Microsatellite Instability Is Uncommon in Lymphoepithelioma-like Carcinoma of the Lung

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Abstract

Primary lymphoepithelioma-like carcinoma of the lung (LELC) shares some morphologic and clinical characteristics with malignancies associated with microsatellite instability (MSI). The aims of our study were to determine the MSI status in LELC and compare these findings with stage I non–small cell lung carcinoma (NSCLC) with marked lymphocytic host response (MLHR). We assessed MSI by a DNA-based polymerase chain reaction assay using mononucleotide (BAT25 and BAT26) and dinucleotide (D2S123, D5S346, and D17S250) repeats. MSI was detected in 2 (29%) of 7 LELC cases with only 1 marker (D17S250), and in 3 (19%) of 16 NSCLC cases with MLHR with only 2 markers (1 D2S123 and 2 D17S250). Loss of heterozygosity (LOH) was detected at 1 or 2 of 3 dinucleotide repeats in 11 NSCLC cases (69%) with MLHR and 3 LELC cases (43%) (P = .36). The overall frequencies of LOH in NSCLC with MLHR were 29% and 19% in LELC (P = .55). MSI is very uncommon in LELC, indicating that MSI is not an important event in carcinogenesis for this tumor subtype. The presence of LOH suggests a probable role of tumor suppressor genes in LELC carcinogenesis.

The majority of tumors have evidence of mutational inactivation of tumor suppressor genes and activation of oncogenes. Microsatellite instability (MSI) is a somatic event that was initially described in colorectal cancer, representing an alternative genetic pathway in the development of cancer.1 It has been shown that patients with hereditary nonpolyposis colon cancer (HNPCC) carry germline mutations, primarily in the mismatch repair (MMR) genes hMSH2 and hMLH1 and, to a lesser extent, in hMSH6 and hPMS2.2-4 The defective MMR system then allows for errors in the genetic sequence to be propagated as MSI. MSI+ colon tumors are often poorly differentiated and have a lymphocytic infiltrate.5,6 They also have a lower incidence of lymph node metastases. MSI has also been reported in a number of noncolonic sporadic cancers such as endometrial, gastric, and lung cancer.7 There are conflicting data on the frequency of MSI in lung cancer, ranging from 0% to 75% in non–small cell lung carcinoma (NSCLC) and 45% in small cell lung carcinoma.8-12

Primary lymphoepithelioma-like carcinoma of the lung (LELC) is a rare neoplasm of uncertain tumorigenesis. A strong association between Epstein-Barr virus (EBV) and LELC has been reported in Asian patients, but studies failed to demonstrate this association in Western patients.13-19 Similar to HNPCC and associated malignancies demonstrating MSI, LELC shows a poorly differentiated epithelial component admixed with lymphocytic infiltrate.20 It is also associated with a lower incidence of nodal disease or distant metastases and a better prognosis than other NSCLCs.21-25 The mean age of patients with LELC is 10 years younger than that of the patients with other histologic types of NSCLC. In contrast with other lung carcinomas, LELC tends to affect nonsmokers more frequently.
At the molecular level, few studies have explored the potential role of tumor suppressor genes in carcinogenesis of LELC of the lung. They failed to demonstrate a significant role of \( \text{p53} \) or \( \text{bcl-2} \) in tumorigenesis in these rare tumors. These findings indicate that other genetic mechanisms may have a role in the pathogenesis and prognosis of LELC.

Because of the overlapping morphologic and clinicopathologic features between LELC and cancers associated with MSI, we postulated that an alternative molecular pathway of DNA MMR defect and resultant MSI may be present in LELC. This study examined the MSI status of LELC and NSCLC.

Materials and Methods

We obtained 7 cases of LELC and 16 cases of stage 1 NSCLC with MLHR from the paraffin-block archives of the University of Pittsburgh Medical Center, Pittsburgh, PA. Cases were selected using conventional histologic examination and criteria of the World Health Organization histologic typing of lung and pleural tumors. LELCs were composed of large tumor cells with abundant cytoplasm, vesicular chromatin, and prominent nuclei admixed with a heavy lymphocytic infiltrate and occasional plasma cells. The NSCLC group was composed of 10 poorly differentiated adenocarcinomas and 6 poorly differentiated squamous cell carcinomas. The lymphocytic host response in NSCLC was subjectively graded by 2 pathologists (S.D. and S.A.Y.). There was variation in the pattern of lymphocytic infiltration in selected cases of NSCLC: 14 cases showed peritumoral infiltrate confined to the stroma, and 2 cases of adenocarcinoma showed intratumoral lymphocytes dispersed among the tumor cells. Image II. All LELC cases were EBV– as demonstrated by immunohistochemical and in situ hybridization studies. Immunoperoxidase studies demonstrated that all LELCs were positive for cytokeratin 7 and negative for cytokeratin 20, cytokeratin 5/6, and p63. Only 1 case of LELC was positive for thyroid transcription factor.

In each case, normal lung parenchyma and tumor tissues were microdissected from two to three 4-µm-thick unstained histologic sections under visualization using a stereoscopic microscope. DNA was extracted by Proteinase K digestion and a DNEasy DNA extraction column (Qiagen, Valencia, CA).

Five standard sets of polymerase chain reaction primers were used to amplify the short tandem repeat units recommended by the National Cancer Institute for detecting MSI. Two of these loci (\( \text{BAT25} \) and \( \text{BAT26} \)) are mononucleotide repeats, and 3 (\( \text{D2S123} \), \( \text{D5S346} \), and \( \text{D17S250} \)) are dinucleotide repeat units. Amplification products were detected with capillary electrophoresis and fragment analysis (Prism 3100 and GeneScan software, Applied Biosystems, Foster City, CA). A positive control sample from a patient with hereditary colon carcinoma was run in tandem. Dinucleotide heterozygous markers were also examined for evidence of clonal loss of heterozygosity (LOH), which would be apparent if the allele peak ratio from the tumor and normal tissue samples was less than 0.7 or greater than 1.43.

A microsatellite marker was scored as MSI+ when there was a different allele pattern for the microsatellite locus in tumor tissue as compared to normal control DNA. MSI-high was defined as more than 30% of the examined markers had

![Image II](https://academic.oup.com/ajcp/article-abstract/127/2/282/1760146)

**Image II**

A, Lymphoepithelioma-like carcinoma of the lung. Large tumor cells with abundant cytoplasm, vesicular chromatin, and prominent nuclei admixed with heavy lymphocytic infiltrate (H&E, original magnification, ×40). B, Adenocarcinoma of the lung with marked lymphocytic host response (H&E, original magnification, ×40).
MSI (≥2 markers of 5). Tumor samples were considered microsatellite stable if no unstable microsatellites were found and considered to have low frequency MSI (MSI-low) when MSI was present in fewer than 30% of the markers (1 marker of 5).28

Statistical analyses were performed by using the χ² test, Fisher exact probability test, and t test. Statistical significance was defined as a P value of less than .05.

**Results**

The mean age of 5 women and 2 men with LELC was 63 years (range, 63-76 years) and of 9 women and 7 men with stage I NSCLC was 67 years (range, 50-79 years). The mean size of LELCs was 3.1 cm and of stage I NSCLCs, 3.3 cm.

Only 2 (29%) of 7 cases of LELC were MSI-low. Both tumors showed MSI at 1 locus (D17S250) [Image 2]. Of 16 cases of NSCLC with MLHR, 3 (19%) showed MSI at only 2 markers (1 at D2S123 and 2 at D17S250) and were considered MSI-low.

LOH was detected at 1 or 2 of the 3 dinucleotide repeat markers in 11 cases (69%) of NSCLC with MLHR and 3 cases (43%) of LELC (P = .36). The overall frequency of LOH in NSCLC with MLHR was 29% and was 19% in LELC (P = .55) [Figure 1]. LOH was most frequently identified at the dinucleotide repeat D5S346, which is located at 5q23, in both groups of tumors (9 cases of NSCLC with MLHR and 3 cases of LELC).

**Discussion**

Most patients with LELC of the lung have early-stage disease at their initial examination. The etiologic and molecular events responsible for occurrence of these tumors are almost entirely unknown. Tobacco smoking has been indicated as a major risk factor in the carcinogenesis of lung carcinomas.29 Only 26% to 40% of patients with LELC of the lung have a tobacco smoking history, in contrast with more than 60% of patients with other types of lung carcinomas.30 The low frequency of an association with cigarette smoking suggests that smoking may not be the most important etiologic factor in LELC. It seems that association of EBV and LELC in the lung depend on racial and geographic factors.9 Primary pulmonary LELC has a higher incidence in the Asian population and is strongly associated with EBV in this population. However, this association was not found in Western patients. Furthermore, the absence of the EBV genome in the LELC in the Western population suggests that EBV is not an important factor in its pathogenesis.

The activation of oncogenes, the loss of function of tumor suppressor genes, and impaired DNA MMR function are mechanisms of tumorigenesis known to be involved in the development of solid tumors, including lung carcinomas. There are very limited data about these processes in LELC of the lung. Chan et al.21 in a study of 23 LELCs of the lung, demonstrated by immunohistochemical analysis a
very low frequency of expression of p53 in 4 cases. In the same study, no tumors showed c-erb-B2 oncprotein expression. These results were in contrast with conventional NSCLC, in which p53 and c-erb-B2 can be detected in more than 50% of cases.31,32

In our study, we used a standard polymerase chain reaction–based assay to assess for MSI at the DNA level. Only 2 cases of LELC were considered to have evidence of MSI at the DNA level, and both of these were low levels. Similar to previous reports, we also confirmed that smoking-related primary lung cancers had infrequent MSI. Only 3 cases of NSCLC in our study were considered MSI-low. We also looked at the dinucleotide markers used for evidence of LOH. In stage I NSCLC with MLHR, we found significant detectable LOH, which is similar to previous reports. It is interesting that almost half of the LELCs showed LOH at the same markers. Both groups of tumors most frequently showed LOH at D5S346, suggesting the possibility of a contribution of pathogenesis from a tumor suppressor gene in this chromosomal region (5q23).

Our results suggest that despite differences in clinical manifestations and risk factors, carcinogenesis of LELC is most likely very similar to other types of NSCLC. These results are similar to those reported for medullary carcinoma of the breast, which has morphologic and clinical characteristics similar to those of LELC of the lung and is not commonly associated with MSI.33,34 Because only rare tumors in our study displayed MSI-low, our data suggest that the DNA MMR system is not frequently altered in LELC. The presence of LOH indicates that inactivation of tumor suppressor genes is the most likely mechanism of tumorigenesis in LELC. Because cancer development requires an accumulation of genetic alterations, it would be of interest to further explore other critical chromosomal loci involved in lung carcinogenesis that may have a role in the development of LELC. Although there was no statistical difference in overall frequency of LOH between the 2 analyzed groups in our study, a lower frequency of LOH in LELC may be responsible for its relatively different clinical and biologic behavior.

These molecular results suggest that the carcinogenesis of LELC is likely to be similar to that of other types of NSCLC. We showed that the DNA MMR system is not frequently altered in LELC and that LOH is seen in LELC and NSCLC. Additional molecular studies of tumor suppressor genes frequently involved in lung carcinogenesis are needed to elucidate the mechanisms responsible for the development of LELC.

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References


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