ORIGINAL ARTICLE

BRCA1-Associated Protein 1 (BAP1) Immunohistochemical Expression as a Diagnostic Tool in Malignant Pleural Mesothelioma Classification: A Large Retrospective Study

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ABSTRACT

Introduction: Malignant pleural mesothelioma (MPM) is a highly aggressive disease with limited therapeutic options. Histological subtype remains among the most reliable prognostic factors, because the epithelioid subtype associated with the best prognosis and the sarcomatoid subtype with the worst. The biphasic subtype has an intermediate prognosis, but its definitive histological diagnosis may be challenging owing to the difficulty of assessing the neoplastic nature of the stromal component. Recent data identified BRCA1-associated protein 1 gene (*BAP1*) as one of the most frequently mutated genes in MPM. Immunohistochemical testing for BRCA1-associated protein 1 (BAP1) has been proposed to be predictive for the detection of *BAP1* mutation in neoplastic cells. The aim of the present study was to define the diagnostic usefulness of immunohistochemical determination of BAP1 in MPM, with clinicopathological correlation.

Methods: A series of 143 MPMs were investigated for BAP1 protein expression in correlation with clinical and pathological data, including with a newly proposed nuclear grade. A pilot series of 20 selected cases were also investigated for *BAP1* mutational status.

Results: Negative nuclear staining for BAP1 occurred in 62% of MPMs (including 27% with a cytoplasmic pattern) and was significantly associated with the presence of *BAP1* mutation, epithelioid subtype, and a better prognosis. In a subgroup of cases, the pattern of expression of BAP1 in stromal cells supported their distinction as reactive versus neoplastic, thus helping achieve the correct classification of biphasic histological subtype.

Conclusions: We showed that BAP1 protein determination is a diagnostic tool to correctly distinguish biphasic MPM from epithelial subtypes with an atypical/activated reactive stroma and an independent prognostic parameter in MPM.

Keywords: Malignant mesothelioma; Pleura; BAP1 mutation; Prognosis; Histology

Introduction

Malignant pleural mesothelioma (MPM) is a rare, highly aggressive, relatively chemotherapy- and radiotherapy-resistant type of cancer with limited therapeutic options.[1] In patients with advanced-stage disease treated with cisplatin and pemetrexed, median survival time is

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approximately 12 months, long-term survivors are seen only occasionally,[2,3] and disappointingly, there is no approved agent for second-line chemotherapy.[4] In MPM, proposed prognostic factors include clinical variables, radiological parameters at presentation, and molecular/pathological findings, but the vast majority of them are not fully validated[5] and the proposed scoring systems (Cancer and Leukemia Group B and European Organization for Research and Treatment of Cancer[6,7]) are not widely used. Histological subtype remains among the most reliable prognostic factors because the epithelioid subtype is associated with the best prognosis and the sarcomatoid subtype with the worst.[8] Although the biphasic/mixed subtype usually has an intermediate prognosis, sometimes its definitive histological diagnosis may be cumbersome owing to the sometimes problematic grade assessment of nuclear atypia in the stromal component. Furthermore, high-grade MPM with pleomorphic features has a controversial histological classification; although according to the guidelines, it is classified as epithelioid MPM,[8,9] clinical and pathological findings suggest an association with the sarcomatoid subtype.[10,11]

Recently, in the epithelioid subtype only, a nuclear grading system based on nuclear atypia and mitotic count has been proposed and shown to be associated with prognosis.[12]

Next-generation sequencing data indicate cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*), neurofibromatosis 2 gene (*NF2*), and BRCA1-associated protein 1 gene (*BAP1*) as the most frequently mutated genes in MPM.[13–15] BAP1 is a nuclear deubiquitinating enzyme[16] that was recently suggested to be a tumor suppressor gene with a role in cell proliferation and growth inhibition.[17] *BAP1* gene is located on chromosome 3p21, a region that harbors germline mutations associated with an inherited multicancer syndrome with a dominant autosomal transmission.[18] So far, *BAP1* is the first and only gene that has been proposed as influencing environmental carcinogenesis: when a germline *BAP1* mutation exists, it leads to a higher susceptibility to asbestos, favoring the clinical onset of MPM.[17,19–21] In addition, *BAP1* is the most frequently mutated gene in sporadic MPM[13–15,22]; the mutational status is associated with a less aggressive tumor phenotype and improved prognosis in familial mesothelioma[19] and probably also in sporadic mesothelioma.[23–25]

The loss of *BAP1* gene independently of the underlying mechanism (e.g., gene deletion or insertion, point mutation, gain, or loss) translates into nuclear negativity for BAP1 expression at immunohistochemical (IHC) staining, with a high concordance between the two techniques.[13,22,26] Loss of nuclear BAP1 protein expression is useful in differentiating both malignant mesothelioma versus pleural malignant mimickers (e.g., lung and ovarian cancers) and reactive versus malignant mesothelial proliferation with a high specificity despite the variable sensitivity.[25,27]

The aim of the present study was to (1) clarify the diagnostic usefulness of BAP1 IHC in characterizing MPM biphasic subtype with molecular confirmation and (2) correlate sporadic MPM BAP1 protein expression with clinicopathological and outcome data to validate its prognostic role.

Because of the challenging differential diagnosis between biphasic and epithelioid MPM with atypical reactive stroma[8] and in consideration of the fact that the cellular distribution of BAP1 IHC expression patterns among different MPM histotypes is not clearly established, we investigated the role of BAP1 IHC determination in 143 cases of MPM (including 101 surgically resected cases), aiming to further characterize the current histotypes of MPM. Furthermore, we performed molecular analysis of *BAP1* gene status in a pilot study series of 20 MPMs with different IHC staining patterns and then separately in the epithelial and stromal component of three cases of morphologically biphasic MPM to correlate both BAP1 protein and *BAP1* gene status. Finally, we correlated BAP1 IHC determination with clinicopathological and survival data.

We discovered that (1) BAP1 protein nuclear expression was lost in approximately two-thirds of epithelial and biphasic cases (and in 20% of sarcomatoid MPM), and *BAP1*-mutated tumors showed either a complete loss of the protein expression or a cytoplasmic staining pattern in epithelioid MPM; (2) atypical stromal cells associated with BAP1-negative epithelioid MPMs retained BAP1 expression, and molecular analysis of this stromal cell component confirmed the expected wild-type status; (3) higher disease stage, high nuclear grade, and BAP1 expression are independent predictors of poor prognosis irrespective of the histotype.

Materials and Methods

Tissue Collection

A total of 101 consecutive resected samples of MPM diagnosed between 2000 and 2012 and with enough leftover tissue were retrieved from the pathology files of the pathology units of the University of Torino at San Luigi Hospital (Orbassano, Turin, Italy) and City of Health and Science Hospital (Turin); furthermore, to enrich the study population for sarcomatoid and biphasic MPM cases we also collected 42 consecutive thoracoscopic biopsy samples from pathology unit files of San Luigi Hospital. For all cases, the main clinicopathological data were obtained and analyzed. Relevant clinical pathological findings included a mean age of 60 years and male-to-female ratio of 108:35. Of the surgical