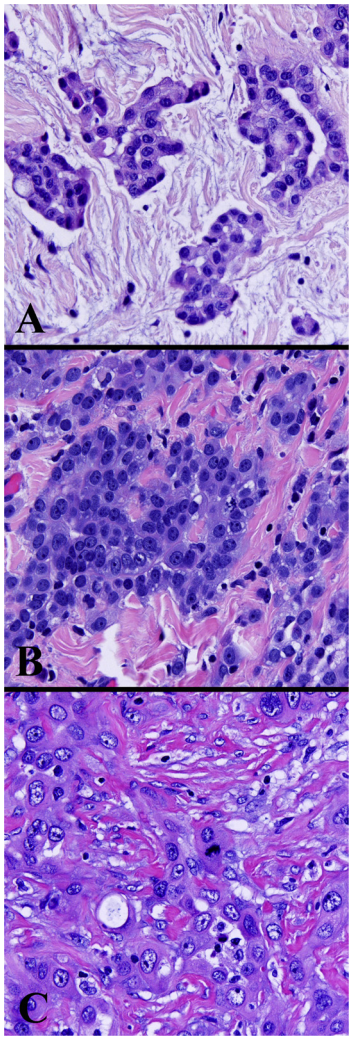

Fig. 1. (A) Epithelioid malignant mesothelioma grading: nuclear grade I with small relatively uniform nuclei and indistinct nucleoli; (B) nuclear grade II with higher nuclear cytoplasmic ratio, more prominent nucleoli, and a mitotic figure; (C) nuclear grade III with large pleomorphic nuclei, very prominent nucleoli, and a mitotic figure.

2. Composite grading of malignant mesothelioma using mitotic count, nuclear atypia, and tumor necrosis in biopsy or resection specimens accurately stratifies patient outcome

Pleural malignant mesothelioma (MM) is an aggressive tumor with poor outcome. Histologic subtype is an important prognostic indicator, with progressively shorter survival in epithelioid MM, biphasic MM, and sarcomatoid MM. Within epithelioid MM, however, there is variation in survival, which correlates with a recently described nuclear grading system [8].

Husain and colleagues designed a study to validate this nuclear grading system, and to identify additional prognostic factors. They analyzed cases of epithelioid MM (including biopsies and resections) from 17 institutions across the globe, diagnosed between 1998 and 2014. Nuclear atypia and mitotic count were combined using the published system [8] to give a nuclear grade of I, II, or III (Fig. 1A–C). One pathologists assessed nuclear grade for three institutions; the remaining cases were assessed independently at each institution. The presence or absence of necrosis and predominant growth pattern were also evaluated. Two additional scoring systems were evaluated, one combining nuclear grade and necrosis and the other mitotic count and necrosis. Median overall survival was the primary endpoint.

A total of 776 epithelioid MM cases were studied, including 301 (39%) nuclear grade I tumors, 354 (45%) grade II tumors and 121 (16%) grade III tumors. The median overall survival in all cases was 16 months, and correlated independently with age ($p = 0.006$), sex ($p = 0.015$), necrosis ($p = 0.030$), mitotic count ($p = 0.001$), nuclear atypia ($p = 0.009$), nuclear grade ($p = < 0.0001$) and mitosis and necrosis score (< 0.0001). The addition of necrosis to nuclear grade further stratified overall survival, allowing classification of epithelioid MM into four distinct prognostic groups: nuclear grade I tumors without necrosis (29 months), nuclear grade I tumors with necrosis and grade II tumors without necrosis (16 months), nuclear grade II tumors with necrosis (10 months) and nuclear grade III tumors (8 months). The scoring system combining mitotic count with necrosis stratified patients by survival, but not as well as the combination of necrosis and nuclear grade. This study confirms that nuclear grade predicts survival in epithelioid MM, identifies necrosis as a factor that further stratifies overall survival, and validates the grading system across multiple institutions and among both biopsy and resection specimens. An alternative scoring system combining mitotic count and necrosis is also proposed [9].

A second study was undertaken to compare the nuclear grade assigned in biopsies of epithelioid MM to the nuclear grade assigned in the paired resection specimen. This study included both pleural and peritoneal tumors. Of 278 patients diagnosed with mesothelioma in a three-year period at one institution, 52 had a biopsy followed by resection. Histologic type, nuclear grade, and necrosis had been documented at the time of diagnosis. Of 52 cases, four biphasic MM cases were excluded, leaving a final cohort of 48 epithelioid MM cases, of which 36 (75%) had the same nuclear grade on both biopsy and resection, while 12 (25%) had different grades. Eleven of 12 (92%) were upgraded in the resection specimen (i.e., from grade I to II or III, or grade II to III). A single case was downgraded from grade II on biopsy to grade I on resection. Of the 48 cases, necrosis was present in 12 (25%), of which four (33%) had necrosis only on resection, seven (58%) had necrosis on both biopsy and resection, and one (8%) was reported to have necrosis only on biopsy. Overall, presence or absence of necrosis was concordant between biopsy and resection in 43 cases (89%). While this study was conducted at a single institution, with grading by a single pathologist (ANH), a confirmatory multicenter study is ongoing.

In conclusion, this study showed fair correlation between biopsy and resection specimens in nuclear grading and presence of necrosis, which we have previously shown to be independent predictors of overall survival in epithelioid MM [9]. Nuclear grading and assessment of necrosis can be performed on routinely stained sections of both biopsy and resection specimens. These factors can be incorporated into a mesothelioma synoptic within the pathology report, and may help guide clinical management.

3. Relevant clinical and pathologic prognostic parameters for malignant peritoneal mesothelioma largely mirror those of pleural mesothelioma

Malignant peritoneal mesothelioma (MPeM) accounts for approximately 10% of new mesothelioma diagnoses each year. While robust prognostic models for pleural mesothelioma have been published [8,9], such models are lacking for MPeM. The current literature on MPeM prognosis is largely limited to studies from single institutions, examining one to few prognostic factors. A survey of available literature on MPeM indicates shorter survival associated with biphasic or sarcomatoid histotype [12–15], higher nuclear grade [15], solid growth, inflammatory infiltrate [15], necrosis [15], higher Ki67 index [13,14], nuclear BAP1 expression [16], patient age and sex [14,15], and asbestos exposure [17].

To further define clinical and pathologic prognostic factors in MPeM, and to incorporate these variables into a single multivariate model of independent predictors of patient outcome, pathologists and surgeons from five institutions assessed 102 peritoneal mesothelial proliferations. Clinical data for each patient (including age, sex, and asbestos exposure) were collected, and at least three H&E-stained slides from each case were reviewed centrally, to assess for histotype (epithelioid, biphasic, or sarcomatoid), nuclear pleomorphism, mitotic rate, necrosis, presence of asbestos bodies, solid tumor growth (focal or diffuse), and presence of a dense inflammatory infiltrate (intratumoral cellularity comprising > 50% inflammatory cells). Assessment of nuclear pleomorphism and mitotic rate were assessed and used to assign a composite nuclear grade, using a model previously described for pleural mesothelioma [8,9] (see also Fig. 1). Immunohistochemistry (IHC) was performed for BAP1 (loss defined by negative tumor nuclei, with positive internal control), 5-hydroxymethylcytosine (5-hmC; tumors grouped by those showing nuclear 5-hmc loss in 0–30%, 31–70%, and > 70% of tumor cells), and Ki-67 index (tumors grouped by those showing 0–9%, 10–17%, and > 17% nuclear staining [13]. Univariate (log-rank test) and multivariate (proportional hazards regression, in a model corrected for year of diagnosis) analyses were performed for overall survival (OS).

The 102 cases included 69 epithelioid MPeM, 14 biphasic MPeM, 7 sarcomatoid MPeM, 6 well-differentiated papillary mesotheliomas, and 6 benign multicystic mesotheliomas. Sixty-one patients were women and 41 were men, with year of diagnosis from 1967 to 2016 and median age at diagnosis of 57 years (range 28–82). Among all MPeM cases, median OS after diagnosis was 16 months (range 2–155), with 26% of patients alive 96 months after diagnosis. Among MPeM patients diagnosed after 1995, median OS was 52 months, with 45% of patients alive 96 months after diagnosis. On univariate analysis of all MPeM cases, shorter OS was significantly associated with older age at diagnosis ($r = -0.49$, $p = 0.002$), male sex (HR 2.4, $p = 0.015$), asbestos exposure (HR 6.5, $p < 0.0001$), presence of asbestos bodies (HR 7.4, $p < 0.0001$), biphasic or sarcomatoid histotype ($p < 0.0001$), at least focal solid growth (HR 3.4, $p = 0.0006$), and necrosis (HR 2.2, $p = 0.01$). Higher nuclear grade ($p = 0.051$), dense inflammatory infiltrate ($p = 0.06$), and BAP1 loss ($p = 0.06$) each showed a strong trend toward shorter OS. On multivariate analysis of all MPeM cases, biphasic or sarcomatoid growth (Fig. 2A), increased nuclear pleomorphism, increased mitotic rate, and increased Ki67 index were independent predictors of shorter OS. In a subcohort comprising only epithelioid MPeM, increased nuclear pleomorphism, increased Ki67 index, and composite nuclear grade [8,9] were independent predictors of shorter OS (Fig. 2B).

Pleural mesothelioma and MPeM differ substantially in their clinical management and prognosis. MPeM occurs more frequently in women and has a significantly longer OS. However, given the relative rarity of a robust prognostic model for MPeM, data from pleural mesothelioma has largely been extrapolated to peritoneal disease. Our data indicate broad overlap between key prognostic factors in pleural and peritoneal mesothelioma, including biphasic or sarcomatoid histotype, nuclear pleomorphism, and mitotic rate [9]. A composite nuclear grade score that has been previously validated for epithelioid mesothelioma of the pleura is also an independent predictor of OS in epithelioid MPeM [8,9]. Refinement of this MPeM prognostic model is ongoing, with future directions including recruitment of additional cases, evaluation of further clinical parameters (including laboratory, oncologic, and surgical variables), and evaluation of novel IHC markers (including MTAP and PD-L1).

4. HEG1 is a new immunohistochemical marker for the pathological diagnosis of malignant mesothelioma

Calretinin, WT1, and D2-40 are the best available mesothelioma markers for histological diagnosis of malignant mesothelioma (MM). However, none of these markers is 100% sensitive or 100% specific for MM. HEG1 is heart development protein with EGF-like domains 1, and suggested to regulate endothelial cell signaling pathway [18]. Tsuji and colleagues recently reported that expression of the protein HEG1 is

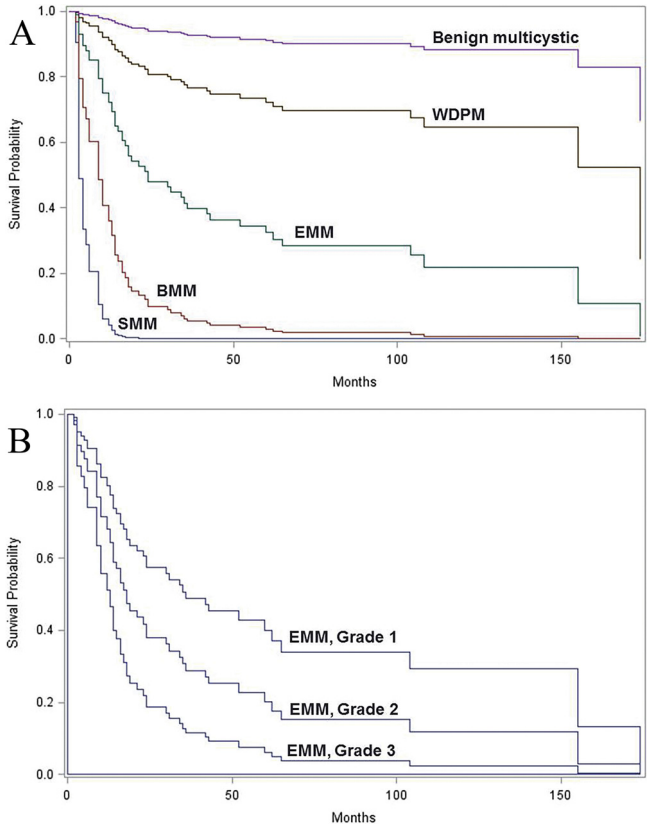

Fig. 2. Overall survival in malignant peritoneal mesothelioma. A) Overall survival was significantly shorter in malignant peritoneal mesothelioma (MPeM), compared to benign multicystic mesothelioma and well-differentiated papillary mesothelioma (WDPM). Survival was significantly shorter in biphasic MPeM (BMM) than in epithelioid MPeM (EMM), and was significantly shorter in sarcomatoid MPeM (SMM) than in biphasic or epithelioid MPeM. B) Among epithelioid MPeM, significantly shorter overall survival was seen with increasing nuclear grade.

highly sensitive and specific for MM [19]. This study was undertaken to further evaluate immunoreactivity of HEG1 as a diagnostic marker for MM.

Tissue microarrays (TMAs) provided by one of the authors (ANH) [20] included 87 pleural MMs (51 epithelioid, 34 biphasic, and 2 sarcomatoid) and 22 peritoneal MMs (19 epithelioid and 3 biphasic). Hiroshima and colleagues collected additional cases, including biopsies of 88 MMs (47 epithelioid, 18 biphasic, and 23 sarcomatoid), 57 pulmonary carcinomas (29 adenocarcinomas, 14 squamous cell carcinomas, 4 large cell neuroendocrine carcinomas, 2 adenosquamous carcinomas, seven pleomorphic carcinomas, and one mucoepidermoid carcinoma), and 11 cases of fibrous pleuritis, as well as pleural effusion cell blocks from 17 patients with MM and 4 patients with reactive pleural effusion.

In the TMA cases, calretinin, WT1, D2-40, and HEG1 were expressed in 73.3%, 62.8%, 60.5%, and 91.4%, respectively, of epithelioid pleural MM; in 81.8%, 68.2%, 68.2%, and 90.9%, respectively, of epithelioid peritoneal MM; and in 48.5%, 42.4%, 33.3%, and 93.9%, respectively, of biphasic pleural MM. In the TMA, both sarcomatoid mesotheliomas expressed HEG1 but were negative for calretinin, WT1, and D2-40. In whole-slide sections of biopsies, all of 47 epithelioid MM, 17 of 18 (94%) biphasic MM, and 18 of 23 (78%) sarcomatoid MM were positive for HEG1. Most epithelioid tumor cells displayed strong, diffuse, membranous reactivity for HEG1, while, sarcomatoid tumor cells showed cytoplasmic HEG1 staining, which was diffuse but more moderate in intensity (Fig. 3).

In contrast, HEG1 was expressed in 0 of 29 pulmonary

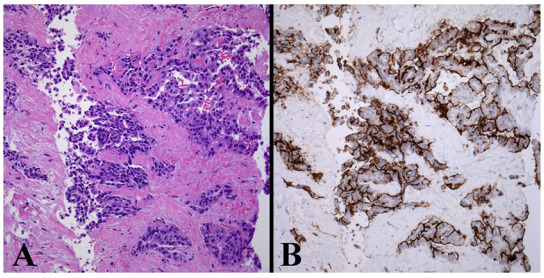

Fig. 3. Epithelioid mesothelioma. A) Atypical cells with round nuclei and eosinophilic cytoplasm proliferate and form nests in the collagenous stroma (hematoxylin and eosin). B) Mesothelioma cells show strong and diffuse membranous staining for HEG1 by immunohistochemistry.

adenocarcinomas, focally in 4 of 14 pulmonary squamous cell carcinomas (29%), 2 of 2 adenosquamous carcinomas (in the squamous component only), and 4 of 7 pleomorphic carcinomas (57%, cytoplasmic staining). Overall, for differentiation between non-sarcomatoid MM and lung carcinoma in histologic specimens, HEG1 shows a sensitivity of 98.5% and specificity of 82.5%. In the differential diagnosis between MM and pulmonary adenocarcinoma, HEG1 is up to 100% specific for MM.

All 11 biopsies of fibrous pleuritis were positive for HEG1, but staining intensity was weak. All 17 pleural effusion cell blocks from MM patients and all 4 cell blocks with reactive mesothelial cells were strongly and diffusely positive for HEG1.

A definitive diagnosis of MM requires a thorough workup, including immunoprofiling. Specifically, guidelines for pathologic diagnosis of MM require two positive MM markers as well as two negative markers for other tumors in the morphologic differential diagnosis [3]. However, not all epithelioid or biphasic MMs stain with 2 or more mesothelial markers, and diagnosis may in some cases rely on a single robust stain. Sensitivity of HEG1 for MM is higher than that of calretinin, WT1, or D2-40. The superior sensitivity of HEG1 is particularly evident in biphasic MM. HEG1 also shows excellent specificity in the differential diagnosis between MM and pulmonary adenocarcinoma. Finally, HEG1 is highly sensitive for detection of mesothelial cells in effusion specimens, with all MM and reactive mesothelial specimens showing HEG1 reactivity in this study.

5. Increased MET protein expression correlates poorly with *MET* amplification status in malignant mesothelioma, and predicts poorer patient outcomes

The hepatocyte growth factor tyrosine-kinase receptor MET is a potential therapeutic target in several cancers, in which it can be activated by various mechanisms, including gene copy number gain /amplification (GCNG/GA) and/or protein overexpression. However, only few studies have investigated the incidence of *MET* GCNG/GA and/or increased protein expression in malignant mesothelioma (MM), and these have shown variable results due to differences in types of samples, sensitivity and specificity of testing methodologies, and scoring methods [21–23]. To further clarify whether and how MET is overexpressed in MM, Santoni-Rugiu and colleagues used fluorescence in-situ hybridization (FISH) and immunohistochemistry (IHC) to investigate *MET* GCNG/GA frequency and expression, using strictly defined diagnostic standards previously applied to lung cancer [24,25].

The study examined 155 consecutive MM cases treated at their institution between 2015 and 2017. The cohort included 110 diagnostic biopsies of treatment-naïve MM (97 pleural and 13 peritoneal) and 45 pleurectomies/decortications (P/D) performed after 3 courses of neoadjuvant cisplatin-pemetrexed. The specimens comprised 77 epithelioid MM, 68 biphasic MM, and 10 sarcomatoid or desmoplastic MM. Thirty-one reactive mesothelial proliferations (RMP) identified in

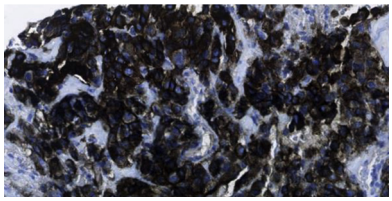

Fig. 4. This epithelioid malignant mesothelioma exhibits overexpression of MET-protein, corresponding to IHC score 3+ (original magnification 320x).

surgical samples from non-malignant pulmonary/mediastinal disease were used as controls. IHC was performed on formalin-fixed paraffin-embedded tissue using the pre-diluted CONFIRM SP44 anti-MET monoclonal antibody (Ventana) in a Ventana BenchMark ULTRA autostainer. MET protein expression was scored (blinded to FISH results) for staining intensity (negative, weak, moderate, or strong) and the percentage of cells staining, yielding four diagnostic "immunoscores": 3+/overexpression (strong intensity in ≥50% of tumor cells); 2+ (moderate intensity in ≥50% of tumor cells); 1+ (weak intensity in ≥50% of tumor cells); and 0 (no staining or < 50% of tumor cells stained with any intensity) [26]. FISH was performed with the ZytoLight SPEC MET/CEN7 dual-color probe (Zytovision), analyzing 100 tumor cell nuclei from 5 random areas of homogenous signal distribution by strict GCNG/GA classification criteria (high-level *MET* GA, intermediate-level *MET* GCNG; low-level *MET* GCNG; no *MET* GA/GCNG [24,25])

By IHC, 22 (17%) of 155 MM specimens had a MET score of 3+ (Fig. 4), 22 (17%) had a MET score of 2+, and the remaining 111 (65%) had a MET score of 1+ or 0. A MET score of 3+ or 2+ was significantly more common in epithelioid MM than in biphasic or sarcomatoid MM ($p < 0.05$ by t-test). Furthermore, in biphasic MM cases with MET score of 3+ or 2+, only the epithelioid component showed 3+ or 2 + MET expression, while the sarcomatoid tumor cells were uniformly 1+ or 0. All 10 sarcomatoid or desmoplastic MM cases had MET score of 1+ or 0. Neoadjuvant chemotherapy produced no significant change in MET expression in P/D specimens as compared to patient-matched diagnostic biopsies.

Only 2 (1.3%) MM cases (both epithelioid) showed 3 + MET IHC score with intermediate-level *MET* GCNG by FISH analysis, while 10 (7%) cases (7 epithelioid, 3 biphasic) showed 3+ or 2 + MET IHC score with low-level *MET* GCNG. None of the analyzed cases showed high-level *MET* GCNG, and no cases with low- or intermediate-level *MET* GCNG had MET IHC score of 1+ or 0. All 31 control RMPs had a MET IHC score of 1+ or 0 and no *MET* GCNG. Finally, in a case-control cohort matched for gender, age, performance status, tumor stage, histotype, asbestos exposure, smoking habit, and surgical resection by P/D, the presence of MET abnormalities (3+ or 2 + MET IHC score, with or without *MET* GCNG) was significantly associated with lower therapy response rate (RR; $p = 0.04$) and shorter overall survival ($p = 0.018$) after first-line platinum-pemetrexed chemotherapy.

These data show that approximately one third of MM have moderately (2+) or strongly (3+) increased MET protein expression, which appears to be restricted to epithelioid tumor cells (either in epithelioid MM or the epithelioid component of biphasic MM). Interestingly, MET overexpression by IHC poorly predicts *MET* GCNG, with low- or intermediate-level GCNG in only 12 of 44 (27%) cases with 3+ or 2 + MET IHC score. This poor correlation between protein expression and gene amplification suggests other mechanisms regulating *MET* expression. We found no high-level *MET* GCNG in our MM cohort, consistent with previous reports [21–23]. Importantly, MET protein overexpression correlates with poorer response to therapy and shorter overall survival in patients receiving platinum-pemetrexed chemotherapy. The predictive significance of MET IHC and *MET* FISH for treatment of MM with targeted MET inhibitors remains to be elucidated.

6. EZH2 overexpression is important in the proliferation, migration, and tumorigenesis of malignant mesothelioma, and predicts shorter overall survival

Malignant mesothelioma (MM) is closely associated with asbestos exposure. Enhancer of zeste homologue 2 (EZH2) is a key component of polycomb repressive complex 2 (PRC2), which catalyzes histone H3 lysine 27 trimethylation (H3K27Me3) and mediates the epigenetic silencing of target genes through posttranslational histone modifications [26]. Aberrant overexpression of EZH2 is found in many malignancies, including carcinomas of breast, prostate, and lung, and is often associated with poor prognosis [27]. It has been recently reported that loss of BRCA1-associated protein 1 (BAP1) function leads to EZH2-dependent transformation [28], but the biological role of EZH2 in MM is not fully understood.

To explore the relation between EZH2 expression and the prognosis in MM patients, Tsujimura and colleagues examined EZH2 expression levels in both MM and reactive mesothelium (RM) by immunohistochemistry. EZH2 expression was significantly higher in MM than in RM. Overall survival (by log-rank test) was significantly shorter in MM patients with high EZH2 expression than in those with low EZH2 expression.

To further clarify the involvement of EZH2 in the oncogenesis and pathogenesis of MM, EZH2 small interfering RNA (siRNA) and EZH2 cDNA were introduced into a MM cell line and a benign mesothelial cell line, respectively. The siRNA-mediated knockdown of EZH2 in the MM cell line decreased H3K27Me3 levels and inhibited the proliferation, migration, and tumorigenicity of MM cells. Conversely, the benign mesothelial cells overexpressing EZH2 showed an increase in H3K27Me3 levels and higher proliferation and migration compared to the parent cells, although they did not acquire complete tumorigenicity. Finally, treatment with an EZH2 inhibitor suppressed the proliferation and migration of MM cells.

EZH2 plays an important role in the proliferation and migration of MM cells, and EZH2 expression level is a prognostic factor in MM. EZH2 overexpression seems to be associated with MM oncogenesis, although EZH2 overexpression alone is not sufficient for complete tumorigenesis in benign mesothelial cells (Fig. 5). These data suggest that EZH2 overexpression may be a potential therapeutic target in MM.

7. Conclusion

Pathologists stand at the nexus of new developments in the diagnosis and management of MM, ranging from novel insights into histomorphology to our ever-growing understanding of the molecular biology of MM, which has given rise to numerous molecular, cytogenetic, and immunohistochemical tests, some of which are already in

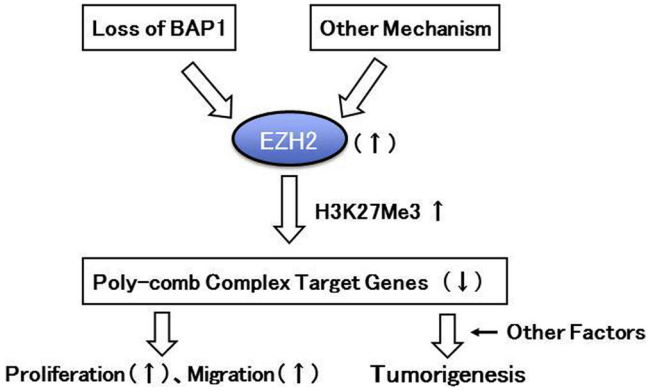

Fig. 5. In malignant mesothelioma, increased EZH2 expression results in increased histone trimethylation, resulting in suppression of polycomb complex genes and increased mesothelial proliferation, migration, and tumorigenesis.

widespread use among pathologists, and others of which stand to enter the clinical diagnostic realm in the coming years. These advances encompass not only increasingly robust diagnostic tools, but also increasingly refined models for risk stratification, which both improve counseling for individual patients and guide clinical trials. Finally, the discovery of novel pathogenic mechanisms in MM provides promising targets for new therapies [29], and valuable predictive tools for guiding personalized medicine in MM.

The central role of the pathologist in diagnosis and management of MM was evident in the "Molecular Pathways and Diagnosis in Malignant Mesothelioma" plenary session at the 2018 iMig conference. To be sure, the road from a scientific discovery to a widely available, clinically relevant, and diagnostically robust test is long. But the multidisciplinary discussions and collaborations that emerge from the iMig conference provide a fast lane for promising pathologic techniques, ensuring both efficient and effective translation of relevant research to life-changing advances in patient care.

Conflict of interest

The authors have no competing interests to declare.

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