Anaplastic Lymphoma Kinase Gene Rearrangement in Children and Young Adults With Mesothelioma

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ABSTRACT

Introduction: Children and young adults diagnosed with malignant mesothelioma may have unique genetic characteristics. In this study, we evaluated for the presence of the anaplastic lymphoma kinase (ALK) translocations in these patients.

Methods: In a prospective study of mesothelioma natural history (ClinicalTrials.gov number NCT01950572), we assessed for the presence of the ALK translocation in patients younger than 40 years, irrespective of the site of disease. The presence of this translocation was assessed by means of fluorescence in situ hybridization (FISH). If the patients tested positive for the ALK translocation, both immunohistochemistry and RNA sequencing were performed on the tumor specimen.

Results: Between September 2013 and December 2018, 373 patients were enrolled in the mesothelioma natural history study, of which 32 patients were 40 years old or younger at the time of their mesothelioma diagnosis. There were 25 patients with peritoneal mesothelioma, five with pleural mesothelioma, one with pericardial mesothelioma, and one with bicompartmental mesothelioma. Presence of an ALK translocation by FISH was seen in two of the 32 patients (6%) with mesothelioma. Both patients, a 14-year-old female and a 27-year-old male, had peritoneal mesothelioma and had no history of asbestos exposure, prior radiation therapy, or predisposing germline mutations. Neither had detectable ALK expression by immunohistochemistry. RNA sequencing revealed the presence of an STRN fusion partner in the female patient but failed to identify any fusion protein in the male patient.

Conclusions: Young patients with peritoneal mesothelioma should be evaluated for the presence of ALK translocations. Presence of this translocation should be assessed by FISH and these patients could potentially benefit from tyrosine kinase inhibitors targeting ALK.

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Keywords: Mesothelioma; Peritoneal mesothelioma; Young patients; ALK mutation; Targeted therapy; FISH testing

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Introduction

Malignant mesothelioma is an uncommon malignancy accounting for 3000 new cases annually.\(^1\) Although most cases are pleural mesothelioma, an estimated 25% involve the peritoneum.\(^2\) Although malignant mesothelioma has had a poor prognosis historically, survival has been substantially improved with current standard of care treatment using cytoreductive operation and hyperthermic intraperitoneal chemotherapy (HIPEC).\(^2\) The pathogenesis of peritoneal mesothelioma is unclear, having weaker associations with asbestos exposure compared with pleural mesotheliomas.\(^3\) Moreover, as there is a long latency period between exposure to asbestos and development of mesothelioma, asbestos is unlikely to play an etiologic role in mesothelioma diagnosed in children and young adults. In addition, predisposing germline mutations in \(BAP1\) and other DNA repair genes are uncommon in patients diagnosed before the age of 40 years.\(^4\)

In patients with no history of asbestos exposure, prior radiation therapy, or predisposing germline mutations, factors such as the presence of oncogenic tumor mutations may play a role. Loharamtaweethong et al. first reported the presence of anaplastic lymphoma kinase (ALK) translocation in the tumor of a 10-year-old female with peritoneal mesothelioma.\(^5\) A study by Hung et al.\(^6\) looked for the presence of ALK rearrangements in 88 consecutive patients with peritoneal mesothelioma seen at their institution. Fluorescence in situ hybridization (FISH) testing confirmed three cases (3.4%) of ALK translocations with novel partners—\(STRN\), \(ATG6L1\), and \(TPM1\). Interestingly, all three patients were young females (median age 36 y, range 17–51 y) with no history of asbestos exposure.\(^6\) As this study evaluated patients of any age and only those with peritoneal mesothelioma, we decided to systematically assess the presence of ALK translocations in young patients with mesothelioma (40 y or younger) regardless of the site of origin of their mesothelioma. These patients were prospectively enrolled in the mesothelioma natural history (MNH) protocol at the National Cancer Institute (ClinicalTrials.gov number NCT01950572).

Materials and Methods

Patients

The patients selected for evaluation of ALK translocations were enrolled in the prospective National Institutes of Health MNH protocol (clinicaltrials.gov # NCT01950572). All the patients with malignant mesothelioma, irrespective of the site of the disease, evaluated at the Thoracic Oncology Clinic of the National Cancer Institute were offered participation in this study. For the purposes of this study, we selected all the patients who were 40 years old or younger at the time of their diagnosis and were enrolled between September 2013 and December 2018, which was the data cutoff point. Clinical characteristics of the patients were obtained from the MNH protocol database and outside physician notes. This included environmental exposures, family history of cancers, and medical history.

Evaluation of ALK Rearrangement

The initial evaluation for ALK rearrangements was done using archival formalin-fixed paraffin-embedded tissue, obtained at diagnosis of mesothelioma. We first performed FISH on the sample using a Vysis LSI Dual Color ALK BA probe (Abbott Molecular, Inc.). Samples were considered positive if more than 15% of cells showed positivity. If positive, samples were evaluated for ALK expression by immunohistochemistry (IHC) and RNA sequencing (RNAseq). IHC was performed using the monoclonal 5A4 antibody (Leica Biosystems Inc.). RNAseq to determine the potential fusion partners in cases with ALK rearrangement was done by constructing RNA sequencing libraries using TruSeq RNA exome kits (TruSeq RNA Access Library Prep Kit) and sequenced using 2 × 75bp paired-end protocol on a NextSeq500 (Illumina).

Results

Characteristics of Patients

Of the 373 patients enrolled in the MNH study, we identified 32 patients who were 40 years old or younger (median 32 y; range 12–38 y) at the time of their diagnosis (Table 1). This included 18 male and 14 female

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patients. There were 25 patients with peritoneal mesothelioma, five with pleural mesothelioma, one with pericardial mesothelioma, and one with bicompartmen-
tal mesothelioma, and almost all had epithelioid histology (96.9%). More than half of the patients were less
than 30 years old at diagnosis. On the basis of the report of the patients, exposure to asbestos and history of ra-
diation were uncommon.

**Presence of ALK Rearrangement Using FISH**

We detected **ALK** translocations in two of the 32 patients evaluated using FISH. Both patients had peri-
toneal mesothelioma with no history of asbestos exposure or radiation therapy. They also did not have germline mutations in DNA repair genes, which would predispose them to mesothelioma. Of note for both patients, IHC staining was negative for **ALK** expression.

**Patient 1.** This female patient was diagnosed with peritoneal mesothelioma (Fig. 1A) at the age of 14 years
and was initially treated with neoadjuvant cisplatin and pemetrexed followed by cytoreductive operation with HIPEC and then adjuvant chemotherapy with cisplatin and pemetrexed. Following disease progression, 4 months after operation, she enrolled in a clinical trial and is continuing on treatment with no evidence of disease since the last follow-up in May 2019. She is more than 5 years from her diagnosis of mesothelioma. FISH testing of her tissue found an **ALK** translocation involving 118 of 118 nuclei (100%) (Fig. 1C).

**Patient 2.** This was a male patient who was diagnosed with peritoneal mesothelioma (Fig. 1B) at the age of 27
years. He was initially treated with cytoreductive operation and HIPEC. After disease recurrence, he received
cisplatin and pemetrexed, followed by antimesothelin therapies with SS1P and anetumab ravtansine and then
cembrolizumab; progressing on all lines of treatment. Evaluation of his tumor by FISH demonstrated an **ALK** translocation involving 36 of 108 identified nuclei (33%) (Fig. 1D). On the basis of these results, he was treated
with crizotinib off protocol but could not complete the recommended treatment owing to the side effect of worsening fatigue. He died from progressive disease at the age of 33 years, 4 months after the initiation of crizotinib.

Identification of ALK Fusion Partners

To identify partners of the ALK gene rearrangement, we performed RNAseq of the tumors from patients 1 and 2. RNAseq of the tumor from patient 1 revealed the presence of ALK-STRN translocation, with the breakpoint involving exon 3 of STRN and intron 19 of ALK (Fig. 2). RNASeq of the tumor from patient 2 did not reveal a fusion partner.

Discussion

In young patients with mesothelioma, 40 years old or younger at diagnosis, we identified two of 32 patients (6.3%) with an ALK translocation. Both patients, a male and a female, had peritoneal mesothelioma of epithelial histology and no known risk factors for mesothelioma. Recent reports have described the presence of ALK translocation in only young female patients with peritoneal mesothelioma. Our finding confirms and extends these reports and demonstrates that such translocations can be present in male patients with peritoneal mesothelioma but are not present in young patients with mesothelioma involving other sites. Therefore, the ALK translocation could potentially represent a novel driver mutation in peritoneal mesothelioma especially in children and young adults. Prior studies have failed to identify an ALK translocation in patients with pleural mesothelioma. Our study confirms this finding although limited by small number of patients with pleural mesothelioma who were 40 years old or younger at diagnosis. Our only patient with pericardial mesothelioma (aged 21 years) did not have the ALK translocation.

Located on chromosome 2, the ALK loci is a frequent site for mutation in a variety of tumors, being involved in translocations with other genes to create fusion proteins with constitutive tyrosine kinase domain activity. Our first patient harbored the ALK-STRN fusion, involving intron 19 of ALK and exon 3 of STRN. This exact mutation was present in one of the patients reported by Hung et al. This translocation has been previously described as having an aggressive nature in both lung and thyroid cancers, though fairly indolent in renal cell carcinomas. Our patient with this fusion has had an indolent course, being alive 5 years from diagnosis. Although our second patient had demonstrated ALK positivity on FISH, he did not have an identifiable fusion partner on RNAseq of his tumor. Possible explanations for this includes limitations of using formalin-fixed paraffin-embedded tissues and the inability of next-generation sequencing to detect fusion partners because breakpoints may occur farther from the sequence being analyzed.

Finally, our study highlights the necessity for FISH testing for the ALK translocation in patients with mesothelioma, as IHC seems to have lower sensitivity. This stands in contrast to Hung et al., who noted ALK positivity by IHC, despite using the same antibody in our study. However previous reports in lung cancer have

Figure 2. Peritoneal mesothelioma with ALK-STRN fusion mapping breakpoint to exon 3 of STRN and intron 19 of ALK. The resulting fusion protein contains domains for the Striatin protein and the kinase domain for ALK.
shown discordance between IHC and FISH in testing, suggesting that both should be performed in parallel.\textsuperscript{13}

Targeted therapy, with tyrosine kinase inhibitors that bind the receptor tyrosine kinase domain of the ALK protein, have been shown to be efficacious in cancers harboring these mutations and are approved for treatment.\textsuperscript{14} Although one of our patients who received crizotinib did not respond to it, a recent case report of a 13-year-old female with peritoneal mesothelioma carrying the ALK-STRN rearrangement found dramatic response to the ALK inhibitor, ceritinib.\textsuperscript{15} Given the benefits in the 5% of patients with lung adenocarcinoma who harbor this mutation, it is routinely recommended that patients undergo testing for this mutation.\textsuperscript{14} Our results and those by Hung et al.\textsuperscript{6} report the presence of the ALK translocation in a similar proportion of patients with peritoneal mesothelioma, with 8% and 3.4% of patients, respectively, harboring this translocation. These patients tend to be younger with no other risk factors for mesothelioma. Given the potential benefits of tyrosine kinase inhibitors against ALK, testing for the ALK mutation should be considered in patients with peritoneal mesothelioma. Based on all the published data thus far and our study, the emerging story is of the presence of ALK translocation mainly in children and young adults.\textsuperscript{5,6,15} Whether all patients with peritoneal mesothelioma should be tested for ALK translocation is an open question and needs further study. However, we would recommend testing all children and young adults with peritoneal mesothelioma for an ALK translocation because it has therapeutic relevance. Finally, to demonstrate the efficacy of ALK inhibitors in peritoneal mesothelioma, multicenter trials are needed given the rarity of this disease.

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**References**