Clinical Characteristics of Patients with Malignant Pleural Mesothelioma Harboring Somatic *BAP1* Mutations

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Introduction: Genomic studies of malignant pleural mesothelioma (MPM) have recently identified frequent mutations in the *BRCA-associated protein 1(BAP1*) gene. In uveal melanoma and clear cell renal cell carcinoma, *BAP1* mutations are associated with poor outcomes but their clinical significance in MPM is unknown. We therefore undertook this study to define the characteristics of patients whose MPM tumors harbor somatic *BAP1* mutation and to examine the relationship between *BAP1* mutation and survival.

Methods: We reviewed the charts of 121 patients with MPM tumors diagnosed between 1991 and 2009 tested for *BAP1* mutation, and extracted the following information: age at diagnosis, sex, histology, stage, smoking status, asbestos exposure, family or personal history of malignancy, and treatment including surgery, chemotherapy, and radiation as well as survival status.

Results: Twenty-four of the 121 tumors (20%) harbored somatic *BAP1* mutations. The percentage of current or former smokers among cases with *BAP1* mutations was significantly higher than in *BAP1* wild-type cases, (75% versus 42%; $p = 0.006$). However, the types of nucleotide substitutions in *BAP1* did not suggest that this association was because of a causative role of smoking in *BAP1* mutations. No other clinical feature was significantly different among those with and without *BAP1* mutations in their MPM. There was also no difference in survival according to somatic *BAP1* mutation status.

Conclusion: There is no apparent distinct clinical phenotype for MPM with somatic *BAP1* mutation. The significance of the more frequent history of smoking among patients with *BAP1*-mutated MPM warrants further study.

Key Words: Mesothelioma, *BRCA-associated protein 1*, Novel therapeutics.

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Despite aggressive multimodality therapy, malignant pleural mesothelioma (MPM) remains almost universally fatal with a median overall survival (OS) of 9 to 18 months from the time of diagnosis. First-line chemotherapy with cisplatin and pemetrexed improves survival by a few months but the benefit, if any, of additional chemotherapy is modest. To improve these poor outcomes, many novel agents are currently under investigation in clinical trials. Unfortunately, despite the activity of these agents in other malignancies, there has been little efficacy in MPM. In an effort to help develop more effective MPM-specific therapeutics, much effort has focused on identifying the key cancer genes driving MPM oncogenesis.

Although it has been known for many years that inactivating mutations in *neurofibromatosis 2* and deletions of *p16* are common in MPM, another commonly mutated gene, *BRCA-associated protein 1* (*BAP1*), was only recently identified.[1] In the initial report, *BAP1* mutations were identified in 23% of the MPM specimens. A variety of alterations were noted, including nonsense mutations, missense mutations, frameshifting indels, and mutations at or near splice sites. Loss of nuclear BAP1 protein expression was confirmed by immunohistochemistry in MPM with *BAP1* mutation. No association was identified between *BAP1* mutation and other commonly identified genetic alterations including *p16* loss and *neurofibromatosis 2* mutation and/or loss.

BAP1 is a 729 amino acid nuclear ubiquitin hydrolase with multiple functional domains and it has, therefore, been implicated in several cellular processes such as cell proliferation, DNA repair response, and chromatin dynamics.[2] Specifically, an interaction between BAP1 and host cell factor 1, which interacts with histone-modifying complexes during cell division, has been described.[1] More recently, it has been shown that BAP1, through an interaction with additional sex like 1 (ASXL1) forms the polycomb group repressive deubiquitinase complex, which affects stem cell pluripotency.[3]

Mutations in *BAP1* have also been described in other cancers. In particular, *BAP1* mutation is common in uveal melanoma (UM), the most frequent ocular tumor in white adults, where it is strongly associated with poor outcomes. About 50% of UMs will metastasize, at which point no effective treatment exists. A multigene expression profiling assay divides UMs into low- and high-metastatic risk with 84% of metastatic UM harboring *BAP1* mutation compared with only 4% of low-risk cases.[4] Likewise, in clear cell renal cell carcinoma, somatic *BAP1* mutations are associated with higher-grade tumors and shorter cancer-specific survival.[5]

Furthermore, recent reports have described germline *BAP1* mutations in families predisposed to MPM and UM[6] as well as atypical melanocytic tumors[7] and renal cell carcinoma.[8] These findings clearly suggest the existence of a new hereditary cancer predisposition syndrome, but the phenotype and penetrance of germline *BAP1* mutations remain unclear as does the role of gene–environment interactions in the development of these tumors.

Although *BAP1* mutation is considered a crucial event in the progression of UM,[4] the clinical impact of *BAP1* mutation in MPM remains unknown. Therefore, the purpose of this study was to characterize the clinical features of MPM patients whose tumors harbor *BAP1* mutations. Additionally, we examined the relationship between *BAP1* mutation and survival.

MATERIALS AND METHODS

With the approval of the Memorial Sloan-Kettering Cancer Center Institutional Review Board, the clinical records of 121 patients whose MPM tumors had been tested for *BAP1* mutation by conventional Sanger sequencing were reviewed. Sanger sequencing was carried out by a commercial vendor on a high-throughput platform (Beckman Coulter Genomics, Brea, CA). Polymerase chain reaction and resequencing were performed to confirm candidate mutations. These 121 tumor specimens were collected over 18 years from 1991 to 2009. Samples were selected based on the availability of frozen tumor specimens that were collected as part of a surgical banking effort. Clinical features were extracted, including age at diagnosis, sex, histology, stage, smoking status, pack-years, asbestos exposure, family or personal history of malignancy, and treatment including surgery, chemotherapy, and radiation, as well as survival status.

The relationship between *BAP1* mutation status and age, sex, histology, stage, smoking status, asbestos exposure, and family or personal history of malignancy was assessed using Fisher's exact tests. To ensure that outcomes stratified by *BAP1* mutation status were not confounded by differences in therapy, the relationship between *BAP1* mutation status and surgery, chemotherapy, and radiation was also assessed using Fisher's exact tests. OS was estimated using Kaplan–Meier method, with patients followed up from the time of diagnosis to death. Patients alive at the end of the study were censored at the time of the last available follow-up. The *BAP1* mutant and *BAP1* wild-type groups were compared with respect to OS, using the log-rank test.

RESULTS

Twenty-four of 121 MPM tumors harbored a somatic *BAP1* mutation, giving a frequency of 20% (95% confidence interval [CI], 13%–27%). Baseline characteristics of all patients included in this analysis are displayed in Table 1. The median age was 64 years (range, 33–81). Seventy percent were men. Histology was distributed with 76% epithelioid, 15% mixed, and 9% sarcomatoid. Stage at diagnosis was 7% stage I, 26% stage II, 42% stage III, and 25% stage IV. Forty-nine percent of patients were former or current smokers with median pack-years of 26 whereas 40% reported known asbestos exposure. Treatment included extrapleural pneumonectomy in 54%, pleurectomy/decortication in 36%, and no surgery in 10%. Fifty-six percent received chemotherapy and 60% received radiation. A family history of mesothelioma was reported in 2% of patients and a family history of any cancer in 55%. Ten percent of patients had a personal history of cancer before the diagnosis of MPM. In addition, 8% of patients had a personal or family history of skin cancer.

As shown in Table 2, with univariate analysis there was no significant difference in age, sex, asbestos exposure, surgery, chemotherapy, radiation, family history of mesothelioma, family history of cancer, and personal history of cancer among patients with *BAP1* mutant and *BAP1* wild-type MPM. However, a smoking history, either former or current, was more common among those with *BAP1* mutant tumors, 75% versus 42% ($p = 0.006$). There was no difference in pack-years among the smokers with respect to *BAP1* mutation status. On the basis of this association, we reviewed the spectrum of nucleotide substitutions in *BAP1*-mutated cases for evidence of a causal link to smoking. Fourteen of the 24 mutations identified in *BAP1* were point mutations. Among those 14 point mutations, only two represented classic smoking-associated nucleotide transversions (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/JTO/A450).

TABLE 1. Patient Characteristics

	% (N = 121)
Age, median (range)	64 (33–81)
Sex	
Male	70 (85)
Female	30 (36)
Histology	
Epithelioid	76 (92)
Mixed	15 (18)
Sarcomatoid	9 (11)
Stage	
I	7 (8)
II	26 (32)
III	42 (51)
IV	25 (30)
Former and current smoking	49 (59)
Pack-years	26 (52)
Known asbestos exposure	40 (48)
Surgery	
EPP	54 (66)
P/D	36 (43)
None	10 (12)
Chemotherapy	56 (68)
Radiation	60 (73)
Family history of mesothelioma	2 (3)
Family history of cancer	55 (67)
Personal history of cancer	10 (12)
Personal or family history of skin cancer	8 (10)

EPP, extrapleural pneumonectomy; P/D, pleurectomy/decortication.

TABLE 2. Clinical Features of Patients with *BAP1* Mutant Versus *BAP1* Wild-Type MPM

	BAP1 Mutant % (n = 24)	BAP1 Wild-Type % (n = 97)	p
Sex (M/F)	79/21	68/32	0.33
Median age, yr	65	63	0.31
Asbestos exposure	54	46	1
Former and current smoking	75	42	0.006
Median pack-years	30	25	0.47
Surgery			
EPP	54	55	1
P/D	38	35	
None	8	10	
Chemotherapy	54	57	0.82
Radiation	71	58	0.35
Family history of mesothelioma	4	2	1
Family history of cancer	38	47	0.49
Personal history of cancer	8	8	0.46

EPP, extrapleural pneumonectomy; P/D, pleurectomy/decortication.

There was no significant difference (p = 0.43) in the stage at presentation for *BAP1* mutant versus wild-type disease (Fig. 1). Similarly, there was no difference (p = 0.28) in the distribution of histologies among *BAP1* mutant versus wild-type disease (Fig. 2). No difference in OS was associated with *BAP1* mutation status. Those with *BAP1* mutant tumors had a median OS of 14.3 months (95% CI, 12.4–19.4) whereas those with *BAP1* wild-type tumors had a median OS of 14.8 months (95% CI, 10.6–37.3), p = 0.81. Adjusted for stage, the p value remains nonsignificant (p = 0.77).

DISCUSSION

Twenty percent of MPM tumors harbor mutations in *BAP1*. We find that a smoking history is significantly more common in MPM patients whose tumors harbor a *BAP1* mutation. The biological significance of this observation is presently unclear. Perhaps, *BAP1* mutation is a sequela of toxic exposure to smoking but the wide variety of mutations is not typical of tobacco smoke mutagenesis (only 2 of 24 mutations were G to T transversions, consistent with classic smoking-associated changes). Alternatively, *BAP1* somatic mutation could be an early event that might impair the response to carcinogenic damage inflicted by smoking in the development of mesothelioma. Other clinical characteristics were similar among those with mutant and wild-type *BAP1* MPM. Although another group reported that *BAP1* mutated cases were more likely to be partly or entirely epithelioid, this difference was not statistically significant in our larger cohort.[9] No difference in survival was observed. Similarly, although the initial report of *BAP1* mutations in MPM found an association between older age and *BAP1* mutation,[1] such association is not confirmed in this larger series.

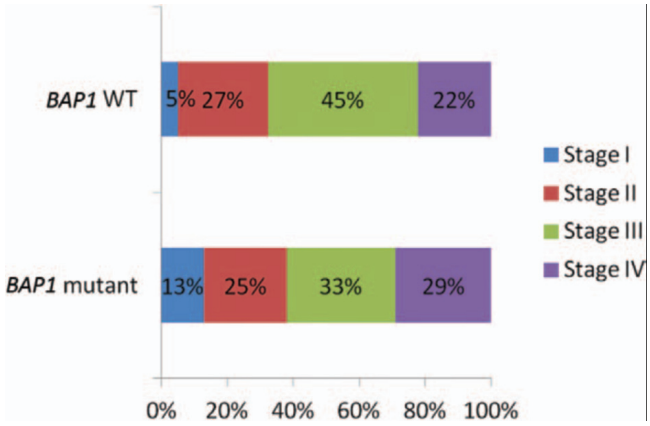

FIGURE 1. The distribution of stage by plotted by *BAP1* mutation status. There is no observed difference (p = 0.43). WT, wild type.

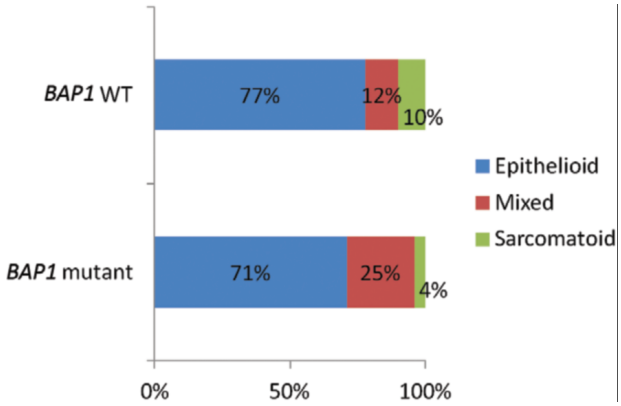

FIGURE 2. The distribution of malignant pleural mesothelioma histology is plotted by *BAP1* mutation status. There is no observed difference (p = 0.28). WT, wild type.

This analysis has some limitations. The retrospective nature of this project precluded thorough investigation of smoking status, asbestos exposure, personal history of malignancy, and family history of mesothelioma and other malignancies. Finally, as these specimens were obtained over an 18-year period, changes in therapy could have obscured differences in survival by *BAP1* mutation status.

Since the initial reports of germline *BAP1* mutations, numerous other neoplasms including meningiomas, renal cell carcinoma, lung cancer, breast cancer, ovarian cancer, pancreas cancer, and leukemia have been associated with *BAP1* mutation.[10–15] Although there is no apparent distinct phenotype for MPM with somatic *BAP1* mutation, further study is needed to identify and characterize patients with germline *BAP1* mutations. To help describe the spectrum of disease associated with germline *BAP1* mutation, we have initiated a prospective clinical protocol for patients with MPM, UM, and choroidal nevus (a premalignant eye tumor). First, patients will provide samples to participate in an anonymous estimate of the prevalence of germline *BAP1* mutations. Additionally, patients whose tumors harbor *BAP1*

mutation or who meet prespecified criteria will be offered identified germline *BAP1* testing. Those patients identified as carrying germline *BAP1* mutations will be asked to invite both potentially affected and unaffected family members for testing through our Clinical Genetics Service. We will also continue to explore the interaction of *BAP1* mutation with other somatic mutations, environmental exposures, and single-nucleotide polymorphisms. As with other malignancies, insights into the biology of MPM may help identify new, disease-specific therapeutic targets.

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