Original Article

Genetic mutations in lung enteric adenocarcinoma identified using next-generation sequencing

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Abstract: Primary lung enteric adenocarcinoma is a rare type of invasive lung carcinoma. Its morphological and immunohistochemical characteristics are similar to those of metastatic colorectal carcinoma, but there is no associated primary colorectal carcinoma. The purpose of this study is to identify mutations by assessing the genetic profile of lung enteric adenocarcinoma with next-generation sequencing (NGS). This study included 11 lung enteric adenocarcinoma patients (5 males and 6 females) from three different centers who received treatment between Feb 2013 and Dec 2016. Immunohistochemical analysis failed to reveal any markers that differentiated this carcinoma from primary gastrointestinal adenocarcinoma. NGS analysis identified ALK/ROS1 primary point mutations in 5 patients (71.42%, 5/7) and MSH2/MSH6 point mutations in 3 patients (42.86%, 3/7). There was no case with drive genes changed, such as EGFR mutation, ALK rearrangement, ROS1 rearrangement, RET rearrangement, MET amplification or 14 exon skipping mutation. The median overall survival of the 11 lung enteric adenocarcinoma patients was 9.0 months. Further, subgroup analysis showed that the median OS of patients with ALK/ROS1 primary point mutations was 6.5 months and that of patients with MSH2/MSH6 primary point mutations was 26.0 months. These two mutations were the most frequent features, but this carcinoma generally showed genetic heterogeneity. Even though we have revealed some hitherto unidentified genetic mutations associated with lung enteric adenocarcinoma, the findings are preliminary and further investigations on more patients will be required to validate our findings.

Keywords: Lung enteric adenocarcinoma, ALK, ROS1, MSH2, MSH6, gene mutation

Introduction

According to the 2015 World Health Organization classification of lung tumors, lung enteric adenocarcinoma is a recognized subtype [1]. Lung enteric adenocarcinoma was first reported in 1991 by Tsao and Fraser [2]. It is described as a rare variant that is predominantly composed of cellular structures that resemble those of intestinal adenocarcinoma. There is also a possibility that it is a tumor that metastasizes from primary gastrointestinal adenocarcinoma [1, 3].

Immunohistochemistry is often used to differentiate between gastrointestinal adenocarcinoma and lung enteric adenocarcinoma: the majority of primary lung adenocarcinomas are positive for cytokeratin (CK7), thyroid transcription factor-1 (TTF-1), and napsin A, whereas among intestinal adenocarcinomas, colorectal tumors are positive for caudal type homeobox 2 (CDX-2), mucin 2 (MUC2) and cytokeratin 20 (CK20) and upper gastrointestinal tract tumors are positive for CDX-2 and CK7. The differentiation of this rare histologic type of lung carcinoma from primary gastrointestinal adenocarcinoma is relevant and has important implications. Indeed, a careful analysis of the clinical history of patients should be conducted to exclude metastatic lesions associated with primary gastrointestinal adenocarcinoma.
Mutations in LEA by NGS

Currently, advanced non-small cell lung cancer (NSCLC) is managed using targeted treatment based on genetic mutations that are present in patients. The most common mutation in lung adenocarcinoma is found in the gene that encodes for the epidermal growth factor receptor (EGFR), and this mutation is found in 60% of Asian patients [4]. In addition, anaplastic lymphoma kinase (ALK) gene rearrangements are found in 2-7% of patients [5]. Wang et al. [6] analyzed the EGFR gene mutation status in 24 cases of small intestinal adenocarcinoma (SIA) by DNA sequencing, and the results revealed that only two patients had EGFR mutations. Moreover, their results indicated that the EGFR mutations in the two cases were minor, and therefore, most patients with SIA may be unsuitable for treatment with the EGFR tyrosine kinase inhibitor (TKI). In addition, another research [7] reported 9 cases of lung enteric adenocarcinoma in which all the tumors expressed wild-type EGFR and KRAS genes. These findings indicate that the phenotype and gene mutations of lung enteric adenocarcinoma are distinct from those of other pulmonary adenocarcinomas. Therefore, the gene mutations associated with lung enteric adenocarcinoma require deeper investigation.

Next-generation sequencing (NGS) is regarded as a sequencing method that allows for the generation of simultaneous reads of a considerable number of DNA sequences in a parallel way [8, 9]. The National Comprehensive Cancer Network guidelines recommend the use of methodologies that can detect multiple molecular alterations simultaneously, as these may hold great promise for clinical testing in the future. NGS, being one such method, may be able to provide us with a better understanding of lung cancer pathogenesis at the genetic level. Thus, NGS-based detection could be used to discover novel molecular alterations in NSCLC.

We performed this study to analyze the pathogenesis and survival of 11 lung enteric adenocarcinoma cases and investigate gene mutations by NGS. To the best of our knowledge, no previous study has applied NGS in the analysis of lung enteric adenocarcinoma patients.

Patients and methods

Patient eligibility

Eleven lung enteric adenocarcinoma patients, who received treatment at three medical cen-

Table 1. Clinical profile and outcome of 11 patients with lung enteric adenocarcinoma

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Gender/Age</th>
<th>Smoking status</th>
<th>Stage</th>
<th>Result of targeted NGS</th>
<th>OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>F/53</td>
<td>NO</td>
<td>IV</td>
<td>Failed</td>
<td>17.0</td>
</tr>
<tr>
<td>3</td>
<td>F/48</td>
<td>NO</td>
<td>IV</td>
<td>Failed</td>
<td>9.0</td>
</tr>
<tr>
<td>4</td>
<td>M/59</td>
<td>YES</td>
<td>IV</td>
<td>Failed</td>
<td>6.0</td>
</tr>
<tr>
<td>5</td>
<td>M/65</td>
<td>YES</td>
<td>IV</td>
<td>ALK p.G1326A BRAF p.A308T</td>
<td>6.0</td>
</tr>
<tr>
<td>9</td>
<td>M/56</td>
<td>YES</td>
<td>IV</td>
<td>TP53 p.H193R ALK p.G1474E</td>
<td>4.0</td>
</tr>
<tr>
<td>10</td>
<td>M/64</td>
<td>NO</td>
<td>IV</td>
<td>TP53 p.V173L ROS1 p.E268K</td>
<td>7.0</td>
</tr>
<tr>
<td>11</td>
<td>M/72</td>
<td>YES</td>
<td>IV</td>
<td>Failed</td>
<td>14.0</td>
</tr>
</tbody>
</table>

Table 2. Expression of immunohistochemical markers in the 11 cases of patients with lung enteric adenocarcinoma

<table>
<thead>
<tr>
<th>Case No.</th>
<th>CK7</th>
<th>TTF-1</th>
<th>Napsin A</th>
<th>CDX2</th>
<th>CK20</th>
<th>Villin</th>
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<td>ND</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
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<td>(+)</td>
</tr>
<tr>
<td>3</td>
<td>(+)</td>
<td>(-)</td>
<td>ND</td>
<td>ND</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>4</td>
<td>(-)</td>
<td>(+)</td>
<td>ND</td>
<td>(+)</td>
<td>(+)</td>
<td>ND</td>
</tr>
<tr>
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<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
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</tr>
<tr>
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<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
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<td>(+)</td>
<td>(-)</td>
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</tr>
<tr>
<td>9</td>
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<td>(-)</td>
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<td>(+)</td>
<td>(+)</td>
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<tr>
<td>10</td>
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<tr>
<td>11</td>
<td>(-)</td>
<td>(+)</td>
<td>ND</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

ND: not done.
Mutations in LEA by NGS

Targeted NGS

Targeted region capture and NGS of the 11 tumor specimens was performed. Genomic DNA sequencing libraries were prepared using the protocols recommended by the Illumina TruSeq DNA Library Preparation Kit. For samples that did not meet the minimum input requirement, additional pre-capture PCR cycles were performed to generate sufficient PCR products for hybridization. The libraries that were generated were hybridized with custom-designed probes (Integrated DNA Technology), including all exons of 170 genes and select introns of ALK, RET and ROS1, for the detection of genomic rearrangements. DNA sequencing was performed on a HiSeq3000 sequencing system (Illumina, San Diego, CA) with 2 × 75-bp paired-end reads. The reads were aligned to the human genome build GRCh37 using a Burrows-Wheeler aligner. Somatic single nucleotide variants and indel calls were generated using MuTect and GATK, respectively. Somatic copy number alterations were identified with CONTRA. Genomic rearrangements were identified by the software developed in house for analyzing chimeric read pairs.

Follow-up examinations

Follow-up examinations were conducted every 3-6 months after treatment, and the last follow-up was on December 31, 2016.

Statistical analysis

Categorical variables were compared using the X² test. The Kaplan-Meier method was employed for survival analysis, and the log-rank test was used for comparison between different groups.

Overall survival (OS) was defined as the period from confirmed diagnosis of advanced stage disease to the date of death or the last follow-up. P < 0.05 was considered to indicate statistical significance. Analyses were conducted using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

A total of 11 patients with histologically confirmed lung enteric adenocarcinoma were recruited from three medical centers. The clinical characteristics and immunohistochemical findings of all the patients are shown in Tables 1 and 2, respectively. The age of the patients ranged from 25 to 78 years, and 54.55% (6/11) of the patients were older than 60 years. The majority of the patients with lung enteric adenocarcinoma were male (63.64%) and had a hist-
Mutations in LEA by NGS

SMOKE of smoking (54.55%). The percentage of patients with lung enteric adenocarcinoma stage IIIB was 18.18%; stage IIIB, 9.09%; and stage IV, 72.72%. Immunohistochemical analysis for the markers CK7, TTF-1, Napsin A, CDX2, CK20 and Villin showed that they were expressed in 3 out of 10 (30.00%), 3 out of 11 (27.27%), 1 out of 5 (20.00%), 9 out of 10 (90.00%), 3 out of 10 (30.00%) and 8 out of 10 (80.00%) cases, respectively (Figure 1).

Molecular analysis

Targeted NGS of 7 cases showed that ALK/ROS1 primary point mutations were present in 5 cases (71.42%) and MSH2/MSH6 point mutations were present in 3 cases (42.86%). The mutation was detected at BRCA2 p.C3155S in case 1, BRAF p.A308T in case 5, FBXW7 p.R484Lfs*15 in case 6, NRAS p.Q61R in case 7, PIK3CA p.E545A in case 8 and TP53 in case 6 (p.G245C), 7 (p.R248Q), 9 (H193R) and 10 (V173L) (Figure 2).

Treatment and survival

Complete resection via lobectomy and postoperative chemotherapy were performed in case 9 and 10. Similar treatment was administered in the remaining cases, except for case 1, in which icotinib (a first-generation EGFR TKI) was administered for more than 1.5 months and nivolumab (immunotherapy drug) was administered for 9.5 months. The median OS of the 11 lung enteric adenocarcinoma patients was 9 months. Further, subgroup analysis showed that the median OS of patients with the ALK/ROS1 primary point mutation was 6.5 months, and that the median OS of patients with the MSH2/MSH6 point mutation was 26 months (Figure 3).

Discussion

It has recently emerged that lung enteric adenocarcinoma is a rare subtype of lung adenocarcinoma. The focus of research on this tumor is mainly the differential diagnosis of pulmonary metastases from primary colorectal adenocarcinoma, as some of the morphological characteristics of lung enteric adenocarcinoma resemble those of colorectal adenocarcinoma. However, the treatment strategy and prognosis of these two entities are different. An increasing number of gene mutations associated with lung cancer are being discovered, and drugs that target these mutations have also been developed. Research has also shown that the genetic basis of lung enteric adenocarcinoma may differ from that of other lung adenocarcinomas. Therefore, in the present study, we conducted immunohistochemical and NGS analysis of lung enteric adenocarcinoma and examined the prognosis of this cancer.

Lung enteric adenocarcinoma is described as a metastasis of a gastrointestinal tumor in which the enteric component exceeds 50% and there is no evidence of the primary cancer. The immunohistochemical characteristics of primary lung enteric adenocarcinoma and metastatic colorectal cancer are similar. Moreover, lung enteric adenocarcinoma is typically positive for at least one immunohistochemical marker of enteric differentiation. Generally, the majority of primary lung adenocarcinomas are positive for thyroid transcription factor-1 (TTF-1), CK7 and napsin A, but primary intestinal adenocarcinomas are positive for CDX-2 and CK20 (colorectal tumors) or CDX-2 and CK7 (upper gastrointestinal tract tumors) [10, 11]. Nottegar et al. [12] analyzed 46 lung enteric adenocarcinoma samples, and the results showed that all the samples were positive for CDX-2, 15 samples (32.6%) were positive for CK20, and 35 samples (76.1%) were positive for villin. Wang et al. [13] also explored 9 cases of lung enteric adenocarcinoma in which positive staining for CK7 was observed in 100% of the cases; CK20, 22.2% (2/9); TTF-1, 44.4% (4/9); napsin A, 33.3% (3/9); CDX2, 66.7% (6/9); MUC2, 44.4% (4/9); and villin, 66.7% (6/9). In our study, immunohistochemical analysis showed that CK7 was expressed in 30% of the samples (3/10); TTF-1, in 27.27% (3/11); napsin A, in 20% (1/5); CDX-2, in 90% (9/10); CK20, in 30% (3/10); and villin, in 80% (8/10). Thus, these reports indicate that lung enteric adenocarcinoma has a heterogeneous expression profile and expresses markers of both primary lung adenocarcinoma and intestinal markers. A majority of lung enteric adenocarcinoma cases were positive for CDX-2, which is a transcription factor of the homeobox gene family that is critical for intestinal development [14]. Further, a study compared the expression profiles of lung enteric adenocarcinoma, metastatic colorectal adenocarcinoma, and primary lung adenocarcinoma and reported that CDX-2 was expressed in 71% of enteric lung adenocarcinomas, 100% of metastatic colorectal adenocarcinomas, and
Figure 2. Targeted next-generation sequencing of point mutations. A. ALK p.G1474E; B. ROS1 p.W133R; C. MSH2 p.Q629R; D. MSH6 p.E1254D.
3% (1/30) of primary lung adenocarcinomas [15]. Moreover, our research showed that villin was expressed in many cases. Villin is a component of the brush border membrane that is found lining the intestine, so this finding [16]. Therefore, all these findings imply that it is difficult to identify specific immunohistochemical markers for distinguishing lung enteric adenocarcinoma from primary colorectal adenocarcinoma, and that differential diagnosis of these two entities should be based on the clinical manifestations and pathological features of the tumors.

Wang et al. [17] evaluated mutations of the EGFR and KRAS genes in 9 lung enteric adenocarcinoma cases and found that all the samples were positive for wild-type EGFR and KRAS. In contrast, Lásló et al. [18] demonstrated a KRAS mutation in one case of lung enteric adenocarcinoma, and Stojsic et al. [19] reported two cases of lung enteric adenocarcinoma in which one patient had KRAS mutations. Further, Nottegar et al. [12] found that lung enteric adenocarcinoma had a high frequency of KRAS mutations (60.9%) and a low frequency of EGFR gene mutations (2.2%) in a series of 46 lung enteric adenocarcinoma cases. In our study, we utilized NGS to explore gene mutations in 7 cases of lung enteric adenocarcinoma. All 7 patient had wild-type KRAS, while one patient had an NRAS mutation. NRAS is a rare mutation in NSCLC that is found in 1% of patients with this cancer, and NRAS mutations might indicate sensitivity to treatment with MEK inhibitors [20]. Our report was the first to report mutations of the MSH2/MSH6 gene in three patients, who had a median OS of 26.0 months; the survival of these patients was better than that of patients who had wild-type MSH2/MSH6. We also found ALK gene mutations in 2 patients (28.6%) and ROS1 mutations in 3 patients (42.9%); the prognosis of patients with the ALK/ROS1 gene mutation (6.5 months) was worse than that of patients with the corresponding wild-type genes. Nottegar et al. [12] found that the incidence of EML4-ALK translocation was 13.0%

![Figure 3. Overall survival of lung enteric adenocarcinoma patients.](image)

A. OS of 11 cases of lung enteric adenocarcinoma patients; B. OS of patients with ALK/ROS1 gene mutations and wild-type ALK/ROS1 genes; C. OS of patients with MSH2/MSH6 gene mutations and wild-type MSH2/MSH6 genes.

We do acknowledge, again, the limitations of our study, which are the small sample size and its retrospective nature.

In conclusion, we have described the immunophenotypic and genetic characteristics of lung enteric adenocarcinoma. ALK/ROS1 and MS-H2/MSH6 mutations were found to be frequent in our study sample, but no significant immunohistochemical markers for differential diagnosis of this carcinoma could be identified. Thus, both the immunohistochemical and genetic profile of patients should be considered for distinguishing this subgroup of lung adenocarcinomas. NGS opens new avenues for understanding the development of this type of tumor and identifying potential target genes for personalized therapy.
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Disclosure of conflict of interest

None.

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References


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