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Case Report

CASE REPORT

# Whole-exome sequencing in a patient with synchronous triple primary malignancies involving lung cancer: a case report

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## Abstract

The incidence of multiple primary malignancies (MPMs) has been increasing rapidly in recent years, however, the genetic pathogenesis is largely unknown on account of rare cases, especially for those patients who are diagnosed with three or more tumors. Under these circumstances, whole-exome sequencing (WES) may help to provide more comprehensive genomic information and guidance to proper therapeutic strategies. Here, we presented a rare case of a 66-year-old Chinese male patient who was diagnosed with synchronous triple primary malignancies: esophageal squamous cell carcinoma (ESCC), lung adenocarcinoma (LA), and hepatocellular carcinoma (HCC). Tumors were surgically removed within 3 months. WES was performed when the patient suffered from cancer recurrence and tumor-specific neoantigens were predicted. Each tumor displayed a distinct somatic mutation profile, providing direct evidence of independent origins. No shared driver gene mutation or neoantigen was detected among the three tumors. Two germline alterations of cancer susceptibility genes—*SPINK1* c.194 + 2T>C and *JAK3* c.425G>A were identified. This case is the first report of synchronous primary triple cancers covering the esophagus, lung, and liver. Our findings highlight the complexities of MPMs that even when under identical germline genetic backgrounds, the occurrence of MPMs can be a random event and driven by distinct somatic gene mutations. Synchronous multiple primary cancers that originated from different organs may not have common therapeutic gene targets, and it can be difficult to find a treatment to cover all the tumors.

**Key words:** triple primary malignancies; whole-exome sequencing; lung adenocarcinoma; esophageal squamous cell carcinoma; hepatocellular carcinoma; somatic mutation; germline mutation; neoantigen

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## Background

The incidence of multiple primary malignancies (MPMs) has been increasing for the past few years, among which the incidence of lung cancer-related MPMs was estimated to range from 2.5% to 3.4% of total lung cancer patients.[1,2] Although frequently taken as an indicator of hereditary cancer, fewer than 25% of MPM patients had an identified pathogenic germline variant,[3] and there is a paucity of information on the genetic mechanisms underlying pathogenesis. It can be quite challenging to understand the etiology and find a therapeutic regimen to cover each type of cancer simultaneously, especially for those patients who were diagnosed with three or more tumors. Compared with next-generation sequencing (NGS)-based target gene panel, whole-exome sequencing (WES) may provide more comprehensive genomic information to guide diagnosis and therapy.

Here, we report the first case of a patient with synchronous occurrence of triple primary malignancies: esophageal squamous cell carcinoma (ESCC), lung adenocarcinoma (LA), and hepatocellular carcinoma (HCC). Each tumor harbored a distinct somatic mutation profile and was driven by different gene mutations. Tumor-specific neoantigens were predicted. We describe two novel germline cancer susceptibility gene alterations—*SPINK1* c.194 + 2T>C and *JAK3* c.425G>A mutations in MPMs.

## Case Presentation

In June 2017, a 66-year-old Chinese male patient presented at our hospital due to progressive dysphagia. Contrast-enhanced chest CT showed thickening of the esophageal wall, obvious lumen stenosis, and a blurred peripheral fat gap. Enhanced scan showed marked enhancement (Fig. 1A). Upper gastrointestinal X-ray barium meal examination further confirmed mid-esophageal luminal stenosis (Fig. 1B). CT also showed a 1.9 cm × 1.2 cm soft tissue mass shadow in the posterior segment of the left upper lobe, with peripheral burrs, pleural indentation, and mild dilatation of the bronchus (Fig. 1C, D). In July 2017, the patient received excision of left thoracic esophageal tumor and wedge resection of left upper lobe. Histopathological evaluation revealed distinct tumor morphologies. The esophagus tumor was identified as a moderate-to-poorly differentiated ESCC (T4N0M0; stage III) (Fig. 2A). The lung tumor was diagnosed as a moderate-to-poorly differentiated LA (T1bN0M0, stage IA2) (Fig. 2B). Immunohistochemistry (IHC) of LA revealed positive expression of CK7, TTF-1, Ki67 (MlB-1), with negative expression of CK20, CDX2, P63, ALK-V (D573), ROS-1. This patient was a former smoker with a 10-pack per year history, but quit 3 years ago. There was no remarkable family or medical history.

In August 2017, chest CT re-examination showed no signs of recurrence. However, abdomen MRI revealed a 2.4 cm × 2 cm massive shadow in the right anterior superior segment of the liver (Fig. 1E, F). In September 2017, the patient underwent liver tumor resection. Postoperative pathological showed a moderate differentiated HCC (T2N0M0, stage II) with focal hyaline degeneration (Fig. 2C). IHC revealed positive expressions of Hepa, GPC-3, and GS, while negative expressions of AFP, P63, P40, TTF-1, and CK7. The patient suffered from swallowing obstruction again 7 months after surgery. PET-CT showed anastomotic recurrence of esophageal cancer and a new hepatic lesion. Obstruction was alleviated after radiochemotherapy. Unfortunately, the liver lesion soon progressed, and hepatic transcatheter artery chemoembolization (TACE) was performed. In October 2018, this patient died of respiratory failure due to pulmonary infection.

To investigate the underlying genetic etiology and at the same time explore for a personalized therapeutic regimen that can cover all types of tumor, WES were performed when the patient suffered cancer relapse. For identification of somatic gene alterations, DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor sections and sequenced on Illumina HiSeq platform. All tumors were tumor mutation burden (TMB) moderate and microsatellite instability (MSI) low (Supplementary Table S1), and each harbored a distinct somatic mutation profile (Fig. 3A). A total of 134 nonsynomous mutations were identified in ESCC, including four potential driver mutations: *AJUBA* c.1008_1009insTTCTCTTCTCAGGC, *KMT2D* c.8719dupT, *TP53* c.844T>C and c.727C>T. For LA, 216 nonsynomous mutations were identified, including three potential driver mutations: *RB1* c.1723C>T stop_gain, *TP53* c.851delT, and *EGFR* c.2573T>G (L858R). For HCC, 103 nonsynonymous were identified, but none of the driver mutations were detected (Table 1). The detailed information of somatic mutations was listed in Supplementary Table S2. These results provided direct evidences that each tumor originated independently, which were consistent with the histopathological assessment.

To further study the germline gene alterations, DNA was extracted from peripheral white blood cells and sequenced via WES. Three high-confidence germline heterozygous variants of cancer susceptibility genes were detected, including *SPINK1* c.194 + 2T>C splice site, *JAK3* c.425G>A, and *UGT1A1* c.1091C>T mutations, which displayed different variant allele frequencies (VAFs) in each tumor (Fig. 3B). Additionally, tumor neoantigens were predicted based on WES results of tumor tissues and normal cells. Target neoantigens were predicted and scored based on a predefined set of criteria. Each tumor had a moderate tumor neoantigen burden. No shared neoantigen was identified among the three tumors. The detailed information about the tumor neoantigens is listed in Supplementary Table S3.

## Discussion

To our knowledge, this is the first report of synchronous esophagus, lung, and liver cancers that happened in one

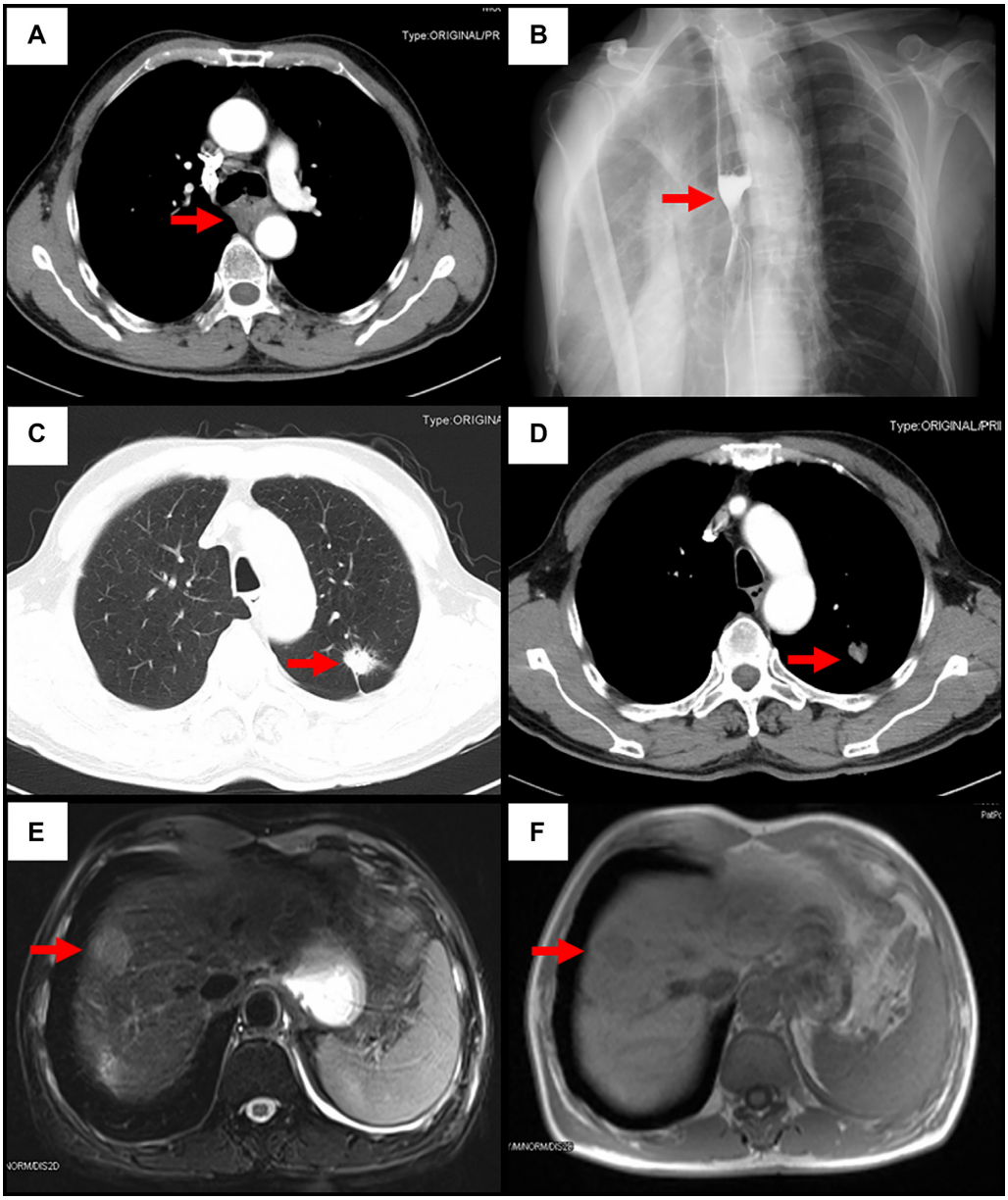


**Figure 1.** Radiographic images of three primary tumors. Contrast-enhanced chest CT (**A**) and upper gastrointestinal X-ray barium meal (**B**) show a left thoracic esophageal tumor. (**C, D**) Chest CT shows a 1.9 cm × 1.2 cm soft tissue mass shadow in the posterior segment of the left upper lobe. (**E, F**) Abdomen MRI reveals a 2.4 cm × 2 cm massive shadow in the right anterior superior segment of the liver. The arrow shows the location of tumor.

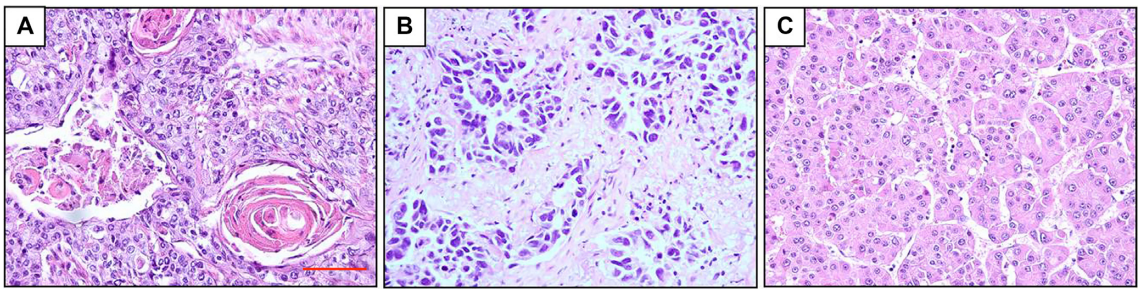


**Figure 2.** Pathologic subtype of different lesions. Hematoxylin and eosin staining of three primary cancers: (**A**) esophageal squamous carcinoma, (**B**) lung adenocarcinoma, and (**C**) hepatocellular carcinoma. Scale bar, 100 $\mu$m.

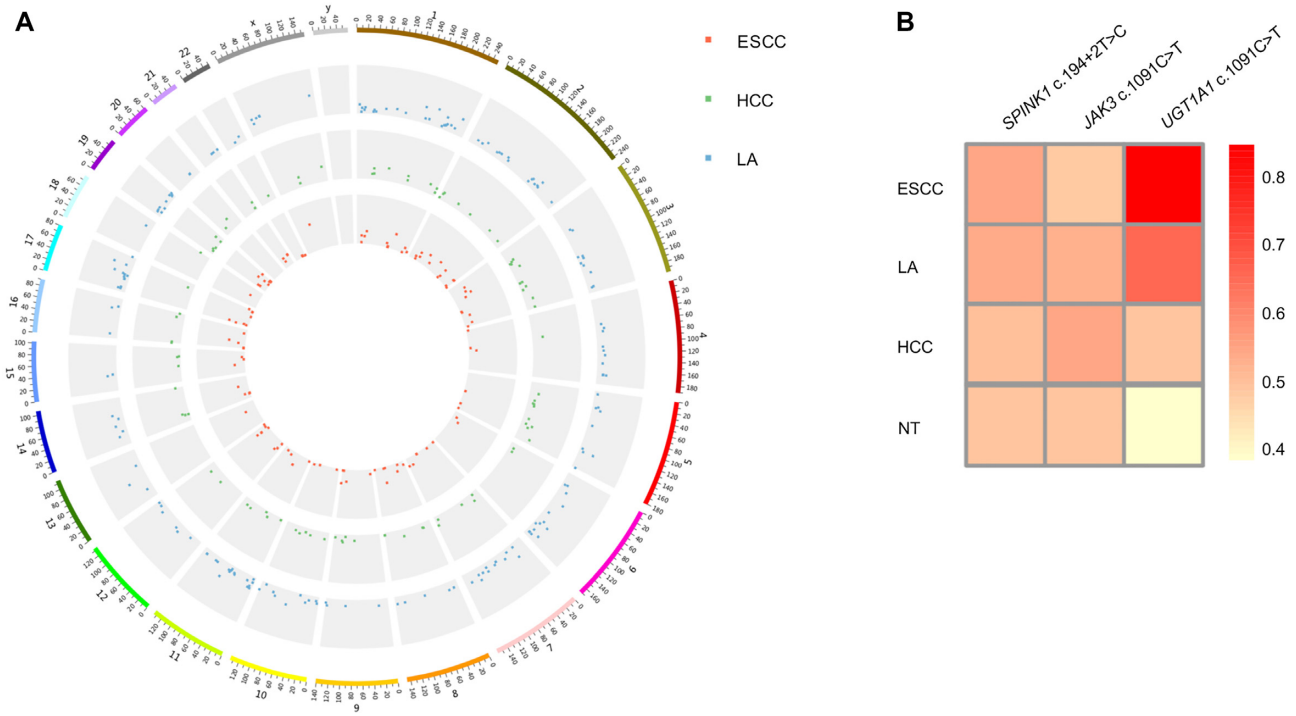


**Figure 3.** Nonsynonomous mutations of three primary tumors. (A) Circus plots of somatic nonsynonomous mutations. The inner ring displays the mutations in esophageal squamous cell carcinoma (ESCC). The middle ring displays the mutations in hepatocellular carcinoma (HCC). The outer ring displays the mutations in lung adenocarcinoma (LA). Nonsynonomous mutations were detected by whole-exome sequencing (WES) and included both single nucleotide variations (SNVs) and frameshift indels. (B) Germline variant allele frequencies (VAFs) of three primary tumors and normal tissue (NT). ESCC, esophageal squamous cell carcinoma; LA, lung adenocarcinoma; and HCC, hepatocellular carcinoma.

**Table 1.** Nonsynonomous mutations identified in synchronous triple primary malignancies by whole-exome sequencing (WES).

| Primary cancer site | No. of nonsynonomous mutations | No. of SNV | No. of InDel | CNV | No. of fusion gene | Driver mutation |
|---|---|---|---|---|---|---|
| Esophageal | 134 | 103 | 31 | *ARHGEF10* gain (3.61), *KAT6A* gain (3.63), *EIF3H* gain (3.7), MYC (4.32) | 0 | *AJUBA* c.1008_1009insTTCTCTTCTCAGGC, *KMT2D* c.8719dupT, *TP53* c.844T>C and c.727C>T |
| Lung | 216 | 200 | 16 | 0 | 0 | *RB1* c.1723C>T stop_gain, *TP53* c.851delT, *EGFR* c.2573T>G |
| Liver | 103 | 93 | 10 | 0 | 0 | N/A |

Abbreviations: SNV, single nucleotide variant; InDel, insertion or deletion mutation; CNV, copy-number variation; N/A, not available.

patient. According to previous studies, only 3.9% to 5.4% of lung cancer-related MPMs were diagnosed with three or more tumors.[1,2] Although germline alterations such as *BRCA1/2* and *TP53* mutations have been demonstrated as a strong genetic predisposition toward MPM, only fewer than 25% of patients had an identified pathogenic germline variant.[3] In this situation, it is attractive to find new therapeutic strategies that can cover all types of cancer in the same patient, especially for those originated from different organs.

According to current guidelines, it is difficult to find a therapeutic regimen to cover all the tumors in our patient. Furthermore, the present standard relies on histopathology to distinguish MPMs, which can hardly provide comprehensive information. Although several studies have used NGS-based gene panel to classify MPMs, most of those are confined to focal changes and provide limited information. There is no systemic genomic evaluation for MPMs that represent a variety of tumor types. In this case, WES was used to detect somatic and germline alterations when the patient suffered from cancer recurrence, and tumor neoantigens were predicted for future design of an individualized cancer vaccine. Unfortunately, no shared therapeutic gene target or neoantigen was identified. Each tumor showed a distinct somatic mutation profile and was driven by different oncogenic event. The tumors were further evaluated as a moderate or low TMB, indicating current immunotherapies such as PD-L1/PD-1 checkpoint inhibitors may not be applicable. Interestingly, three unreported deleterious heterozygous germline variants were identified: *SPINK1* c.194 + 2T>C splice site,

*JAK3* c.425G>A, and *UGT1A1* c.1091C>T. Serine protease inhibitor Kazal-type 1 (SPINK1) encodes human pancreatic secretory trypsin inhibitor, and *SPINK1* c.194 + 2T>C is the most frequent mutation in Chinese patients with chronic pancreatitis, which causes the skipping of entire *SPINK1* exon 3 and leads to a loss of function phenotype.[4] Several clinical studies have suggested a positive correlation between *SPINK1* expression and cancer. Strong SPINK1 expression has been detected in gastric cancer tissues as a sign of favorable outcome.[5] In bladder urothelial carcinoma, SPINK1 expression deficiency has been found to be correlated with biologically aggressive features of cancer and unfavorable outcomes.[6] Heterozygous *SPINK1* c.194 + 2T>C mutation has also previously been reported in a patient with metastatic pancreatic ductal adenocarcinoma.[7] Janus kinase 3 (JAK3) is a protein tyrosine involved in cytokine receptor mediated intracellular signal transduction. JAK3 gene mutation has been identified in ESCC and HCC,[8,9] while germline activating mutation of JAK3 has been detected in 6.7% (62/932) of patients with non-small cell lung cancer (NSCLC).[10] *UGT1A1* c.1091C>T is an common gene polymorphism in the Chinese population and are thus excluded from consideration. Although, in this patient, germline mutations that reported to have a direct relationship with MPMs was not found, germline *SPINK1* c.194 + 2T>C and *JAK3* c.425G>A may function as important background factors in tumorigenesis. However, the specific roles of these two gene mutations in MPMs still need to be further investigated in large population.

In summary, we reported the first case of synchronous esophagus, lung, and liver cancers. This case highlighted the complexity of triple and more primary malignancies, and suggested that the occurrence of MPMs could be a random event. Even under identical genetic background, multiple synchronous tumors can have distinct mutational profiles and be driven by distinct molecular events. This was also supported by one previous study in which a 32-year-old female patient was diagnosed with synchronous triple primary tumors of the lung, kidney, and thyroid. Mutation profiling of this patient was detected by targeted NGS on 416 cancer-related genes, and showed distinct genomic profiles and molecular events.[11] It can be difficult to find an individualized therapeutic regimen to cover all cancer types.

## Supplementary data

Supplementary data is available online at *PCMEDI*.

## Author contributions

DL collected, analyzed, and interpreted the patient data, and wrote the manuscript. MY collected the clinical data. PZ performed histological examinations. JY was responsible for WES and neoantigen prediction. YSW designed the study, revised the article, and approved for the final version to be submitted. All authors read and approved the final manuscript.

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## Conflict of interest statement

Jie Yang was employed by the company YuceBio Technology Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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