Lesions in patients with multifocal adenocarcinoma are more frequently in the right upper lobes

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Abstract

OBJECTIVES: Opportunities to treat multifocal lung cancers, mostly adenocarcinoma, are increasing due to the development of imaging technologies. The optimal therapy modality to treat multifocally growing lung cancers remains obscure. To determine the features of multifocal lung cancers, we retrospectively reviewed patients with multiple lung lesions.

METHODS: Clinical, pathological and genetic characteristics of 31 patients with multifocal lesions were compared with those of patients who had had radical lung resection for solitary lung cancer. Gene mutation analyses for EGFR, KRAS and P53 were performed on three tumours of each of the patients who had four or more lesions.

RESULTS: Of the 31 patients, 17 had double tumours, 4 had triple tumours and 10 had 4 or more lesions. Patients with four or more lesions were significantly more likely to be females and never smokers. All of the histologically confirmed tumours of the cases with four or more lesions were adenocarcinoma in situ or lepidic predominant adenocarcinoma. The number of lesions in the right upper lobes when compared with the right lower lobes was significantly higher in patients with four or more lesions than in patients with double or triple lesions (P = 0.013). Five of the 12 tumours were positive for the EGFR mutation L858R in exon 21. No KRAS mutation was found.

CONCLUSIONS: Lesions in patients with multifocal adenocarcinoma are more frequently in the right upper lobes. Genetic analysis suggested that the specific EGFR mutation L858R in exon 21 might be the main factor contributing to lung carcinogenesis in multiple lung cancers. Further investigation of the right upper lobe in those patients compared with the lower lobes might provide more insights into lung carcinogenesis.

Keywords: Lung cancer • Adenocarcinoma • Multiple lung tumour • EGFR mutation

INTRODUCTION

Opportunities to treat multifocal lung cancers, mostly adenocarcinoma, are increasing due to the development of imaging technologies. Recent accumulating data based on pathological-radiological correlation show that most cases of atypical adenomatous hyperplasia (AAH), adenocarcinoma in situ and lepidic predominant adenocarcinoma can be detected by ground-glass opacity (GGO)—the radiographical appearance of hazy lung opacity not associated with the obscuration of the underlying vessels [1]. As observed in colon carcinogenesis, recent reports support an AAH—adenocarcinoma sequence in lung carcinogenesis [2, 3]. Nowadays, in clinical practice, careful interpretation of high-resolution computed tomography for GGO areas or solid parts around GGO areas provides an approximate prediction of the pathological findings based on the adenocarcinoma with lepidic growth. Synchronous or metachronous multiple tumours sometimes pose difficulty in decision making regarding the required treatment. A number of retrospective studies have demonstrated that the well-selected use of sublobar resection can offer survival and recurrence rates comparable with those of lobectomy [4]. Therefore, particularly for multiple (for example, double or triple) primary lung cancer, sublobar resection has been considered to provide adequate oncological management [5]. However, the optimal therapy modality (local or systemic) to treat more multifocally growing lung cancers, for example, those with four or more synchronous lesions, remains obscure. In fact, no standard management for the diagnosis and treatment of more multifocal lung adenocarcinomas has been established.

To determine the features of patients with multifocal lung cancers, we retrospectively reviewed 31 patients with synchronous or metachronous multiple lung lesions and compared them with those patients with single lung cancer. Here, we considered lesions with radiological findings similar to pathologically confirmed tumours as lesions with the same pathological histology. We also analysed the mutational status of the epidermal growth...
factor receptor (EGFR), V-Ki-ras2 Kirsten rat sarcoma viral onco-
gene homolog (KRAS) and P53 in four patients with four or
more lung lesions.

PATIENTS AND METHODS

We conducted a retrospective review of patients with lung
cancer on whom pulmonary resection was performed between
January 2006 and December 2010 at the Kansai Medical
University Hirakata Hospital. Patients who had been surgically
evaluated for at least two tumours were included in this study as
cases of multifocal lesions. When the pathological examination
of tumours revealed AAH, the number of lesions was not deter-
mained. When multiple tumours spread to both sides of the
lungs, lesions with similar radiological appearance, usually
showing peripheral GGOs, even in the lung opposite to that of
the surgical evaluation site, were included in the total number of
lesions. Radiologically pure GGO lesions smaller than 5 mm were
not included in the total number of lesions because they were
highly suspected to be AAH [6].

Clinical and pathological characteristics of patients with multi-
focal lesions were compared with those of patients who had had
radical lung resection for solitary lung cancer. To particularly
focus on multiple lesions, we included patients with four or
more lesions in a separate group. Overall, patients were divided
into three groups, those with: one lesion, patients with double
or triple lesions and patients with four or more lesions. To deter-
mine the number of lesions per lobe, we calculated the tumour
accumulation index by dividing the number of lesions located
on a lobe by the number of patients with a single lesion, double
or triple lesions or four or more lesions.

Histological examination

All surgically resected lung tumour specimens were embedded in
paraffin, and serial 5-μm-thick sections were prepared. The
 pathological examination was based on standard haematoxylin
and eosin-stained slides from all blocks of tissues. In this
study, the definition of lung adenocarcinoma follows
International Association for the Study of Lung Cancer/American
Thoracic Society/European Respiratory Society (IASLC/ATS/
ERS) International Multidisciplinary Classification of Lung
Adenocarcinoma [6]. Histological findings were represented as
the predominant subtype that was the most dominant classifica-
tion, such as lepidic, papillary and acinar and minor subtypes of
carcinomas other than the predominant subtype that were the
remaining components of the tumour.

Mutational analysis of EGFR, KRAS and P53

Gene mutation analyses for EGFR, KRAS and P53 were per-
formed on three tumours of each of the four patients who had
four or more lesions. Because most tumours were small in size,
specific histological fields of each tumour specimen were
selected and microdissected under light microscopy to minimize
any normal tissue contamination. DNA was extracted from
formalin-fixed paraffin-embedded tissues. Mutations in the EGFR
(exons 18–21) gene were analysed by the peptide nucleic acid
locked nucleic acid PCR clamp (PNA LNA PCR clamp) technique
[7] and in the KRAS (codons 12 and 13) and P53 (exons 5–9)
genes by direct sequencing [8, 9]. Mutation analysis was per-
formed on the 12 tumours, for which informed consent was
obtained from patients. The ethics committee of the Kansai
Medical University approved the genetic analyses in the present
study.

Statistical analysis

Data are expressed as the mean or the number of patients.
Student’s t-tests were used for continuous data, and χ² tests
were used for categorical data. Calculated tumour accumulation
indexes of the upper lobes were compared with those of the
lower lobes on the same side. A P-value < 0.05 was considered
to indicate statistical significance; all tests were two-tailed. All
statistical analyses were performed using the JMP software
(version 7.0.2; SAS Institute, Inc., Cary, NC, USA).

RESULTS

A total of 31 patients had multiple lesions of lung cancer. Of the
31 patients, 17 had double lesions, 4 had triple lesions and 10
had 4 or more lesions (range: 5–9; mean: 5.5). The clinical and
pathological characteristics of the 31 patients with multifocal
lesions were compared with those of the 298 patients who had
had radical lung resection for solitary lung cancer (Table 1).

Patients with four or more lesions were significantly more
likely to be females and never smokers. All of the histologically
confirmed tumours of the cases with four or more tumours were
adenocarcinoma in situ or lepidic predominant adenocarcin-
omas. In the patients with solitary lung cancer, 27.5% of the
tumours were located in the right upper lobe. In turn, the 21
patients who had double or triple tumours had a total 46 lesions
(mean: 2.2). The 10 patients who had 4 or more lesions had a
total of 55 lesions (mean: 5.5). The tumour accumulation index
in the right upper lobe was 0.6 in patients with double or triple

<table>
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<tr>
<th>Table 1: Clinicopathological characteristics of the patients as a function of the number of lesions</th>
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<td>Number of lesions/case</td>
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<tr>
<td>Number of cases</td>
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<tr>
<td>Age</td>
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<td>Female [%]</td>
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<td>Confirmed histology [%]</td>
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<tr>
<td>Adenocarcinoma</td>
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<tr>
<td>Squamous cell carcinoma</td>
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<td>Large cell carcinoma</td>
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<td>Smoking status [%]</td>
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*One of the patients had double lesions with different histology,
resembling adenocarcinoma and large cell carcinoma.
P-value between number of females with 1 and ≥4 lesions was 0.027.
lesions, and 2.3 in patients with 4 or more lesions (Table 2). In fact, the number of tumours in the right upper lobes when compared with the right lower lobes was significantly higher in patients with four or more lesions than in patients with double or triple lesions ($P = 0.013$). A detailed tumour location analysis of the 10 patients who had four or more lesions is shown in Table 3, and the radiological and pathological findings of cases number 7 and 8 are shown in Figs 1 and 2. The tumour accumulation indexes of the right upper lobes were significantly higher than those of the lower lobes (Fig. 3). Regarding the patient outcomes, all of the patients who had four or more lesions were alive within a mean follow-up period of 35 months (range: 6–54 months).

Three tumours of each of the four patients with four or more lesions were selected for an analysis of gene mutations in the \textit{EGFR}, \textit{KRAS} and \textit{P53} genes. The results of the gene mutation analysis are summarized in Table 4. Five of the 12 tumours were positive for the \textit{EGFR} mutation L858R in exon 21. No \textit{KRAS} mutation was found in any of the 12 tumours. Only one patient had a \textit{P53} mutation in codon 262 of exon 8. Four of the seven

![Figure 1: Radiological (A–C) and pathological (D–F, corresponding to A–C, respectively) findings of the three tumours in Case number 7.](image-url)
tumours located in the right upper lobe and one of the five tumours located in the left lower lobe had the \textit{EGFR} \textit{L858R} mutation in exon 21. There was no obvious difference in the gene mutations between the right upper lobes and the left lower lobes.

\textbf{DISCUSSION}

This study demonstrated that patients with four or more lesions were more frequently females and never smokers. The result is consistent with previously reported features of patients with lepidic predominant adenocarcinoma and those whose adenocarcinomas have \textit{EGFR} mutations [10–13]. Histological analysis of tumours from patients with four or more lesions revealed that they were all adenocarcinomas, and most of the tumours had components of lepidic growth. Moreover, we demonstrated that lung lesions in patients who had four or more lesions were more frequently located in the upper lobes, particularly in the right upper lobes, than those of patients with solitary, double or triple lesions. This tendency is consistent with the study by Maeshima et al. [3], who reported that multiple (five or more) AAHs are predominantly present in the upper lobe (86%). Therefore, it seems that when lung cancers occur more multifocally, the lesions appear more in the upper lobes, particularly in the right upper lobes. This phenomenon suggests that carcinogenesis is preferentially triggered in the right upper lobe as opposed to the other lobes of the lungs. We previously reported that some GGOs that occur secondarily in patients who undergo pulmonary resection for invasive lung cancer more rapidly progress to invasive adenocarcinoma than other slow-growing GGOs [14]. In the lungs of some patients, adenocarcinoma \textit{in situ} or lepidic predominant adenocarcinoma appear multifocally and progress rapidly.

The multiplicity of distribution of lung cancer and the rapid growth of the tumours reveal that carcinogenesis and cancer progression are activated in the lung field of those patients. The phenomenon is well expressed by the idea of field cancerization, which was first introduced by Slaughter et al. [15]. Recent molecular findings support the carcinogenesis model in which the development of a field with genetically altered cells plays a central role [16]. Therefore, to seek reasons why multiple lung lesions occur more frequently in the upper lobes, we investigated the presence of gene mutations of \textit{EGFR}, \textit{KRAS} and \textit{P53} that have been previously reported to have a clinical impact on lung cancer patients [17]. For this, we examined four patients who had four or more lesions. With regard to the analysis of \textit{EGFR} mutations, 5 (42\%) tumours of a total of 12 were positive for the \textit{L858R} mutations in exon 21. None of the tumours analysed had a \textit{KRAS} mutation, and only one in which both \textit{EGFR} and \textit{KRAS} were wild type had a \textit{P53} mutation in codon 262 of exon 8. Chung et al. [1] reported the mutational analysis of the \textit{EGFR} and \textit{KRAS} genes for 24 patients with multifocal lung tumours. In their analysis, in which most patients had only two tumours and the counted lesions included AAH, \textit{EGFR} mutations were found in 19 (exon 19 deletion) and 7 (\textit{L858R} mutation in exon 21) of a total of 56 lesions. In our analysis, mutations in the \textit{EGFR} gene were all in exon 21 (\textit{L858R}). This type of mutation might be specifically related to
cancerogenesis of lung tumour occurring more multifocally. Mutational analysis of a total of 56 lesions from 24 patients reported by Chung et al., revealed that only 2 tumours from different patients who had double adenocarcinomas had the G12V KRAS mutation; for the remaining 54 tumours, the genotype was wild type. The KRAS gene is reported to undergo relatively late genetic alteration in pathogenesis [18]. Considering that all our patients with four or more lesions had the wild-type genotype for KRAS, mutations in KRAS might not be involved in the carcinogenesis of more multifocal lung tumours. In the more multifocally arising lung adenocarcinomas, EGFR would be the only factor that strongly affects carcinogenesis.

The reason why the upper lobe, especially the right upper lobe, had more lesions than the other lobes in patients with more multifocal lung cancer requires clarification. Carcinogenesis and tumour progression have been suggested to be related to chronic inflammation [19, 20]. Chronic inflammation may cause genetic alterations during carcinogenesis [21]. Several studies, using cytological and molecular techniques, have demonstrated that cigarette smoking creates a field injury in airway epithelial cells and have suggested a relationship between the cigarette smoking-related inflammation and lung field carcinogenesis [21, 22]. However, the results from our study indicate that this model might not be suitable in the case of patients with multifocal lung cancer. Generally, many pathological conditions, such as Mycobacterium tuberculosis infection, are associated with specific inflammation in the upper lung field. Yet an explanation as to why the right upper lobe is the most affected is unknown, but it might be associated with the anatomy of the right upper lobe bronchus, which diverges more at the head side than other bronchi.

In conclusion, lesions in patients with multifocal adenocarcinoma are more frequently in the right upper lobes. The clinical characteristics of patients with lung lesions that occur more multifocally were more frequently female sex and never smoking. Pathological examination of those patients showed that all of the tumours were adenocarcinoma in situ or lepidic predominant adenocarcinomas. Genetic analysis suggested that the specific EGFR mutation L858R in exon 21 might be the strong factor contributing to lung carcinogenesis in those patients. Nevertheless, we failed to identify any genetic difference between the right upper lobe and the other lobes. Further genetic and more evolving examinations of the right upper lobe compared with the lower lobes could provide key insights into lung carcinogenesis.

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Conflict of interest: none declared.

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