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Case Report

Combined large cell neuroendocrine carcinoma of the lung associated with low-grade fetal adenocarcinoma without *β-catenin* mutation: a case report

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Abstract: We report a case of combined large cell neuroendocrine carcinoma of the lung associated with low-grade fetal adenocarcinoma (L-FLAC) without *β-catenin* mutation in exon 3. A 33-year-old man presented at the hospital with a more than 5-month history of cough with no obvious cause. Computed tomography revealed a large, solid, round mass located in the upper lobe of the right lung. Microscopic examination showed two tumor components: large cell neuroendocrine carcinoma and low-grade fetal adenocarcinoma. Immunohistochemistry ofthe fetal adenocarcinoma showed abnormal nuclear/cytoplasmic expression of *β-catenin*, but no exon 3 mutation in *β-catenin*. Our findings provide further insight into the pathologic mechanism of FLAC.

Keywords: Combined large cell neuroendocrine carcinoma, fetal adenocarcinoma, mutation in *β-catenin*

Introduction

Large cell neuroendocrine carcinoma (LCNEC) is defined as a non-small cell carcinoma with neuroendocrine morphology. The incidence of LCNEC is very low. Fetal adenocarcinoma (FLAC) of the lung is also an extremely rare malignant tumor that is often associated with gene mutation in *β-catenin*. Due to the rarity of LCNE and FLAC, much of the literature on LCNE or FLAC comes from respective case reports, making combined large LCNE with FLAC of the lung is even more rare. Patients with LCNEC and/or FLAC are are more likely to be older and heavy smokers. It is worth noting that the patient in this case was a nonsmoker, young male, and no exon 3 mutation of *β-catenin* was observed by DNA polymorphism sequencing in the low-grade (L-FLAC).

Materials and methods

The clinical sample used in the present study was obtained from a patient at the Affiliated Hospital of Southwest Medical University (Luzhou, Sichuan, China). The diagnosis was confirmed by histopathology. The specimens were fixed with 4% formaldehyde solution, and paraffin embedding was routinely performed. After enzymatic digestion, continuous sections were stained by using hematoxylin, eosin, and Alcian blue (AB) (pH 2.5). For immunohistochemistry, a traditional EnVision method was used. Mutations in KRAS, EGFR, and BRAF were detected by RT-qPCR. Diverse point mutations in KRAS were as follows: G12C, G12S, g12r, G12V, G12D, G12A and G13D in codons 12 and 13 of exon 2 (Human KRAS Gene Seven Mutations Detection Kit, Wuhan YZY Medical Science & Technology Co., Ltd., China). The eleven point mutations in EGFR were G719S, G719C, G719A in exon 18, Glu746-Ala750del in exon 19, S768I, T790M, V769-D770insASV, H773-V774insH and D770-N771insG in exon 20, and L858R and L861Q in exon 21 (Human EGFR Gene Mutations Detection Kit, Wuhan YZY Medical Science & Technology Co., Ltd., China). The point mutation in *BRAF* was V600E. Mutations in β-catenin were detected by DNA nucleotide sequencing. The primers for *β-catenin* were as follows: forward primer: 5'-CAATGGGTCATATCACAGATTCT-3' and reverse primer: 5'-CTAAGTATTTGCTATCCTAAATGGT-3. The sequencing primers were provided by Guang-

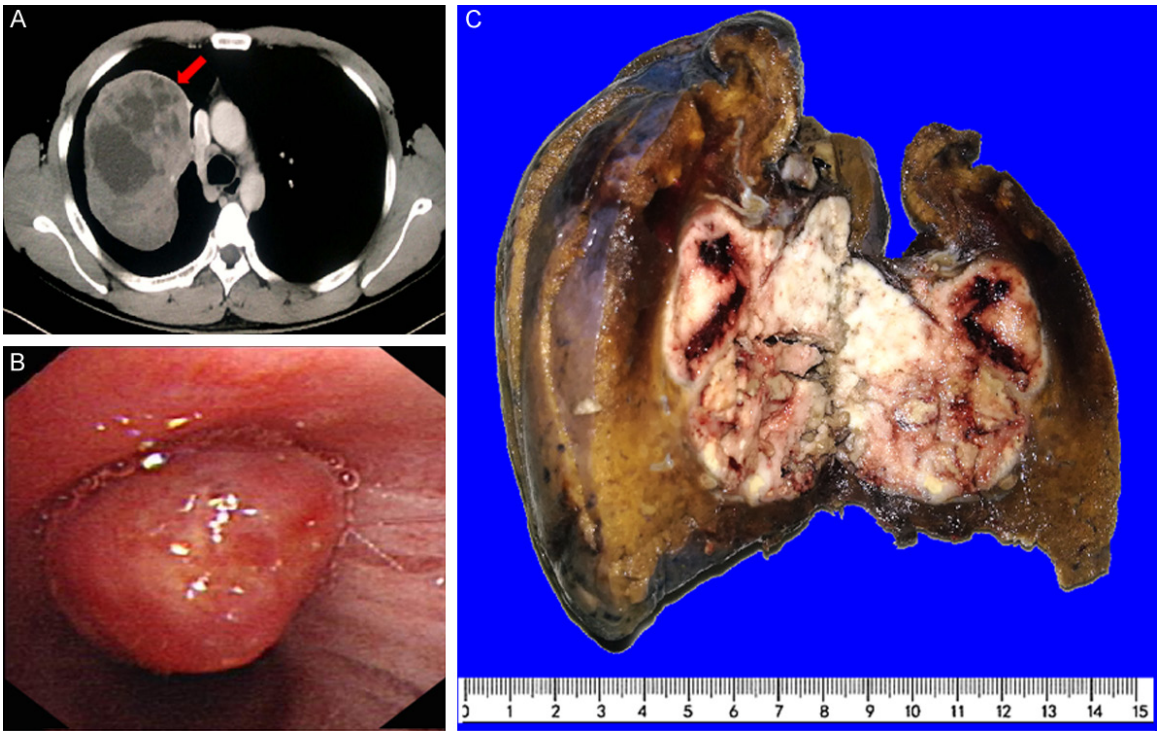


Figure 1. Gross images and radiology. A. Computer tomography image. A large, solid, round mass was revealed in the upper lobe of the right lung (red arrow). B. Bronchoscopy image. A new growth caused the bronchial lumen to narrow completely and occlude. C. Gross examination. The cut surface of the mass was grayish white, solid, medium in texture, brittle, and accompanied by bleeding and necrosis. It invaded the bronchi.

zhou LBP Medicine Science and Technology Co., LTD., China. All procedures were supervised and granted approval by the Ethics Committee of the Affiliated Hospital of Southwest Medical University (No. KY2019181). Written informed consent was obtained from the patient in the present study.

Results

Clinical findings

A 33-year-old man presented to the hospital with a more than 5-month history of cough with no obvious cause and without symptoms of fever, sputum, chest pain, hemoptysis, and dyspnea. The patient was a nonsmoker and had no previous medical history. Computed tomography revealed a large, solid, round mass located in the upper lobe of the right lung (**Figure 1A**). No lymph node swelling or other organ metastases were observed. Bronchoscopy revealed new growth at the branch of the bronchus in the upper right lobe, which caused the bronchial lumen to be completely occluded (**Figure 1B**). The patient underwent right upper lobectomy and lymph node dissection.

Histopathologic and immunohistochemical findings

A 10 cm × 9 cm × 8 cm mass was observed in the lung tissue during gross examination. The cut surface was grayish white, solid, medium in texture, and brittle, accompanied by bleeding and necrosis. The boundary between the mass and surrounding lung tissue was unclear. The mass invaded the bronchus, which was immediately adjacent to the lung capsule (**Figure 1C**).

Microscopically, the tumor cells showed two different components. Most (approximately 70%) were L-FLAC, and the remaining components (approximately 30%) were LCNEC. There was a small amount of fibrovascular stroma between the two components, and no intermigration was observed. Morphologically, the L-FLAC component had tumor cells that formed branched glandular duct structures, which were covered with pseudostratified columnar epithelium. The nuclei were small and relatively uniform, with mild atypia. The cytoplasm was transparent to weakly eosinophilic. Perinuclear vacuoles could be seen. The tumor glands were separated by a loose fibrous interstitial sub-

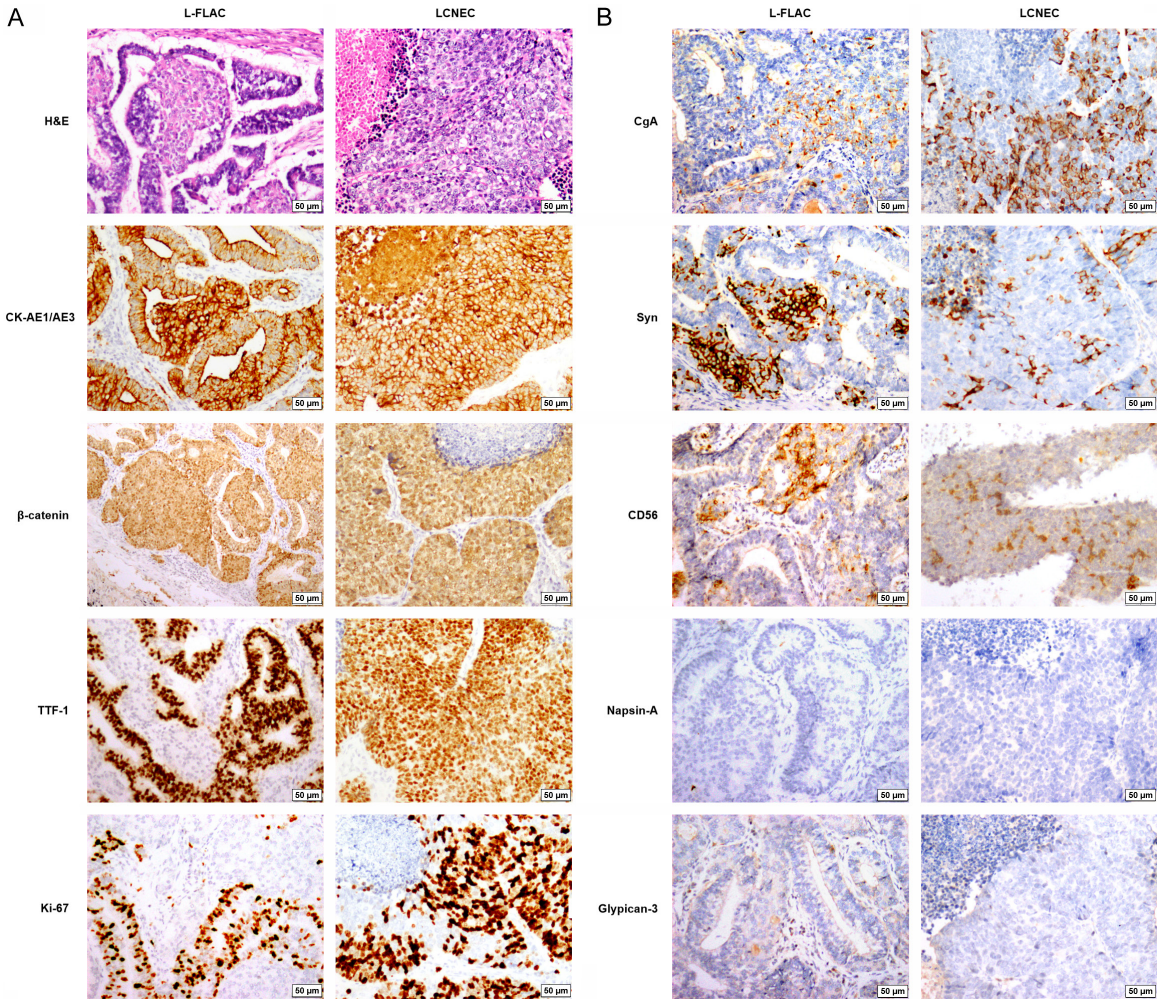


Figure 2. Results of hematoxylin-eosin (H&E) and immunohistochemical staining; (×200). A. Histology of L-FLAC: branched glandular duct structures and morule structures. Histology of LCNEC: tumor necrosis (upper left), peripheral palisading and rosettes, slightly eosinophilic cytoplasm, and nuclei with vesicular chromatin and frequent mitosis. The tumor cells of the two different components were diffusely positive for CK-AE1/AE3, β-catenin and TTF-1. The expression of β-catenin was showed aberrantly nuclear/cytoplasmic, and was more prominent in the morular component of the tumor. The proliferation indexes of Ki-67 were approximately 40% and 70% respectively. B. The tumor cells partially expressed CgA, Syn, and CD56, while neither L-FLAC nor LCNEC expressed Napsin-A or Glypican-3.

stance, and showed a prominent morule structure. The morphology resembled the pseudoglandular stage of a fetal lung. The tumor cells showed aberrant nuclear/cytoplasmic expression of β-catenin, which was more prominent in the morular component of the tumor, with no p53 overexpression, which is one of the characteristics of L-FLAC. In addition, tumor cells also widely expressed CK-AE1/AE3 and thyroid transcription factor-1 (TTF-1) and partially expressed chromogranin-A (CgA), synaptophysin (Syn) and CD56, while neither the glandular duct structure nor the prominent morule structure expressed CK5/6, P40, CK7, CK20, Napsin-A, AFP, and Glypican-3. MIB1 (Ki-67) staining was approximately 40% (**Figure 2**). This histologic pattern and immunohistochemical profile were consistent with L-FLAC.

Morphological characteristics of the LCNEC component included tumor cells arranged in solid nests and pieces with large necrosis, and cells around the cancer nests showed peripheral palisading and rosettes. There were slim fibrovascular stromal cores between cancer nests. The tumor diameter/quiescent lymphocyte size (T/L) ratios were greater than three. The cytoplasm was medium and slightly eosinophilic. Nuclei with vesicular chromatin were clearly observed, with mitotic rates exceeding

40 mitoses per 2 mm^2. Almost all tumor cells showed expression of TTF-1, CK-AE1/AE3 (membrane), and β-catenin (aberrant nuclear/cytoplasmic expression), with partial expression of neuroendocrine markers (Syn, CgA and CD56). The MiB1 (Ki-67) staining was approximately 70% (**Figure 2**). CK5/6, P40, CK7, CK20, Napsin-A, AFP, Glypican-3, and p53 were negative. These histologic patterns and immunohistochemical profiles were consistent with LCNEC of the lung.

Then, we further measured whether KRAS, EGFR, and BRAF were mutated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and whether β-catenin was mutated by DNA nucleotide sequencing in L-FLAC. The results presented no mutations in *EGFR*, *KRAS* or *BRAF* (**Figure 3A-C**). In the present case, the results of immunohistochemical tests confirmed heterogeneous patterns of β-catenin overexpression. Surprisingly, no mutations in exon 3 of *β-catenin* were found (**Figure 3D**, **3E**).

Pathologic diagnosis

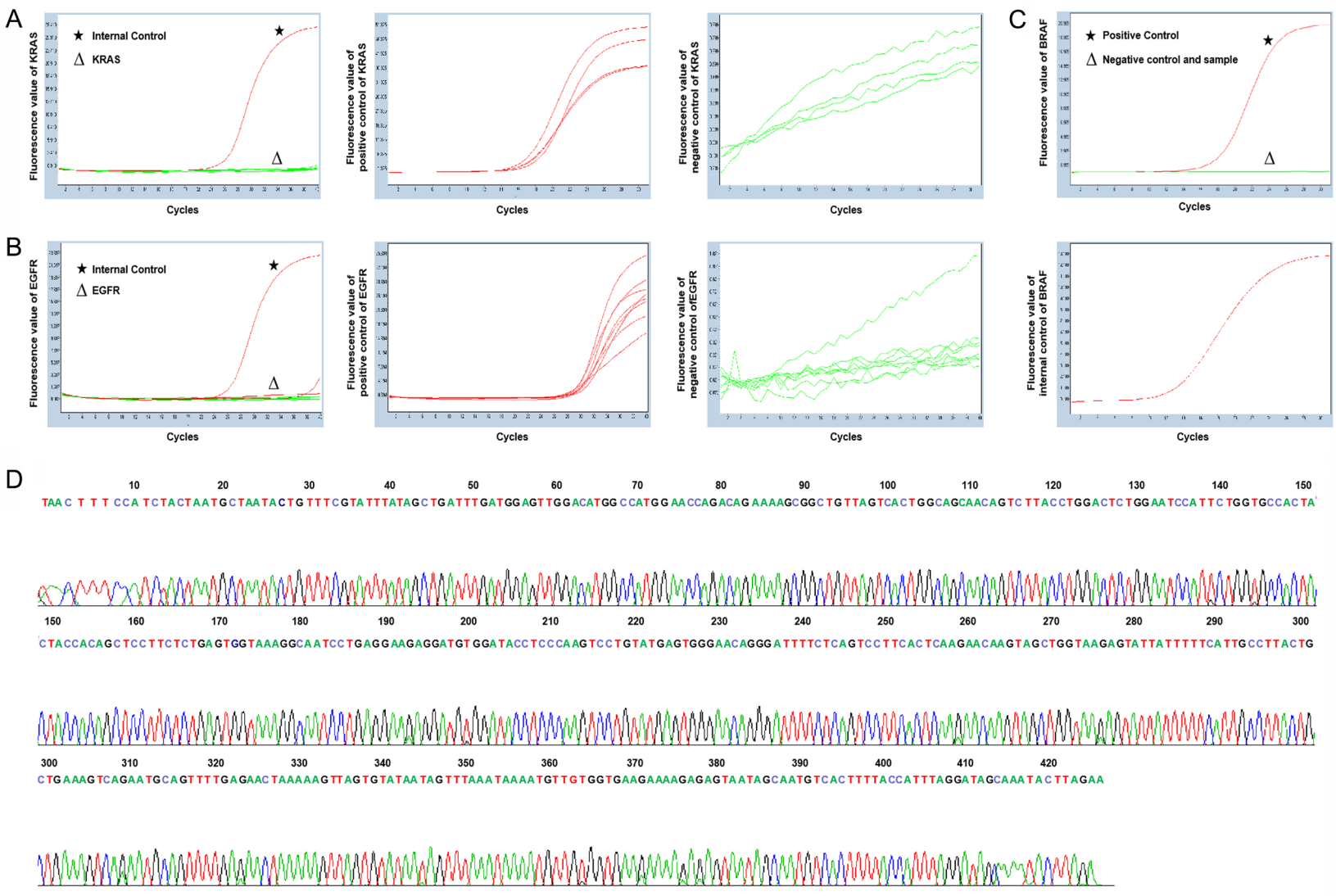
In summary, although mutations in several genes were not detected, the pathological diagnosis in this case was still "combined LCNEC of the lung associated with L-FLAC without mutations of *β-catenin*", due to the classic morphological findings and immunohistochemical results. Fortunately, the patient did not have lymph node or other organ metastases.

Discussion

Large cell neuroendocrine carcinoma (LCNEC) is a highly aggressive non-small cell carcinoma with neuroendocrine morphology that was first defined in 1991 [1]. The incidence of LCNEC is very low and has been reported to range from 2.4% to 3.1% among resected lung cancers [2]. Patients with LCNEC are predominantly male, older, and heavy smokers [1]. The diagnostic criteria for LCNEC include observation of neuroendocrine morphology (nesting, peripheral palisading, rosettes), abundant mitoses (>10 mitosis/2 mm^2), and expression of at least one neuroendocrine markers (Syn, CgA, CD56) [3], all of which were seen in our case. Combined LCNEC is an LCNEC that comprises adenocarcinoma, squamous cell carcinoma, spindle cell carcinoma or/and giant cell carcinoma, with an incidence of 10.6% of all LCNECs [4]. The present case is combined LCNEC associated with FLAC.

FLAC is also an extremely rare adenocarcinoma, accounting for 0.1%-0.5% of all pulmonary neoplasms [5]. Its morphology is similar to that observed in the fetal lung during the pseudoglandular stage (8-16 weeks of gestation) [5, 6]. Fetal adenocarcinoma was first described as a subtype of pulmonary blastoma in 1982 [7], and in 1984, it was described as "fetal adenocarcinoma" [8]. The classification by the World Health Organization in 1999 has removed FLAC tumors from the pulmonary blastoma category and classified them as a variant of adenocarcinoma [9]. Due to the differences in histopathology and clinical processes, FLAC has been further divided into low-grade (L-FLAC) and high-grade (H-FLAC) types. One review summarized the histopathological, immunohistochemical, and genomic characteristics of L-FLAC and H-FLAC [10]. At present, there are several gene mutations have been studied in FLAC: *EGFR*, *KRAS*, *BRAF* and *β-catenin*. Usually, *KRAS* and/or *EGFR* gene mutations are related to most lung adenocarcinomas. However, FLAC is mainly related to gene mutations of *β-catenin* and rarely involves *EGFR* and *KRAS* [5]. A large number of studies have reported that mutations in exon 3 of *β-catenin* play an important role in L-FLAC [1, 2]. Mutations in exon 3 of *β-catenin* will lead to the activation of oncogenic target genes such as *c-myc* and *cyclin D1*, interfere with the degradation of *β*-catenin, and allow it to accumulate in the nucleus and cytoplasm [7]. Since *β-catenin* mutations are rare among lung tumors, this distinctive genetic feature is of great significance in the diagnosis of FLAC. From a genetic point of view, FLAC is thought to be different from conventional lung adenocarcinoma. Interestingly, the immunohistochemical results of the present case showed abnormal nuclear/cytoplasmic expression of β-catenin, but no exon 3 mutation was detected in *β-catenin*. Some studies have reported that most, but not all L-FLAC patients have mutations in exon 3 of *β-catenin* [11, 12]. The authors proposed that mutational inactivation of the *APC* gene and the tumor suppressor gene *PTEN* may also result in abnormal nucleocytoplasmic expression of *β-catenin* [11, 12].

At present, FLAC is currently treated with radical surgery, which can be combined with radiotherapy and/or chemotherapy. The prognosis of L-FLAC is often favorable, with a 5-year survival rate of 85%, which is much higher than the 15-25% survival rate of LCNEC. The tumor and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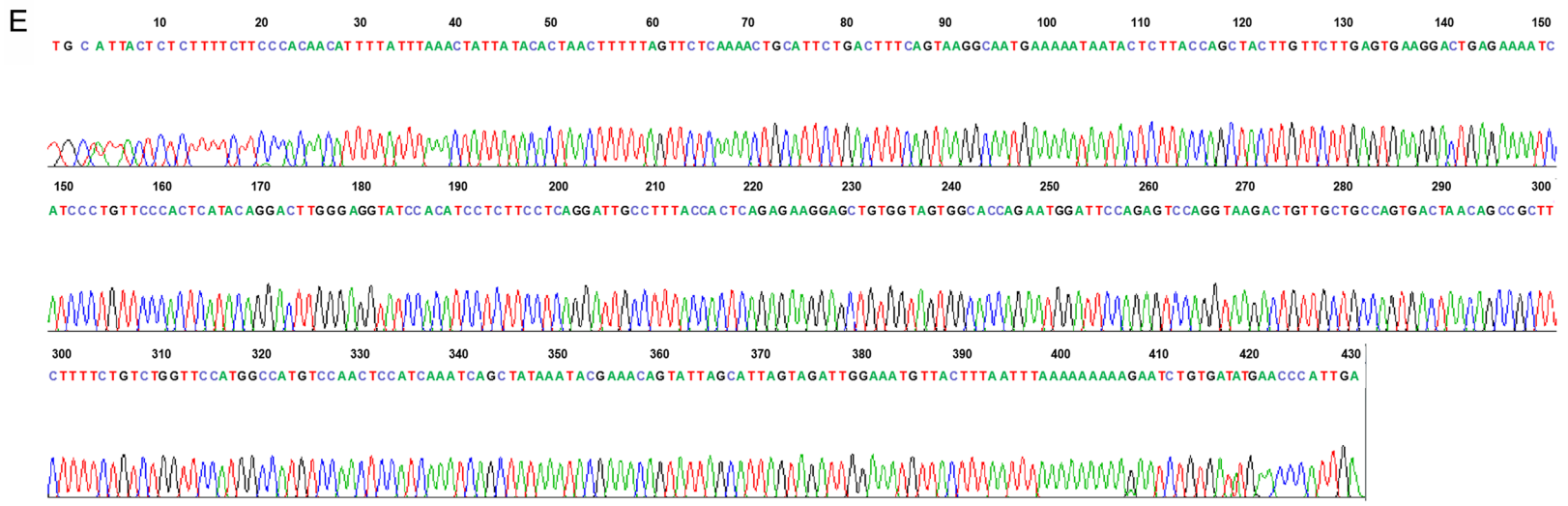

Figure 3. The Study of mutations in *KRAS*, *EGFR*, and *BRAF* by RT-qPCR and *β-catenin* by DNA nucleotide sequencing. (A) The most common mutations of KRAS were wild-type. (B) Common mutations of the EGFR gene were not detected. (C) No V600E mutation of BRAF gene was detected. (D and E) No mutations in exon 3 of β-catenin were found. Sequence analysis for exon 3 of the β-catenin gene in L-FLAC by DNA nucleotide sequencing with forward primer (D) and reverse primer (E).

associated lung lobes of the patient in this case were completely removed by surgery. No regeneration or metastasis was observed within 14 months after surgery. Investigations into the long-term effects are ongoing.

Conclusion

Combined LCNEC of the lung associated with L-FLAC is extremely rare. The pathologic characteristics, gene mutations and prognosis of L-FLAC are different from those of other lung adenocarcinomas. In most but not all patients, *β-catenin* mutation in exon 3 can be detected.

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Disclosure of conflict of interest

None.

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