Review

Marie Brevet*

Comparative genetics of diffuse malignant mesothelioma tumors of the peritoneum and pleura, with focus on BAP1 expression

DOI 10.1515/pap-2016-0007
Received February 26, 2016; accepted May 4, 2016; previously published online May 27, 2016

Abstract: Malignant mesothelioma (MM) is a malignancy arising from the mesothelial cells lining the thoracic and abdominal serosal cavities. The pleural space is the most commonly affected site, accounting for about 80% of cases, while peritoneum makes up the majority of the remaining 20%. The different types of mesotheliomas are generally considered as distinct diseases with specific risk factors, therapeutic strategies and prognoses. Epidemiological and clinical differences between pleural and peritoneal MM raise questions about the involvement of different molecular mechanisms. Since the BAP1 gene is involved in the BAP1 cancer syndrome and seems to be a prognostic factor in MM, this review presents an overview of BAP1 alterations in mesothelioma comparing pleural and peritoneal localizations.

Keywords: BAP1, hereditary syndrome, mesothelioma, molecular alteration, prognostic factors

Introduction

Malignant mesothelioma (MM) is a rare disease that results from unregulated proliferation of the mesothelial cells lining the pleural, peritoneal and pericardial cavities. Localized MMs are rare and appear as solitary circumscribed nodular tumors, attached either in a sessile or pedunculated manner to the surface of the pleura or peritoneum. In opposition, diffuse MMs are characterized by diffuse spread over the serosal surface. By definition, localized and diffuse MMs are histologically and immunohistochemically identical [1]. Most MMs are related to exposure to mineral fibers, especially asbestos and erionite. The percentage of asbestos exposure differs depending on the cavity involved: from 70% in pleural MM to only 30% in peritoneal MM [2]. Other clinical differences include younger age of incidence in peritoneal MM and sex ratio [3].

Histologically, according to the 2015 WHO classification [4], MM can be categorized into epithelioid, sarcomatoid and biphasic subtypes. Localized malignant mesotheliomas, well-differentiated papillary mesothelioma and adenomatoid tumors have mainly been observed in the pleura, which is why they were excluded from this review. Differences of incidence have been observed for each histological subtype depending on MM location. Sarcomatoid and biphasic mesotheliomas are very rare in peritoneal MMs, while they represent 20–25% of pleural MMs [4].

The biochemical mechanisms responsible for the genesis of MM are not completely understood and most data come from pleural MM analyses. As a result of asbestos exposure, the inflammatory response generates resistance to apoptosis and accumulation of DNA damage [5]. In parallel, various cellular pathways such as receptor tyrosine kinase (RTK), Hippo signaling or PI3K/AKT pathways are altered [6, 7]. Promoter methylation of known tumor suppressor genes has also been observed, suggesting that epigenetic inactivation may be involved in the development and progression of MM tumors [8]. Finally, the main genetic alterations described in MM include cyclin-dependent kinase inhibitor 2A (CDKN2A)/alternative reading frame (ARF), neurofibromatosis type 2 (NF2) and BRCA1-associated protein-1 (BAP1) gene alterations [9–11]. Interestingly, the BAPI gene, located on chromosome 3p21.1, has been shown to be an important tumor suppressor gene in MM. Altered BAP1 was found in 42% of mesothelioma tumors through combining array CGH analysis and sequencing data [10]. Initially identified as a BRCA1-binding protein, BAP1 is a deubiquitinating enzyme with a C-terminal
active hydrolase domain (UCH) and N-terminal nuclear localization signals (NLS1, NLS2). It has been postulated that BAP1 functions as a tumor suppressor through different mechanisms that all require nuclear localization of the BAP1 protein. Somatic BAP1 mutations have been reported in other malignancies including uveal melanoma and renal cell carcinoma. The most exciting discoveries of the past years were the description of germline BAP1 mutations and the existence of family cases of mesothelioma tumors [12, 13].

In the scientific literature on mesothelioma carcinogenesis, most papers include pleural mesothelioma tumors, while only few papers focus on the peritoneal localization of the disease. The molecular pathogenesis of peritoneal mesothelioma has mainly been described through the extrapolation of findings from pleural mesothelioma. The main objective of the present review is to provide an overview of BAP1 alterations in mesothelioma. We will compare DNA loss, BAP1 mutation and BAP1 protein expression in both pleural and peritoneal MMs.

**BAP1 DNA loss in malignant mesothelioma**

DNA copy number or DNA rearrangement can be investigated through different techniques including array-comparative genomic hybridization (CGH), multiplex ligation-dependent probe amplification assay (MLPA), next generation sequencing (NGS) or conventional cytogenetic analysis. Using these methods, 3p21 rearrangement has been reported in 30 to 65% of mesotheliomas [10, 14–20]. Until recently, the biological differences between pleural and peritoneal mesothelioma had not been investigated in relation to DNA copy number, since most CNA studies focused on pleural disease. In 2015, Alakus et al. published an analysis on 12 peritoneal mesotheliomas and identified loss of BAP1 DNA in five cases (42%) through targeted sequencing [17]. A recent paper published by Columbia University compared genomic imbalances between 48 peritoneal epithelioid mesotheliomas and 41 pleural epithelioid mesotheliomas [21]. The authors described similar recurrent genomic imbalances between pleural and peritoneal mesotheliomas although their frequencies differed depending on the site of tumor origin. DNA loss on the 3p chromosome arm including the BAPI gene was observed for both localizations, but with higher frequency in pleural mesothelioma (the difference was not statistically significant). Based on these results, peritoneal and pleural MMs show a similar genomic profile through array-CGH analysis. DNA loss in 3p21 occurred in both pleural and peritoneal mesotheliomas without statistically significant differences (Table 1).

**BAP1 mutations in malignant mesothelioma**

Since the discovery of BAPI alterations in mesotheliomas and uveal melanomas, the number of articles describing BAPI mutations has increased (Table 2). In 2011, Bott et al. described somatic mutations in 23% of pleural mesotheliomas, and Testa et al. described germline mutations in two families with multiples MMs and/or uveal melanomas [10, 12]. Since 2011, somatic BAPI mutations have been described in 20 to 61% of pleural mesotheliomas [10, 15, 16, 22–25] (Table 2) whereas

---

**Table 1:** Frequency of 3p21 DNA loss in pleural and peritoneal malignant mesothelioma.

<table>
<thead>
<tr>
<th>Source</th>
<th>Pleural MM, n (%)</th>
<th>Peritoneal MM, n (%)</th>
<th>Total number of MMs, n (%)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taguchi et al. [14]</td>
<td>13/23 (57)</td>
<td>0</td>
<td>13/23 (57)</td>
<td>Cytogenetic analysis</td>
</tr>
<tr>
<td>Bott et al. [10]</td>
<td>16/53 (30)</td>
<td>0</td>
<td>16/53 (30)</td>
<td>CGH</td>
</tr>
<tr>
<td>Yoshikawa et al. [15]</td>
<td>11/18 (61)</td>
<td>NA</td>
<td>11/18 (61)</td>
<td>CGH</td>
</tr>
<tr>
<td>Nasu et al. [16]</td>
<td>9/22 (41)</td>
<td>NA</td>
<td>9/22 (41)</td>
<td>MLPA</td>
</tr>
<tr>
<td>Alakus et al. [17]</td>
<td>0</td>
<td>5/12 (42)</td>
<td>5/12 (42)</td>
<td>NGS</td>
</tr>
<tr>
<td>Cigognetti et al. [18]</td>
<td>36/51 (71)</td>
<td>NA</td>
<td>36/51 (71)</td>
<td>Cytogenetic analysis</td>
</tr>
<tr>
<td>Taylor et al. [19]</td>
<td>0</td>
<td>1/1 (100)</td>
<td>1/1 (100)</td>
<td>Cytogenetic analysis</td>
</tr>
<tr>
<td>Emi et al. [20]</td>
<td>11/17 (65)</td>
<td>NA</td>
<td>11/17 (65)</td>
<td>MLPA</td>
</tr>
</tbody>
</table>

CGH, array comparative genomic hybridization; MLPA, multiplex ligation-dependent probe amplification assay; NA, not available. *The exact localization of the disease was not specified. By default, the data are completed in the “pleural MM” part of the Table. The study included MM peritoneal cases but results on these specific cases were not specified. By default, the data are completed in the “pleural MM” part of the Table.*
27.3% of peritoneal mesotheliomas included somatic BAP1 mutations [17, 26]. Such mutations have been found in a substantial proportion of epithelioid mesotheliomas (71%) and to a lesser extent in sarcomatoid mesotheliomas (<5%) [25]. A recent publication by Bueno et al. [27] described BAP1 as the most frequently mutated gene in malignant pleural mesothelioma before NF2 and TP53. BAP1 mutations include base substitution leading to nonsense and missense mutations, frameshift insertions or deletions, splice-site mutations, truncations or rearrangements. Truncating mutations frequently result in the loss of the NLS and/or C-terminal protein-binding domain. Missense mutations affect the ubiquitin hydrolase function of BAP1.

Germline BAP1 mutations are considered as a predisposing factor for mesothelioma and uveal melanoma, as well as basal cell carcinoma, cutaneous melanoma or renal cell carcinoma [12, 28–31]. Germline mutations in BAP1 increase patient susceptibility to asbestos-induced malignant mesothelioma [32] and may contribute to MM development through the same mechanisms as somatic mutations, regulating cell cycle progression, chromatin modulation and DNA double-strand break repair via the BRCA1/BARD1 complex [33]. Nevertheless, germline BAP1 mutations are not frequent in sporadic MM, with a prevalence of about 1–2% [12, 15, 34–36]. Ohar et al. identified germline BAP1 mutations in nine out of 150 MM cases (6%) with personal or family history of cancer [37]. Out of these nine indexed MM cases, five (55.6%) were peritoneal and four (44.4%) were thoracic, while 116 MM cases without germline BAP1 mutation (82.3%) were thoracic and 25 (17.7%) were peritoneal. All nine MM cases had histories of asbestos exposure. In accordance with the hypothesis, the authors did not find any germline BAP1 mutations in the 153 individuals with significant exposure to asbestos without mesothelioma. Battaglia et al. made an exhaustive description of every published germline BAP1 mutation depending on tumor origin. Like somatic mutations, germline mutations included missense, nonsense or deleterious frameshift mutations, truncations or rearrangements. In some cases, one BAP1 allele was lost via monosomy 3 and the other was non-functional due to an inactivating BAP1 mutation, in accordance with Knudson’s two-hit model [33]. Finally, Cheung et al. reported an asbestos-exposed family with high incidence of cancer, including eight cases of pleural MM. BAP1 tumor sequencing of four cases in this family did not show any BAP1 mutation nor DNA loss in 3p21 (n = 2). This suggests that another susceptibility locus may contribute to high MM incidence in this family [38].

To summarize, both pleural and peritoneal mesotheliomas include germline and somatic BAP1 mutations. Such mutations result in loss of protein function, confirming the role of BAP1 as a tumor suppressor gene.

### BAP1 protein expression in malignant mesothelioma

A large number of immunohistochemical markers have been proposed to support the diagnosis of mesothelioma. In addition to two positive (i.e. calretinin, cytokeratin 5/6 or HBME1) and two negative markers (i.e. TTF-1, BerEP4, Napsin A) [4], auxiliary markers such as epithelial membrane antigen, p53 or GLUT1 are also useful tools. Although these markers may be helpful in borderline cases, they do not show enough sensitivity or specificity.

---

**Note:** The table below provides frequency of somatic BAP1 mutations in pleural and peritoneal malignant mesothelioma.

<table>
<thead>
<tr>
<th>Source</th>
<th>Pleural MM, n (%)</th>
<th>Peritoneal MM, n (%)</th>
<th>Total number MMs, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bott et al. [10]</td>
<td>24/121 (20)</td>
<td>0</td>
<td>24/121 (20)</td>
</tr>
<tr>
<td>Yoshikawa et al. [15]a</td>
<td>11/18 (61)</td>
<td>NA</td>
<td>11/18 (61)</td>
</tr>
<tr>
<td>Zauderer et al. [25]</td>
<td>24/121 (20)</td>
<td>0</td>
<td>24/121 (20)</td>
</tr>
<tr>
<td>Nasu et al. [16]a</td>
<td>6/22 (27)</td>
<td>NA</td>
<td>6/22 (27)</td>
</tr>
<tr>
<td>Lo Iacono et al. [22]</td>
<td>78/123 (63)</td>
<td>0</td>
<td>78/123 (63)</td>
</tr>
<tr>
<td>Alakus et al. [17]</td>
<td>0</td>
<td>5/12 (42)</td>
<td>5/12 (42)</td>
</tr>
<tr>
<td>Guo et al. [23]</td>
<td>8/22 (36%)</td>
<td>0</td>
<td>8/22 (36)</td>
</tr>
<tr>
<td>Sheffield et al. [26]</td>
<td>0</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>De Rienzo et al. [24]</td>
<td>31/147 (21)</td>
<td>0</td>
<td>31/147 (21)</td>
</tr>
<tr>
<td>Bueno et al. [27]</td>
<td>46/202 (23)</td>
<td>0</td>
<td>46/202 (23)</td>
</tr>
</tbody>
</table>

NA, not available. a The exact localization of the disease was not specified. By default, the data are completed in the “pleural MM” part of the Table. b The study included MM cases but results on these specific cases were not specified. By default, the data are completed in the “pleural MM” part of the Table.
Table 3: Frequency of BAPI protein loss by immunohistochemistry in pleural and peritoneal malignant mesothelioma.

<table>
<thead>
<tr>
<th>Source</th>
<th>Pleural MM, n (%)</th>
<th>Peritoneal MM, n (%)</th>
<th>Total number of MMs, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bott et al. [10]</td>
<td>27/47 (57)</td>
<td>0</td>
<td>27/47 (57)</td>
</tr>
<tr>
<td>Yoshikawa et al. [15]</td>
<td>NA</td>
<td>NA</td>
<td>11/17 (65)</td>
</tr>
<tr>
<td>Arzt et al. [43]</td>
<td>74/123 (60)</td>
<td>NA</td>
<td>74/123 (60)</td>
</tr>
<tr>
<td>Nasu et al. [16]</td>
<td>61/91 (67)</td>
<td>NA</td>
<td>61/91 (67)</td>
</tr>
<tr>
<td>Farzin et al. [39]</td>
<td>106/229 (46)</td>
<td>0</td>
<td>106/229 (46)</td>
</tr>
<tr>
<td>Sheffield et al. [40]</td>
<td>7/26 (27)</td>
<td>NA</td>
<td>7/26 (27)</td>
</tr>
<tr>
<td>Cigognetti et al. [18]</td>
<td>139/212 (66)</td>
<td>NA</td>
<td>139/212 (66)</td>
</tr>
<tr>
<td>Singh et al. [42]</td>
<td>0</td>
<td>49/86 (57)</td>
<td>49/86 (57)</td>
</tr>
<tr>
<td>McGregor et al. [43]</td>
<td>65/111 (59)</td>
<td>0</td>
<td>65/111 (59)</td>
</tr>
<tr>
<td>Sheffield et al. [26]</td>
<td>0</td>
<td>0/2 (0)</td>
<td>0/2</td>
</tr>
<tr>
<td>Lo Iacono et al. [22]</td>
<td>56/116 (48)</td>
<td>0</td>
<td>56/116 (48)</td>
</tr>
<tr>
<td>Jaouen et al. [44]</td>
<td>12/25 (48)</td>
<td>0</td>
<td>12/25 (48)</td>
</tr>
<tr>
<td>Hwang et al. [47]</td>
<td>8/13 (62)</td>
<td>2/2 (100)</td>
<td>10/15 (67)</td>
</tr>
</tbody>
</table>

NA, not available. *The exact localization of the disease was not specified. By default, the data are completed in the “pleural MM” part of the Table. #The study included peritoneal MM cases but results on these specific cases were not specified. By default, the data are completed in the “pleural MM” part of the Table.

Discussion

The pleura is the most common MM localization before the peritoneum. Consequently, the molecular pathogenesis of MM is mostly derived from descriptions of pleural MM. While these tumors arising from different sites have similar morphologies, peritoneal MM presents with unique characteristics such as younger median age and higher frequency in female patients [49]. In this review, we would like to compare molecular pathogenesis in pleural and peritoneal MM with focus on the BAPI gene.
The discovery of an inherited cancer syndrome caused by germline BAP1 mutations, the correlation between genetic alterations and BAP1 expression, the availability of a specific antibody that could be used in histopathological examination and the specificity of BAP1 loss for the diagnosis of malignant mesothelioma may explain the dramatic increase in the number of articles that have reported analyses of BAP1 expression in mesothelioma since 2011. Although most articles deal with pleural mesothelioma, some focus on peritoneal MM. The published data suggest similar molecular pathogenesis of pleural and peritoneal MM, including DNA loss in 3p21, 9p21 and 22q12 [21]. Somatic and germline BAP1 mutations have been described in both sites and systematically led to BAP1 inactivation.

The discovery of the BAP1 cancer predisposition syndrome has had a number of clinical implications. If BAP1 mutations are identified in the tumors, testing for germline BAP1 mutations should be preferably considered within the framework of a family cancer genetic screening unit. Patients with confirmed germline BAP1 mutations will need appropriate genetic counseling for themselves and their families. As a consequence, these patients will require close monitoring for the early detection and curative resection of uveal and cutaneous melanoma, and should be informed of the risk for MM and other malignancies.

BAP1 IHC is a cheap and quick way to screen tumors with BAP1 mutations, since paraffin-embedded tumor tissue is readily available. Tumors that present with loss of nuclear BAP1 expression may then undergo subsequent confirmatory sequencing. The correlation between immunohistochemical and sequencing data in MM and other tumors is strong. Therefore, the immunohistochemical screening for BAP1 should become a standard histopathological test for MM or melanocytic tumors [10, 15, 16, 22, 50]. More specifically, BAP1 IHC is a highly specific marker to differentiate mesothelioma from reactive mesothelial proliferations. In case of noninvasive mesothelial proliferation, loss of BAP1 expression is highly suggestive of a malignant disease [18, 40, 45, 47].

The role of BAP1 mutations and BAP1 expression as prognostic biomarkers is still emerging. It should be noted that results differ depending on tumor origin. Multivariate analysis demonstrated that BAP1 expression was an independent prognostic factor for colorectal cancer (CRC) [51]. The clinicopathological significance and oncologic outcomes of BAP1 loss in renal cell carcinoma (RCC) have been investigated. IHC for BAP1 loss was performed in 559 non-metastatic RCC using tissue micro-array. Cox regression indicated significantly worse disease-free and overall survival for patients with BAP1-negative (82/559, 14.7%) than patients with BAP1-positive tumors [52]. Different results have been observed in pleural MM, and BAP1 protein loss is paradoxically associated with improved survival [39, 41, 43]. No data are available for peritoneal mesothelioma, although it is known to be associated with longer survival than pleural MM. To date, the way to consider this prognostic histopathological marker in ordinary clinical practice is still unclear.

Currently, the therapeutic strategy in pleural and peritoneal MM is to provide specific standard protocol for each MM localization. Patients with pleural MM are mostly treated with systemic chemotherapy recently added with anti-VEGF drug [53]; patients with peritoneal MM undergo surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) [54, 55]. If future studies are able to define a therapeutically accessible synthetic lethal target in the setting of BAP1 loss, it could eventually benefit patients with BAP1-negative or mutation tumors, regardless of the site of tumor origin.

To conclude, the latest reports on peritoneal mesothelioma confirmed the data observed in pleural mesothelioma, which suggests that both localizations have similar genetic background. BAP1 IHC should be used in ordinary diagnosis to help the pathologist separate MM from reactive mesothelial proliferations in the pleural and peritoneal cavities, in tissue samples and effusion cytology specimens. For clinical practice and in case of family cancer history, the absence of BAP1 staining in tumor cells may suggest the presence of germline BAP1 mutations. These patients should be considered for BAP1 genetic testing to identify those who may undergo further screening. Indeed, early diagnosis and treatment may be partly responsible for significantly improved mesothelioma prognosis in germline BAP1 mutation carriers.

Acknowledgments: The author would like to thank Laure Gallay for critical review of the paper.

Author contributions: The author has accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: This work is supported by the Auvergne Rhone-Alpes Region administration, France (Groupement Interrégionaux de Recherche Clinique et d’Innovation [GIRCI] and Plateforme d’Aide à la Recherche Clinique en Cancérologie – Auvergne Rhones-Alpes [PARCC-ARA]), AMARAPE, La Ligue contre le cancer and Lyon Research Innovation for Cancer (LYRIC) (Grant no: INCa-DGOS-4664).

Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
Conflict of interest: The authors have declared conflicts of interest with Astra Zeneca, Novartis, Roche and BMS.

References


