BRIEF REPORT

Mismatch Repair Protein Defects and Microsatellite Instability in Malignant Pleural Mesothelioma

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ABSTRACT

Introduction: Malignant pleural mesothelioma is an aggressive malignancy with limited systemic therapy options. Promising results have been reported with use of anti–programmed cell death 1 therapy; however, its benefits appear to be confined to a subgroup of patients. Microsatellite instability (MSI) results from the inactivation of DNA mismatch repair genes and results in a high tumor mutational burden, a phenomenon that has not been seen with mesothelioma. MSI and protein absence have been shown to correlate in colorectal cancer, such that most centers have adopted immunohistochemistry (IHC) to screen for MSI-high colorectal cancers. We profiled a large cohort of patients with mesothelioma to determine the rate of negative IHC staining results the four common mismatch repair proteins.

Design: A tissue microarray comprising 335 patients with malignant pleural mesothelioma were used. IHC for the four common mismatch repair proteins (mutL homolog 1; PMS1 homolog 2, mismatch repair system component; mutS homolog 2; and mutS homolog 6) was performed. Programmed death ligand 1 IHC staining with the E1L3N clone was also performed. DNA was isolated from IHC equivocal samples and analyzed for microsatellite instability by using the Promega MSI Analysis System (version 1.2, Promega, Madison, WI).

Results: Of the patients profiled, 329 had intact mismatch repair proteins by IHC. Six samples with IHC testing results indicating absent mismatch repair protein were analyzed for MSI and confirmed to be negative. Of the six IHC-negative samples, five were negative for programmed death ligand 1 staining and one sample had more than 5% staining.

Conclusion: In this large retrospective series, we were unable to identify any patients with malignant pleural mesothelioma with microsatellite instability. Response to anti–programmed cell death 1–based immunotherapy may be driven by other mechanisms.

Keywords: Malignant pleural mesothelioma; Microsatellite instability; Mismatch repair protein; Immunohistochemistry; PD-L1

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Introduction

Microsatellite instability (MSI) arises from inactivation of any of several mismatch repair (MMR) genes—mutL homolog 1 gene (*MLH1*); PMS1 homolog 2, mismatch repair system component gene (*PMS2*); mutS homolog 2 gene (*MSH2*); and mutS homolog 6 gene (*MSH6*)—resulting in failure to repair the routine errors that occur during replication of short repeats in DNA sequences.[1,2] These translated proteins form heterodimers that repair DNA damage, with the most common and relevant to tumorigenesis being mutL homolog 1 (MLH1)/PMS1 homolog 2, mismatch repair system component (PMS2) and mutS homolog 2 (MSH2)/mutS homolog 6 (MSH6).[3] Immunohistochemistry (IHC) for MMR has a specificity of 88.8%, which is comparable to the specificity of 90.2% with MSI testing by polymerase chain reaction, making IHC a valid screening method for deficient MMR.[4]

Deleterious germline mutations occur in the MMR genes in families with Lynch syndrome, in which a second hit in the unaffected wild-type allele through loss of heterozygosity, mutation, or hypermethylation leads to cancers.[3] The classical association of Lynch syndrome is the development of colorectal cancers; however, it is also linked with other cancer types, especially endometrial cancer, but also including ovarian, gastric, and prostate cancers and glioblastoma.[5] It is also now recognized that MSI can occur in the absence of germline MMR mutations on account of epigenetic inactivation of *MLH1* by promoter methylation.[6]

Determining the MSI status in tumors has become clinically relevant because of its prognostic and predictive implications. From a prognostic value, mutations arising from MSI have been observed to drive tumorigenesis by inactivating tumor suppressor genes.[7] MSI-high colorectal carcinomas have improved clinical outcomes, such that chemotherapy is not recommended for stage II MSI-high colon cancers.[8] Moreover, MSI-high tumors elicit a high tumor mutational burden and are associated with responses to programmed cell death 1 (PD-1) inhibitors, presumably from a higher neoantigen load leading to enhanced T-lymphocyte recognition.[9]

Malignant pleural mesothelioma (MPM) is an aggressive thoracic malignancy with a median survival of 12 months.[10] After platinum doublet chemotherapy with an anti–vascular endothelial growth factor agent, there is no standard of care second-line systemic option. The Keynote-028 phase 1B study included a cohort of 25 heavily pretreated patients with MPM with a level of programmed death ligand 1 (PD-L1) staining higher than 1%. Pembrolizumab, an anti–PD-1 antibody, resulted in an objective response rate in five patients (20%) and disease stability in 13 patients (52%), with an associated median response duration of 12 months.[11] Although encouraging, this response rate was modest given the treated population, which was biomarker and biology selected (32% with two or more lines of therapy). A subsequent retrospective study of 46 PD-L1–unselected patients who had received a median of two previous lines of therapy and had been treated with pembrolizumab showed a similar objective response rate of 15%, with the median duration of response not yet reached.[12]

We hypothesized that this minor group of anti–PD-1 responders might have either a sporadic or germline defect in MMR. There are only two reported cases in the literature of MSI in MPM[13] and only one report of a patient with peritoneal mesothelioma who was found to have a defect in MMR (loss of *MLH1/PMS2*).[14] We sought to profile a large cohort of MPM archival tumor samples to investigate whether there was any loss of MMR proteins suggesting an MSI-high phenotype.

Methods

Under a human research ethics committee–approved protocol, archival tissue specimens from 335 patients that were collected from December 1989 to December 2013 were obtained from the Austin Hospital pathology department. All patients had histological confirmation of MPM by a pathologist. Clinicopathological information, including age, sex, smoking history, asbestos exposure, and histological subtype, was gathered.

Tissue microarrays (TMAs) were created by using a Mark II TMA arrayer (Beecher Instruments, Sun Prairie, WI) from formalin-fixed, paraffin embedded blocks with three cores per patient, each with a size of 1×1.5 mm. The primary monoclonal antibodies used on these TMAs were MLH1 (clone ES05 [Dako, Carpinteria, CA]), MSH2 (clone G219-1129 [Cell Marque,]), MSH6 (clone 44; Cell Marque, Rocklin, CA), and PMS2 (clone EP51 [Dako]). Bound primary antibodies were detected by using a Ventana UltraView Universal DAB detection kit (Ventana Medical Systems, Tucson, AZ) for MLH1, MSH2, and MSH6 and a Ventana Optiview DAB IHC Detection Kit (Ventana) for PMS2. Slides were scanned by using an Aperio ScanScope XT system (Leica Biosystems, Nussloch, Germany). Normal expression was defined as nuclear staining within tumor cells, with infiltrating lymphocytes used as a positive internal control. Negative protein expression was defined as complete absence of nuclear staining within tumor cells in the context of positive labeling in internal noncancerous tissue.

Samples that were negative for MMR according to IHC staining were analyzed for MSI. DNA was isolated from two areas on each TMA, one containing tumor and the other containing nonmalignant epithelial tissue, by using the DNeasy Blood and Tissue kit (Qiagen, Valencia, Ca).