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# Paired genetic analysis by next-generation sequencing of lung cancer and associated idiopathic pulmonary fibrosis

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## Abstract

The pathogenesis of lung cancer associated with idiopathic pulmonary fibrosis (IPF) has remained largely uncharacterized. To provide insight into this condition, we undertook genomic profiling of IPF-associated lung cancer as well as of adjacent fibrosing lung tissue in surgical specimens. Isolated DNA and RNA from 17 IPF-associated non-small cell lung cancer and 15 paired fibrosing lung tissue specimens were analyzed by next-generation sequencing with a panel that targets 161 cancer-related genes. Somatic genetic alterations were frequently identified in *TP53* (n = 6, 35.3%) and *PIK3CA* (n = 5, 29.4%) genes in tumor samples as well as in *EGFR* (n = 7, 46.7%), *PIK3CA* (n = 5, 33.3%), *ERBB3* (n = 4, 26.7%), and *KDR* (n = 4, 26.7%) in IPF samples. Genes related to the RAS-RAF signaling pathway were also frequently altered in tumor (n = 7, 41.2%) and IPF (n = 3, 20.0%) samples. The number of somatic alterations identified in IPF samples was almost as large as that detected in paired tumor samples (81 vs 90, respectively). However, only 6 of the 81 somatic alterations detected in IPF samples overlapped with those in paired tumor samples. The accumulation of somatic mutations was thus apparent in IPF tissue of patients with IPF-associated lung cancer, and the RAS-RAF pathway was implicated in lung tumorigenesis. The finding that somatic alterations were not frequently shared between tumor and corresponding IPF tissue indicates that IPF-associated lung cancer does not develop through the stepwise accumulation of somatic alterations in IPF.

**KEYWORDS**

cancer-related gene, idiopathic pulmonary fibrosis, lung cancer, next-generation sequencing, somatic alteration

## 1 | INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease characterized by the destruction of alveolar architecture associated with the proliferation and differentiation of myofibroblasts.[1] Idiopathic pulmonary fibrosis has been implicated as an independent risk factor for the development of lung cancer, with a cumulative incidence rate of lung cancer ranging from 9.8% to 38% in

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IPF patients and lung cancer being a major contributing factor to the death of such patients.[2-8] The prognosis for individuals with IPF-associated lung cancer is worse than that for lung cancer patients without IPF, mainly as a result of complications such as exacerbation of preexisting interstitial lung disease during cancer treatment.[9]

Although several somatic genetic alterations targetable by specific drugs have been identified in non-small cell lung cancer (NSCLC), with such targeted agents having a pronounced therapeutic efficacy,[10-18] the pathogenesis and genetic basis of IPF-associated lung cancer have remained largely unknown, and optimal treatment for this condition is still lacking. The frequency of *KRAS* or *TP53* mutations has been found to be higher in lung tissue from patients with IPF and lung cancer than in lung tissue from those with IPF but without lung cancer,[19] suggesting that somatic alterations occur more frequently in patients with IPF complicated by lung cancer than in those with IPF alone. It has remained unknown, however, whether somatic alterations are shared by IPF-associated lung cancer and the fibrosing lung tissue and whether IPF-associated lung cancer develops through a stepwise accumulation of somatic alterations in fibrosing lung tissue.

To investigate the etiology of IPF-associated lung cancer, we evaluated the genomic profile of lung tumors and adjacent fibrosing lung tissue from patients with IPF-associated lung cancer with the use of targeted next-generation sequencing (NGS).

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

Seventeen patients who underwent surgical resection for NSCLC associated with pathologically diagnosed IPF at Kyushu University Hospital between January 2012 and December 2017 and for whom tumor tissue specimens were available were enrolled in the study. The study was approved by the institutional review board of Kyushu University Hospital (approval number 30-62) and was carried out in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical Research Involving Human Subjects in Japan. We obtained the following information from clinical records of the patients: age, sex, tumor histology, clinical stage, smoking status, and the high-resolution computed tomography (HRCT) imaging pattern of IPF.

### 2.2 | Next-generation sequencing analysis

DNA and RNA were isolated from formaldehyde-fixed, paraffin-embedded specimens of lung tumor tissue and those of tumor-adjacent fibrosing lung tissue with the use of an AllPrep DNA/RNA FFPE Kit (Qiagen). Isolated DNA (10 ng) was subjected to multiplex PCR-based amplification with the use of an Ion AmpliSeq Library Kit Plus (Thermo Fisher Scientific). Isolated RNA (10 ng) was subjected to reverse transcription with the use of SuperScript IV VILO Master Mix (Thermo Fisher Scientific) followed by library generation with the use of an Ion AmpliSeq Library Kit Plus (Thermo Fisher Scientific). The Oncomine Comprehensive Panel version 3 (Thermo Fisher Scientific) was used to target clinically relevant single nucleotide variants, insertions/deletions, copy number variations, and gene fusions affecting 161 unique cancer-related genes (Table S1). The library generated from RNA was used for the detection of gene fusions. The amplification products were ligated to Ion Xpress Barcode Adapters (Thermo Fisher Scientific) and purified with the use of Agencourt AMPure XP beads (Beckman Coulter). The purified libraries were pooled and then sequenced with an Ion S5 XL instrument, Ion 540 Kit-Chef, and Ion 540 Chip Kit (Thermo Fisher Scientific). DNA sequencing data were accessed through the Torrent Suite version 5.2.2 program (Thermo Fisher Scientific). Reads were aligned with the hg19 human reference genome, and potential mutations and copy number alterations were called with the use of Ion Reporter software version 5.2. Raw variant calls were filtered with a quality score of less than 100 and were manually checked with the use of the Integrative Genomics Viewer (Broad Institute). Somatic alterations reported in the Catalogue of Somatic Mutations in Cancer database were collected. Germline mutations were excluded with the use of the Human Genetic Variation database (http://www.genome.med.kyoto-u.ac.jp/SnpDB) and Exome Aggregation Consortium database (http://exac.broadinstitute.org).

## 3 | RESULTS

### 3.1 | Patient characteristics

Tumor samples were obtained from 17 patients who underwent surgical resection of NSCLC associated with pathologically diagnosed IPF. The clinical characteristics of the patients are shown in Table 1. The HRCT of the lung showed the usual interstitial pneumonia (UIP) pattern in 16 (94%) patients and a probable UIP pattern in 1 (6%) patient. The median age was 73 years (range, 62-78 years). Fourteen (82%) patients were male. Tumor histology was adenocarcinoma in 9 (53%) patients and squamous cell carcinoma in 8 (47%) patients. All patients had a smoking history, with 14 (82%) being former and 3 (18%) current smokers.

### 3.2 | Somatic alterations in NSCLC

Analysis of tumor samples from the 17 patients with IPF-associated lung cancer uncovered somatic alterations in a total of 97 genes (including repeat counts of those affected in more than 1 patient), with the median number of affected genes per patient being 4 (range, 1-24) (Table S2). Such alterations were frequently detected in *TP53* (n = 6, 35.3%) and *PIK3CA* (n = 5, 29.4%) genes (Figure 1). Although 1 or more somatic alterations were identified

in *BRAF*, *RET*, *EGFR*, *ALK*, *ROS1*, and *NTRK1* (Table S2), these changes did not include those—such as the Val600Glu (V600E) mutation of *BRAF*, activating mutations of *EGFR*, and fusions of *RET*, *ALK*, or *ROS1*—that confer sensitivity to molecularly targeted drugs (Table S3). Non-V600E mutations of *BRAF* and mutations of *ARAF*, *KRAS*, *NRAS*, or *HRAS* were detected in 4, 1, 2, 1, and 2 patients, respectively, with alterations that affect the RAS-RAF signaling pathway being identified in 7 (41.2%) of 17 tumor samples. No gene fusions were identified in tumor samples.

**TABLE 1** Characteristics of patients with idiopathic pulmonary fibrosis (n = 17)

| Characteristic | No. of patients (%) |
|---|---|
| Median age (range), y | 73 (62-78) |
| Sex | |
| Male | 14 (82) |
| Female | 3 (18) |
| Clinical stage | |
| I | 8 (47) |
| II | 6 (35) |
| III | 2 (12) |
| IV | 1 (6) |
| Histology | |
| Adenocarcinoma | 9 (53) |
| Squamous cell carcinoma | 8 (47) |
| Smoking status | |
| Never | 0 (0) |
| Previous | 14 (82) |
| Current | 3 (18) |
| HRCT pattern | |
| UIP | 16 (94) |
| Probable UIP | 1 (6) |

### 3.3 | Somatic alterations in fibrosing lung tissue

Fibrosing lung tissue located adjacent to tumor tissue and resected at the time of surgery was available for 15 of the 17 patients enrolled in the study. All such tissue samples were pathologically confirmed as positive for fibrosis with the UIP pattern and to contain no tumor cells. Next-generation sequencing analysis of these 15 IPF samples detected somatic alterations in 69 genes (including repeat counts of those affected in more than 1 patient), with the median number of genes in which somatic alterations were identified per sample being 2 (range, 0-16) (Table S2). Such alterations frequently occurred in *EGFR* (n = 7, 46.7%), *PIK3CA* (n = 5, 33.3%), *ERBB3* (n = 4, 26.7%), and *KDR* (n = 4, 26.7%) (Figure 1). No somatic alterations—such as the V600E mutation of *BRAF* or activating mutations of *EGFR*—that confer sensitivity to molecularly targeted drugs for NSCLC and no gene fusions were identified in the IPF samples (Table S3). Somatic alterations that affect the RAS-RAF pathway were identified in 3 (20.0%) of the 15 IPF samples (a non-V600E mutation of *BRAF* as well as mutations in *KRAS* and *ARAF* in 1 patient each).

### 3.4 | Overlap of somatic alterations in paired samples

In the case of the 15 patients for whom paired tumor and fibrosing lung tissue samples were available, 90 and 81 somatic alterations were detected in 81 and 69 genes in tumor and IPF samples, respectively, with a total of 19 genes overlapping in 8 of the sample pairs (Figures 2 and 3). Among these 19 genes, only 6 somatic alterations

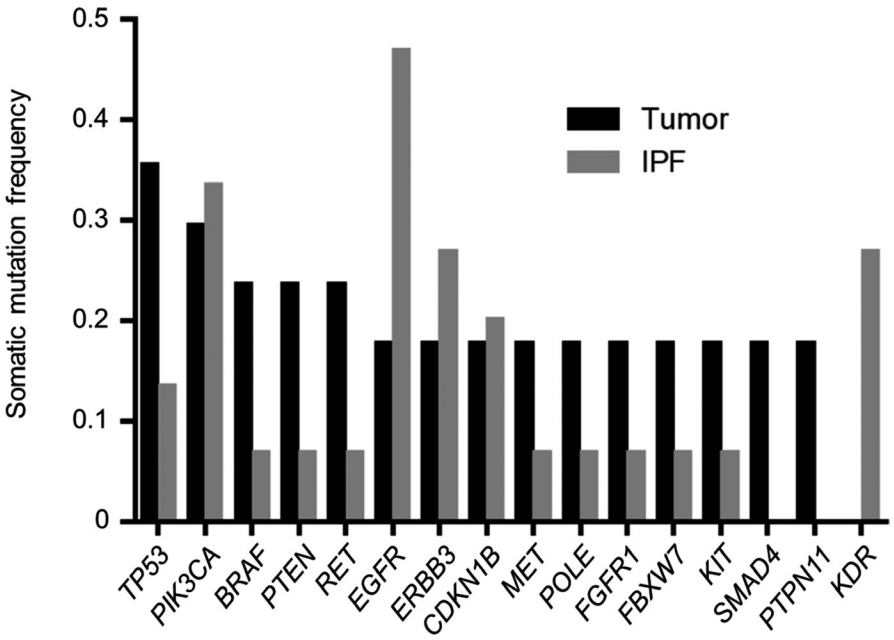


**FIGURE 1** Frequently detected genes with somatic alterations in tumor or idiopathic pulmonary fibrosis (IPF) tissue specimens of patients with IPF-associated lung cancer. Those genes with a somatic alteration frequency of 0.15 or higher, as detected by next-generation sequencing, are shown

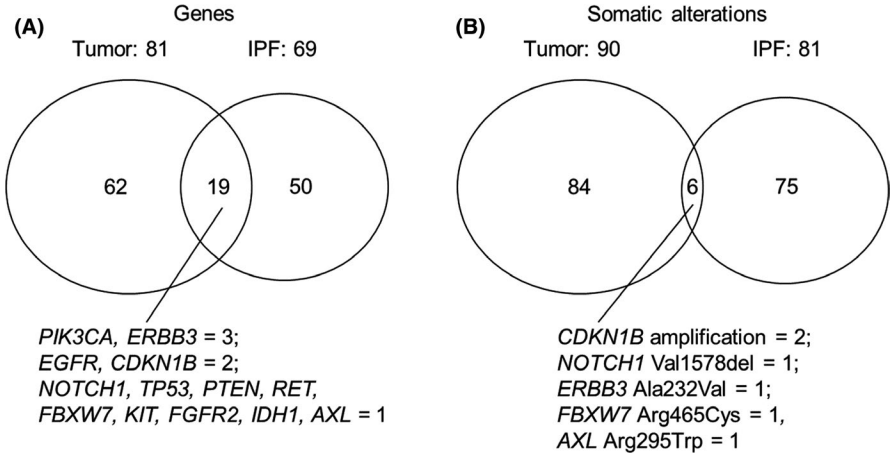


**FIGURE 2** Overlap in genes with somatic alterations (A) or in somatic alterations themselves (B) between tumor and idiopathic pulmonary fibrosis (IPF) tissue in paired samples from patients with IPF-associated lung cancer

(*CDKN1B* amplification in 2; Val1578del of *NOTCH1*, Ala232Val of *ERBB3*, Arg465Cys of *FBXW7*, and Arg295Trp of *AXL* in 1 each) overlapped in 4 paired samples. Most somatic alterations, such as those affecting *TP53*, *PIK3CA*, and *RET*, were thus not shared by corresponding tumor and IPF tissue (Tables S2 and S3).

## 4 | DISCUSSION

With the use of NGS with a panel that targets 161 cancer-related genes, we have here investigated somatic alterations in both tumor and adjacent fibrosing lung tissue from patients with IPF-associated lung cancer. Somatic alterations were identified in 97 genes in 17 tumor samples. Although somatic alterations—such as activating mutations of *EGFR*, rearrangement of *ALK*, or the V600E mutation of *BRAF*—that confer sensitivity to molecularly targeted drugs for NSCLC were not identified, non-V600E mutations of *BRAF* were frequently detected in IPF-associated tumor samples (4 of 17, 23.5%), consistent with previous results.[20,21] All of these 4 mutations of *BRAF* were located in the kinase domain (Thr599Ile in 2; Gly464Arg and Gly466Glu in 1 each). The Thr599Ile and Gly464Arg mutants of BRAF were found to have increased kinase activity compared with the WT protein.[22,23] Although the kinase activity of the Gly466Glu mutant of BRAF is impaired, this mutant increases signaling by the downstream kinase ERK as a result of an increased affinity for the GTP-bound form of RAS compared with WT BRAF.[24] This type of BRAF mutant has been found to coexist with RAS mutants.[24] Consistent with this previous observation, we identified a tumor with both the Gly466Glu mutation of *BRAF* and a mutation in *HRAS*. Somatic alterations that affect the RAS-RAF pathway were identified in 7 (41.2%) of the 17 tumor samples in the present study, suggesting that this pathway plays an important role in tumorigenesis in patients with IPF.

Although genomic profiling of IPF-associated lung cancer by NGS has been undertaken in previous studies,[20,25] that of IPF tissue in patients with IPF-associated tumors has not. In the present study,

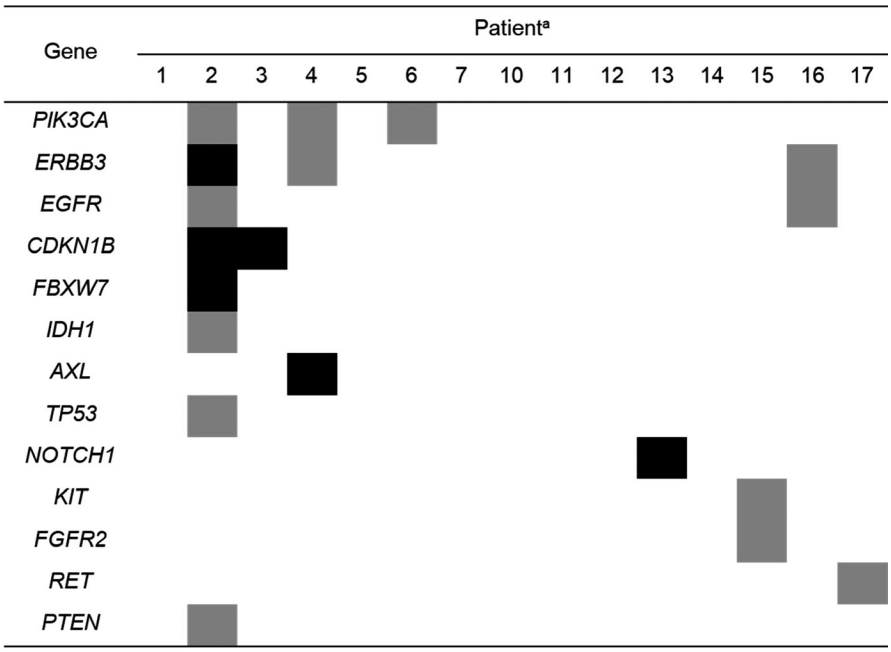


**FIGURE 3** Overlapping genetic alterations in tumor and idiopathic pulmonary fibrosis (IPF) samples from the 15 patients for whom paired samples were available. The presence of any or the same genetic alterations of each gene in both tumor and IPF samples is indicated by gray or black shading, respectively

somatic alterations in cancer-related genes were identified almost as frequently in IPF samples (n = 15) as in paired tumor samples (81 vs 90 alterations), suggestive of susceptibility to somatic mutation in the IPF component of individuals with IPF-associated lung cancer and suggesting that such susceptibility might contribute to the development of lung cancer.

Among the 81 somatic alterations identified in IPF samples, 6 changes overlapped with those detected in paired tumor samples. These 6 alterations occurred in 5 genes that encode receptor tyrosine kinases (*ERBB3* and *AXL*), a cell cycle regulator (*CDKN1B*), an activator of oncogene expression (*NOTCH1*), and a protein that contributes to the degradation of oncoproteins (*FBXW7*), all of which regulate cell differentiation, growth, proliferation, or survival.[26-30] *CDKN1B* amplification was apparent in 2 of the sample pairs. Although *CDKN1B* encodes a cyclin-dependent kinase inhibitor ($p27^{Kip1}$), which plays a role in tumor suppression, its amplification is thought to contribute to oncogenesis by promoting tumor cell migration, invasion, and metastasis.[31,32] *CDKN1B* amplification might therefore also contribute to the pathogenesis of IPF-associated lung cancer.

Although 6 overlapping somatic alterations were apparent in 4 of the 15 paired tumor and IPF samples, the remaining sample pairs did not show overlap in somatic alterations. *TP53* mutations were frequently detected in both tumor and IPF samples in the present study, consistent with previous findings.[19,33,34] Although all of the *TP53* mutations were located in the DNA binding domain of the encoded protein, which recognizes a consensus sequence in the promoters of several genes related to DNA repair and apoptosis,[35,36] they were diverse and did not overlap between tumor and IPF tissue. Somatic alterations in most of the genes, including *TP53*, *PIK3CA*, and *RET*, identified in both tumor and IPF samples thus differed between these 2 types of sample. These results suggest that most cases of IPF-associated lung cancer do not develop through the stepwise accumulation of somatic alterations in fibrosing lung tissue.

There are several limitations to the present study. First, the sample size was relatively small. Second, we analyzed only surgical samples obtained from patients with early-stage lung cancer. There is thus a possibility that our findings might not be applicable to advanced lung cancer. However, surgical or transbronchial biopsy of lung tissue is not recommended for diagnosis of IPF when HRCT of the lung demonstrates the UIP pattern.[1] It is therefore difficult to obtain paired biopsy samples of IPF-associated lung cancer and IPF, which limited us to the use of only surgically resected specimens containing both lung cancer and fibrosing lung tissue. Third, NGS analysis of IPF samples was carried out for IPF tissue in only 1 location for each patient. We thus cannot exclude the possibility that somatic alterations were shared between tumor samples and IPF tissue in locations other than those examined in the present study. Finally, the gene panel adopted for the present study targets only cancer-related genes. Analysis of other genes, such as those related to the pulmonary surfactant system, in paired IPF-associated tumor and fibrosing lung tissue might also provide insight into the etiology of IPF-associated lung cancer.[25]

In conclusion, as far as we are aware, our study is the first to investigate somatic genetic alterations in paired tumor and tumor-adjacent fibrosing lung tissue specimens by targeted NGS. Although somatic alterations were frequently detected in several genes in both sample types, such alterations were not frequently shared within the sample pairs. Further comprehensive NGS analysis of paired tumor and IPF samples is warranted to provide further insight into the etiology of IPF-associated lung cancer and to provide a basis for the development of targeted drugs for such tumors as well as for IPF.

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## DISCLOSURE

The authors declare that they have no conflicts of interest.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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