Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.3682-19
Intern Med 59: 1299-1302, 2020
http://internmed.jp

【 CASE REPORT 】

Clinical Application of Next-generation Sequencing for the Diagnosis of Lung Squamous Cell Carcinoma: Is It Primary or Secondary?

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Abstract:
An 80-year-old man with a history of cutaneous squamous cell carcinoma (SCC) was referred to our department for a solitary lung nodule. The nodule was surgically resected and diagnosed as SCC. Because the lung lesion and a previous skin lesion showed similar histological findings, the origin of the lung tumor was uncertain. Next-generation sequencing using a targeted driver oncogene panel was applied for the further examination. The lung lesion was diagnosed as primary lung SCC, as the two tumors possessed distinct somatic mutations in TP53. Recent advances in clinical sequencing have enabled us to obtain an accurate diagnosis in pathologically challenging cases.

Key words: squamous cell carcinoma, massively parallel sequencing, next-generation sequencing, somatic mutation

(Intern Med 59: 1299-1302, 2020)
(DOI: 10.2169/internalmedicine.3682-19)

Introduction

It is still a clinical challenge to distinguish primary lung squamous cell carcinoma (SCC) and pulmonary metastasis of SCC from other organs. Although several immunohistochemistry markers have been reported for identifying the origin of metastatic lung SCC with a history of extrapulmonary SCC, immunohistochemistry markers have not yet been fully established in distinguishing metastasis of lung SCC and skin SCC (1-3).

Recent advances in sequencing technologies, i.e., next-generation sequencing (NGS), have enabled the identification of multiple somatic mutations in individual tumors from a patient. By comparing the mutational panel of each tumor, we are now able to determine whether or not multiple tumors have the same origin.

We herein report a patient with a history of skin SCC who metachronously developed lung SCC. The lung tumor was eventually diagnosed as primary lung SCC using NGS.

Case Report

An 80-year-old man with a heavy smoking history of 59 pack-years was referred to our department for an indefinite lung nodule. He had a history of cutaneous SCC in his right thigh, which had been surgically resected six years ago followed by adjuvant radiotherapy and 5-fluorouracil (5-FU)/cisplatin chemotherapy. Since then, he had been followed up by dermatologists without any evidence of recurrence until chest X-ray showed an enlarging pulmonary nodule suspected of being malignant.

On presentation, he was afebrile, alert, and oriented. Blood tests revealed slightly elevated serum SCC antigen levels of 2.9 ng/mL (>1.5 ng/mL). Chest computed tomography (CT) showed a well-defined, lobulated, and solid nodule with a maximal diameter of 20 mm in the right lower lobe of the lung (Fig. 1). Fluorodeoxyglucose positron emission

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Received: July 18, 2019; Accepted: December 16, 2019; Advance Publication by J-STAGE: February 12, 2020
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tomography (FDG-PET) showed the accumulation of FDG in the nodule (SUVmax=5.2). There was no other significant FDG accumulation.

The lung nodule was suspected of being a malignant tumor, especially primary or metastatic SCC. Because there were no other lesions detected, surgery was indicated for diagnostic and therapeutic purposes. We persuaded the patient to quit smoking. We then performed video-assisted thoracoscopic surgery for partial resection of the right lower lobe of the lung with adequate surgical margins. The postoperative course was uneventful, and the patient was discharged within one week after surgery. Histopathology showed that the lung nodule was composed of well-differentiated SCC. Because the skin lesion resected six years ago had also been well-differentiated SCC, it was difficult to determine whether the lung tumor was primary lung cancer or recurrence of skin cancer (Fig. 2A, B). Immunohistochemical staining for p53 was performed for the further evaluation (monoclonal mouse anti-human p53 antibody, clone DO-7; Roche Diagnostics, Rotkreuz, Switzerland). However, the two lesions showed slightly different staining patterns, and the diagnosis remained equivocal (Fig. 2C, D).

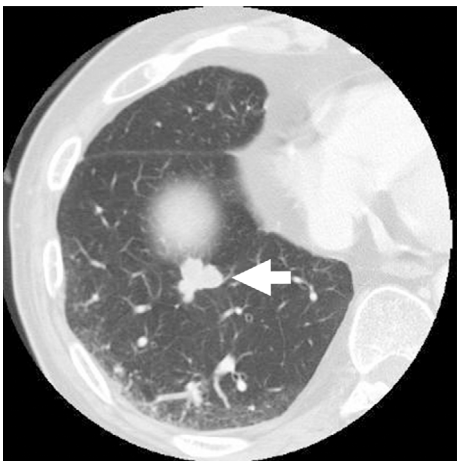

Figure 1. Computed tomography performed six years after resection of cutaneous squamous cell carcinoma revealed a well-defined solid nodule with lobulation in the right lower lobe of the lung (arrow).

We discussed further investigations with the patient, and he decided to enroll in a prospective clinical sequencing study (4). After obtaining his written informed consent, molecular analyses of both the resected lung tumor and previously resected skin SCC were conducted. Approximately 1,176.0 ng DNA was collected from the previous cutaneous cancer sample, and 3,605.0 ng DNA was collected from the formalin-fixed paraffin-embedded lung sample. As a normal control, 2,758.0 ng DNA was collected from the patient's serum. The Todai OncoPanel, developed by The University of Tokyo, was used for the detection of somatic mutations in

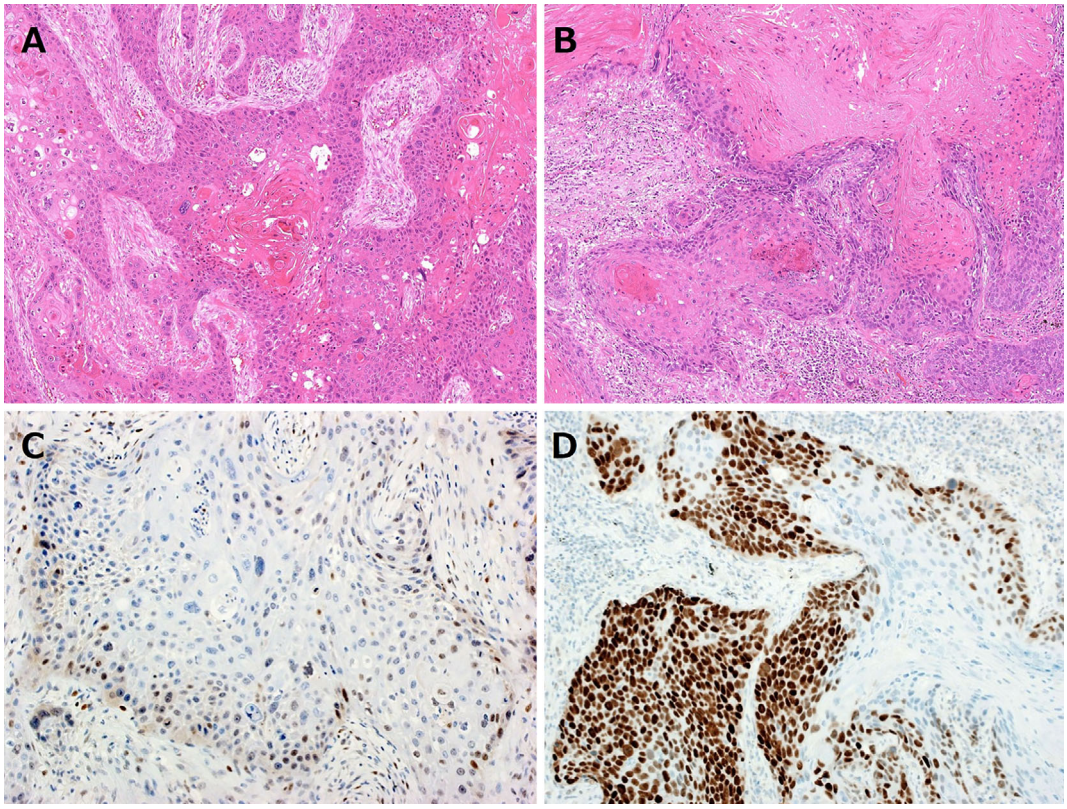

Figure 2. Representative micrographs of skin and lung lesions. Both the (A) skin and (B) lung lesion showed well-differentiated squamous cell carcinoma (Hematoxylin and Eosin staining, ×40 at original magnification). Immunohistochemical staining of p53 in the (C) skin and (D) lung lesion showed a slightly different p53 expression, although the diagnosis remained equivocal (×100 at original magnification).

Table. Panel of Somatic Mutations Obtained by Targeted Next-generation Sequencing.

Skin lesion		Lung lesion	
Somatic mutation	Allele frequency	Somatic mutation	Allele frequency
TP53 R342*	62.0%	TP53 A138P	13.7%
POLD1 Q53H	52.7%	CTLA4 A66S	9.5%
IL7R D122H	10.9%	ZFHX3 N1043D	9.0%
COL22A1 S440L	15.1%	KMT2D G4593E	5.7%

each tumor. In brief, the Todai OncoPanel is a target sequencing panel that covers single nucleotide substitutions, short insertions/deletions, and copy number variations of 464 genes (DNA) and fusion gene detection of 365 genes and expression profiles of an additional 109 genes (RNA) (4). The Phred Quality score (Q score) of each sequenced base in the skin cancer sample was ≥30 (i.e., equivalent to the probability of an incorrect base call 1 in 1,000 times) in 91.6% of the sequenced bases and in 91.4% of the lung cancer sample. The degradation of nucleic acid was minimal in both lesions.

Sequencing analysis results are shown in Table. TP53 R342*, POLD1 Q53H, IL7R D122H, and COL22A1 S440L were detected as somatic mutations from the skin lesion, while TP53 A138P, CTLA4 A66S, ZFHX3 N1043D, and KMT2D G4593E were detected as somatic mutations from the lung lesion. The estimated tumor cell purity of skin and lung lesions was 78.0% and 29.0%, respectively. A germline mutation of BRCA2 (R2318*) was also detected from the patient.

A somatic mutation of TP53 was detected in both lesions; however, the mutation position differed. TP53 R342*, the mutation detected in the skin lesion, is a mutation within the tetramerization motif of tumor protein p53. In contrast, TP 53 A138P, the mutation detected in the lung lesion, is a mutation occurring at the DNA-binding domain. TP53 protein is a negative regulator of cell proliferation and a positive regulator of apoptosis in response to DNA damage. Considering the mutation site and high variant allele frequency of TP53 gene mutation in each lesion, these mutations are likely driver mutations. No other somatic mutations detected from the skin or lung lesions occurred in the functional domain of the tested genes. The variant allele frequency was low compared with the tumor purity of each lesion. Thus, all other somatic mutations, excluding TP53 mutations, were regarded as passenger mutations. The skin and lung lesions did not share any somatic mutations, but they did possess distinct driver mutations, so we diagnosed the lung lesion as primary lung SCC.

We determined the lesion to be primary lung cancer with pathological staging of T1bN0M0-IA2 (The 8th Edition Lung Cancer Stage Classification) (5). The patient did not desire to undergo right lower lobectomy. As the patient had no living blood relatives, he told us that genetic counseling on germline mutation of BRCA2 was not necessary.

At three months after surgery, the patient was alive and doing well at the outpatient clinic without recurrence. However, he died of cerebral hemorrhaging four months after surgery. Head CT taken after the hemorrhaging showed no evidence of brain metastasis.

Discussion

Differentiation between primary lung SCC and pulmonary metastasis of SCC from another organ is clinically important for appropriate treatment; however, the diagnosis is still challenging. Histopathological markers are reported to aid in identifying the origin of metastatic lung SCC with a history of extrapulmonary SCC, including p16 staining for pulmonary SCC versus cervical SCC (6); the combination of CK 19, MMP3, ZNF830, and PI3 for pulmonary SCC versus head and neck SCC (7); and the combination of TTF-1, napsin A, and p16 staining for pulmonary SCC versus esophagus and anorectal SCC (3). However, histopathological diagnostic methods have not yet been fully established for distinguishing pulmonary SCC versus metastasis of skin SCC (1-3). It has been reported that IHC staining for p53 is occasionally used as a surrogate for mutational analyses in the diagnostic workup of carcinomas of multiple sites (8). Strong and diffuse expression or the complete absence of p53 expression may indicate a TP53 gene mutation. However, in the present case, although the two lesions possessed distinct TP53 gene mutations, neither showed such staining patterns.

Advances in sequencing technologies, e.g., NGS, have enabled the identification of multiple somatic mutations in each tumor. Whole-exome sequencing has become a useful tool for investigating disease-causing somatic mutations in various fields. Targeted sequencing has become a popular tool for effectively screening the existence of known driver mutations at a high sequence depth with a relatively low cost (4, 9). Recently, the utility of genomic profiling in multiple lung tumors that are difficult to discriminate pathologically has been reported (10). NGS and the comparison of trunk mutations in each lesion can be used to determine whether a tumor is primary or metastatic with high specificity.

Clinical sequencing using NGS was developed to provide actionable information based on the genomic profile of each tumor. In addition to the detection of targetable driver muta-

tions, clinical sequencing has been used for the assessment of the tumor mutational burden for predicting the efficacy of immune checkpoint inhibitors in non-small cell lung cancer (11). In the present case, clinical sequencing was used to obtain the genomic profile of each tumor and determine whether or not the lung tumor was of primary origin, which would help guide further treatment. Because the tumor was a primary lung cancer, completion right lower lobectomy with lymph node dissection was recommended as a standard of care. However, against our recommendation, the patient resumed smoking and refused to undergo additional treatment, including surgery.

When applying NGS in the clinical setting, e.g., when diagnosing whether a tumor is primary or metastatic lung cancer, it is important to know the appropriate way to interpret sequencing data. The simplest method is to compare the driver mutations of each lesion. Although passenger mutations may differ even if the two lesions have the same origin, the mutation that is important in the early stage of cancerization, also known as the truncal (clonal) mutation, is shared by almost all tumor cells with the same origin (12, 13). Nevertheless, caution must be practiced when comparing driver mutations in multiple lesions, as subclonal driver mutations may also occur later during tumor progression as low-allele-frequency mutations (13, 14).

In the present case, if the lung lesion had been recurrence of skin cancer, then TP53 R342*, the probable driver mutation of skin cancer would have been detected in the lung lesion as well. However, TP53 A138P, a different driver mutation, was detected in the lung lesion. We therefore concluded that the lung tumor had a different origin from the skin lesion and diagnosed it as primary lung cancer. The comparison of driver mutations is important for the evaluation of a tumor's origin; the detection of the driver mutations in each tumor by clinical sequencing is therefore effective and useful.

The authors state that they have no Conflict of Interest (COI).

Financial Support

This study was supported in part by a grant for Program for an Integrated Database of Clinical and Genomic Information from Japan Agency for Medical Research and Development (JP 18kk0205003). A sequencing analysis of the clinical specimens was funded in part by Sysmex Corporation (Kobe, Japan).

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