Case Report

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Next-generation sequencing revealed synchronous double primary lung squamous carcinoma: a case report

De-Song Yang[1,]*, Kai Huang[2,]*, Min Su[1], Ting-Ting Wang[3], Si-Si Liu[3], Yang Xu[3] and Wen-Xiang Wang[1]

Abstract

Synchronous double primary lung squamous carcinoma (sDPLSCC) is rare and difficult to distinguish from metastatic disease, histopathologically. Owing to the heterogeneity of cancer, it is also difficult to select the optimal therapeutic strategy for patients with multiple primary lung cancer (MPLC). The present study reports a rare case of a 61-year-old male patient with sDPLSCC diagnosed using histology and genetic profiling. LSCC-related driver mutations were detected in this patient, and we reported the *TP53* c.475G>C mutation, which has been detected in both breast cancer and hepatocellular carcinoma, but not previously in lung squamous carcinoma. Our findings provide further evidence supporting the necessity of genetic testing for primary tumor diagnosis.

Keywords

Bilateral primary tumor, genomic profiling, heterogeneity, next-generation sequencing (NGS), *TP53* c.475G>C, lung squamous carcinoma

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[1]Department of Thoracic Surgery II, The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University/Hunan Cancer Hospital, Changsha, Hunan, China
[2]Department of Cardiothoracic Surgery, The First People's Hospital of Huaihua, Huaihua, Hunan, China
[3]Department of Research and Development, Nanjing Geneseeq Technology Inc., Nanjing, Jiangsu, China

*These authors contributed equally to this work.

Corresponding author:
Wen-Xiang Wang, Department of Thoracic Surgery II, The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University/Hunan Cancer Hospital, 283 Tongzipo Road, Yuelu District, Changsha, Hunan 410013, P.R. China.
Email: wangwenxiang_hnca@163.com

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Introduction

Multiple primary lung cancer (MPLC) is rare, accounting for 0.26% to 1.33% of all lung cancer cases, and double primary lung cancer (DPLC) is the major type of MPLC.[1] Recently, owing to progress in diagnostic strategies, the MPLC detection rate has gradually increased.[2,3] Nevertheless, the current diagnostic criteria are insufficient to identify some patients with MPLC.[4,5] Furthermore, owing to the heterogeneity of cancer and the lack of evidence-based studies, it is difficult to select the optimal therapeutic strategy for patients with MPLC. Therefore, to better understand the relationships between multiple lesions and to identify more efficient treatment approaches and prognostic biomarkers, molecular testing and genomic profiling have been proposed.[6] In DPLC, the incidence of squamous cell carcinomas is approximately 5.2%.[7] The molecular features of patients with double primary lung squamous cell carcinoma (DPLSCC) remain elusive. Herein, we report a case with synchronous DPLSCC (sDPLSCC), which was diagnosed via imaging and next-generation sequencing (NGS), and which showed completely distinct mutation patterns between tumors. We also identified the *TP53* c.475G>C mutation, which had not been reported in LSCC, previously. Our findings provide meaningful insight into the understanding of driver genetic alterations associated with tumors and underscores the importance of multiple tumor biopsies for genetic testing in patients with concurrent multiple primary tumors.

Materials and methods

Genomic profiling was performed on formalin-fixed paraffin-embedded (FFPE) tumor specimens obtained from patients who provided signed written informed consent. Briefly, tissue blocks with adequate tumor cellularity (>70%, without significant necrosis or inflammation) were selected by pathologists and subjected to DNA extraction with the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Sequencing libraries were constructed with 2000*ng genomic DNA using the KAPA Hyper Prep Kit (KAPA Biosystems, Wilmington, MA, USA) according to the manufacturer's recommendations. Hybridization capture-based targeted NGS with a 425-cancer-relevant-gene panel was performed on the Illumina HiSeq platform (Illumina, San Diego, CA, USA). Bioinformatics analysis was performed as previously described.[8] The original targeted sequencing depth was >700× (8.63 million reads and 7.02 million reads for left and right LSCC, respectively), with a mapping rate of 99.90%, and the somatic variant calls with at least 0.5% mutant allele frequency (MAF) were retained and confirmed manually with Integrative Genomics Viewer (IGV) software.[9]

Case presentation

A 61-year-old male patient was admitted to Hunan Cancer Hospital in September 2018 with a 1-month duration of cough and expectoration. The patient had a 40-year smoking history. Bronchoscopy revealed synchronous bilateral lung lesions in the right and left upper lobes (Figure 1a and 1b). Biopsies of the lesion in the right lobe identified squamous hyperplasia with severe atypical hyperplasia, but no obvious metastases were detected. Additionally, immunohistochemical (IHC) p40 staining was positive in both lesions (Figure 1c and 1d). Subsequently, lobectomy was performed to remove the left upper lobe and right upper lobe lesions in September and December 2018, respectively. Histology from the right lesion showed severe hyperplasia of bronchial squamous epithelium with no

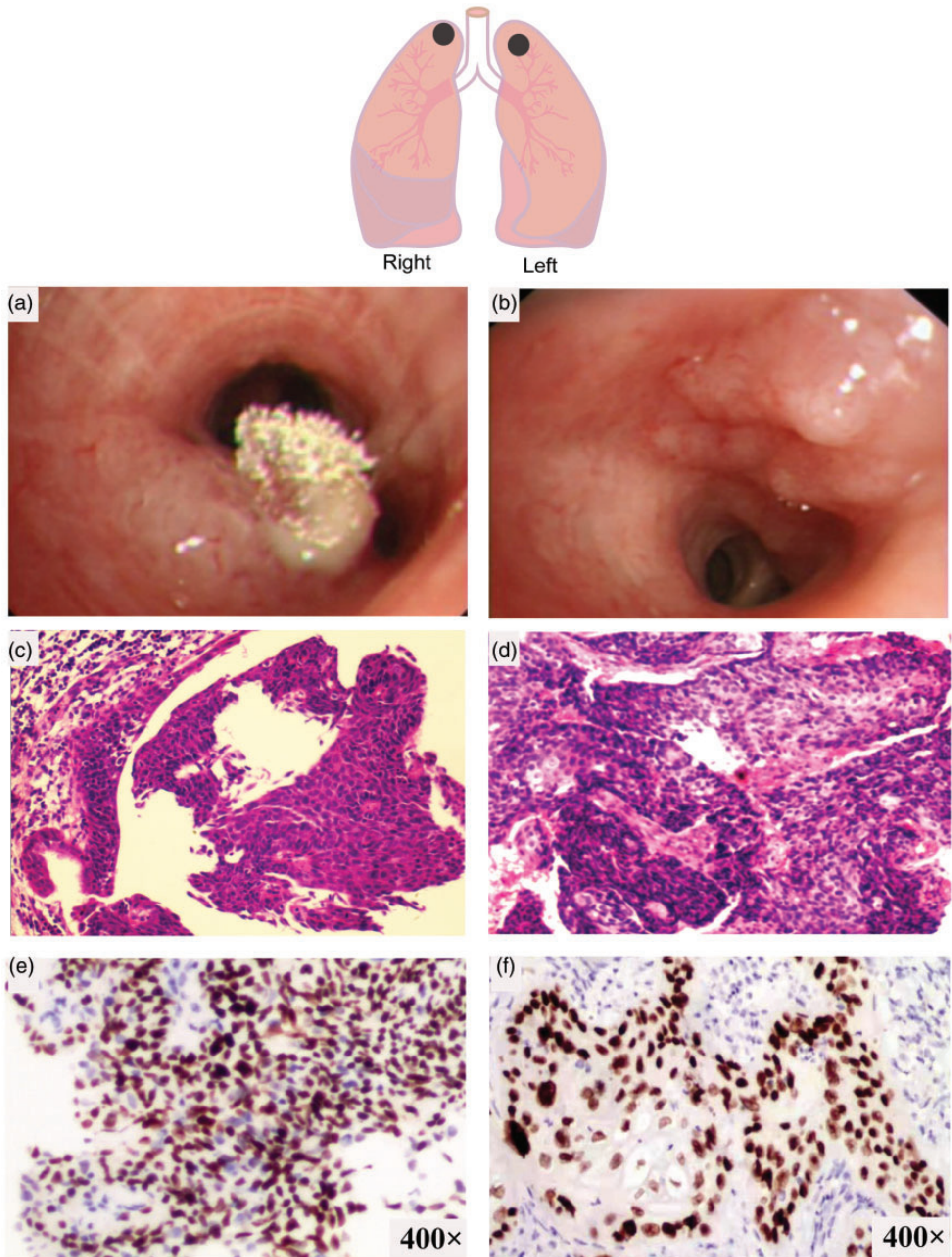

Figure 1. Histopathologic slides from the bilateral lung lesions. Bronchoscopy showing bilateral pulmonary nodules in the right and left upper lobes (a, b), and the biopsies of the right and left lesions were positive for p40 (c, d). Hematoxylin and eosin (HE) staining of the lobectomy samples showing high-grade hyperplasia of bronchial squamous epithelium in the right upper lobe (e) and non-keratinizing squamous cell carcinoma in the left upper lobe (f).

infiltration (Figure 1e). For the left lesion, non-keratinizing squamous cell carcinoma with superficial infiltration was identified (Figure 1f). No metastases were found in the regional lymph nodes or the bronchial stump. These results suggested that the bilateral LSCC was stage IA (tumor, node, metastasis: T1aN0M0). The patient received regular follow-up, with no recurrence or metastasis, and follow-up is on-going.

The bilateral primary tumor lesions were then subjected to NGS targeting 425 cancer-relevant genes[8] (GeneseeqPrime; Geneseeq Technology Inc., Nanjing, China), and their genetic alterations were analyzed and compared (Table 1). Mutated genes in the right upper lobe tumor were as follows: discoidin domain receptor 2 *(DDR2)*, fibroblast growth factor receptor 3 *(FGFR3)*, nuclear receptor binding SET domain 1 *(NSD1)*, chemokine receptor type 4 *(CXCR4)*, and *TP53* (c.341_352delTGCATTCTGGGAinsG), whereas only a *TP53* mutation (c.475G>C) was detected in the left upper lobe tumor (Table 1). Therefore, the mutation pattern was completely different between these two tumors and consistent with sDPLSCC.

Discussion

Synchronous MPLC (sMPLC) was first discovered by Beyreuther in 1924.[10] Despite its rarity, the incidence of sMPLC has been increasing.[11,12] Smoking, lymph node metastasis, and pleural invasion are independent risk factors regarding the prognosis of patients with sMPLC, while early detection and operation can improve treatment outcomes.[13] Owing to the lack of understanding of sMPLC and diagnostic limitations, sMPLC is often ignored or misdiagnosed as tumor metastasis or recurrence. Recently, NGS has played an essential role in understanding the alterations in cancer-related gene pathways, and this technology is now widely used in the diagnosis of primary and metastatic cancers.[14,15]

In this case study, we confirmed that the two bilateral lung lesions were independent primary tumors, inferred by genetic profiling. The tumor suppressor gene, *TP53*, which is the most commonly altered gene in LSCC and which plays crucial roles in cell cycle arrest and apoptosis, was mutated in both lesions, in our patient. In the left

Table 1. Genetic alterations identified in the two primary malignancies.

			MAF (mapped reads/total reads)	
Gene	Alteration	Nucleotide Change	Right LSCC	Left LSCC
TP53	p. L114CfsX31	c.341_352delinsG	10.66% (60/663)	–
DDR2	p. P5S	c.13C>T	9.97% (30/294)	–
FGFR3	p. S249C	c.746C>G	6.03% (27/416)	–
NSD1	p. G2155R	c.6463G>A	5.84% (48/812)	–
CXCR4	p. L165I	c.493C>A	5.37% (77/1432)	–
POLH	p. S459L	c.1376C>T	4.92% (41/785)	–
KMT2A	p. R677*	c.2029C>T	2.83% (30/1033)	–
SDHA	p. R340G	c.1018A>G	1.12% (8/698)	–
TP53	p. A159P	c.475G>C	–	1.28% (10/760)

*, stop codon; –, not applicable; MAF, mutant allele frequency; LSCC, lung squamous cell carcinoma; DDR2, discoidin domain receptor 2; FGFR3, fibroblast growth factor receptor 3; NSD1, nuclear receptor binding SET domain 1; CXCR4, chemokine receptor type 4; KMT2A, lysine (K)-specific methyltransferase 2A; SDHA, succinate dehydrogenase complex, subunit A, flavoprotein.

lesion, a *TP53* c.475G>C (p. A159P) mutation was identified at a mutant allele frequency (MAF) of 1.28% (10/760). This *TP53* mutation was associated with cancer prognosis and recurrence in breast cancer and hepatocellular carcinoma;[16,17] however, this mutation has never been reported in LSCC. The *TP53* p. A159P mutation was located at the DNA-binding domain and was predicted as a pathogenic mutation that disrupts the structure of the β-sandwich.[18,19] In the right lesion, a *TP53* c.341_352delinsG (p. L114CfsX31) mutation was detected with a MAF of 10.66% (60/663). Furthermore, *DDR2* and *FGFR3* mutations were also identified in the right lesion. *DDR2* is a receptor tyrosine kinase that binds collagen to promote cell migration, proliferation, and survival, and *DDR2* mutation-mediated cellular transformation can be blocked by dasatinib treatment or dasatinib in combination with tyrosine kinase inhibitors, in LSCC.[20] *FGFR3* mutations in LSCC drive cellular transformation, which can be reversed by FGFR small molecule inhibitors.[21] Our case study suggests that genetic testing for each sMPLC tumor might be necessary to confirm the diagnosis, elucidate underlying molecular features, and direct treatment decisions.

Conclusion

In this study, we reported a rare case of a 61-year-old male patient with synchronous DPLSCC diagnosed by histology and genetic profiling. Our findings provide further evidence supporting the necessity of genetic testing for primary tumor diagnosis.

Acknowledgments

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Declaration of conflicting interest

Ting-Ting Wang, Si-Si Liu, and Yang Xu are employees of Geneseeq Technology Inc., Nanjing, China. The remaining authors declare no conflict of interest.

Ethics statement

This study was approved by the Ethics Committee of Hunan Cancer Hospital. The patient provided written informed consent for publication.

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ORCID iD

Wen-Xiang Wang https://orcid.org/0000-0002-6902-5359

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