Am J Transl Res 2020;12(10):6689-6693
www.ajtr.org /ISSN:1943-8141/AJTR0114495

Case Report

Molecular diagnosis and clinical outcome of a lung cancer patient with *TP53*-E285K mutated Li-Fraumeni syndrome harboring a somatic *EGFR*-KDD mutation

Dafu Yang[1], Xue Han[2], Dan Li[1], Saiqiong Cui[1], Sisi Liu[2], Xue Wu[3], Zhaoxia Dai[1]

[1]*The Second Department of Thoracic Medical Oncology, The Second Hospital of Dalian Medical University, Dalian, Liaoning, China;* [2]*Department of Research and Development, Nanjing Geneseeq Technology Inc., Nanjing, Jiangsu, China;* [3]*Translational Medicine Research Institute, Geneseeq Technology Inc., Toronto, Ontario, Canada*

Received May 14, 2020; Accepted September 20, 2020; Epub October 15, 2020; Published October 30, 2020

Abstract: Objectives: Li-Fraumeni syndrome (LFS) is an autosomal dominant cancer predisposition, mostly caused by germline *TP53* mutations. Lung adenocarcinoma (ADC) has been identified as the most frequent LFS-related cancer outside the common LFS core spectrum. EGFR-kinase domain duplication (KDD) is rare in lung cancer and the effective therapy for LFS patients with *EGFR*-KDD mutated ADC is unclear. This study reports the first case of a *TP53*-mutated LFS patient with confirmed family history, developing advanced lung ADC harboring *EGFR*-KDD. Materials and methods: The patient's lung tumor, lymph nodes, liquid biopsies and germline control sample at various disease stages were subjected to next-generation sequencing (NGS). The *TP53* germline mutation was confirmed using the peripheral blood of the patient's relatives by Sanger sequencing. Results: A rare *EGFR*-KDD somatic mutation that was missed in the routine *EGFR* hotspots test, and a *TP53*-E285K temperature-sensitive germline mutation were identified by NGS. The patient was diagnosed with breast cancer in 2006 and her family cancer history review revealed that seven out of 13 relatives were diagnosed or died from LFS-spectrum cancers before the age of 45 years. Three of the six relatives were positive for the *TP53*-E285K germline mutation. This patient received multi-line chemotherapy followed by anlotinib, a multi-target tyrosine kinase inhibitor, upon the identification of *EGFR*-KDD, and achieved an overall survival of 18 months. Conclusions: Our study highlights the importance of NGS in discovering rare genetic alterations to guide treatment decision-making, and provides meaningful insight into the potential treatment options for LFS patients with *EGFR*-KDD mutations.

Keywords: Li-Fraumeni syndrome, *TP53* temperature-sensitive mutation, *EGFR*-KDD, lung adenocarcinoma, next-generation sequencing

Introduction

Li-Fraumeni syndrome (LFS) is a rare autosomal dominant cancer predisposition syndrome characterized by the early onset of multiple tumors. LFS was described in 1969 by Li and Fraumeni, and was subsequently shown to be associated with germline *TP53* inactivation mutations [1]. As one of the most prominent tumor suppressor genes, *TP53* plays a key role in DNA damage signaling by stimulating cell cycle arrest, apoptosis and DNA repair mechanisms to protect cellular genomic integrity [2]. Therefore, *TP*53 loss enables the acquisition of additional driver events to accelerate transformation.

Beyond the core LFS spectrum cancers, including sarcoma, breast cancer, brain tumor and adrenal cortical carcinoma, lung adenocarcinoma (ADC) is the most frequently occurring in LFS patients; however, the molecular alteration of LFS-related lung ADC is still unclear and requires further evaluation [3]. Oncogenic mutations of the epidermal growth factor receptor (*EGFR)* gene occur in approximately 45% of Asian lung ADC patients, among which the most frequent are short in-frame deletions in exon 19 (Ex19del) and point mutations in exon 21 (L858R) [4]. Patients with *EGFR*-mutated lung cancers generally achieve better clinical outcomes when treated with EGFR tyrosine kinase inhibitors (TKIs) [5].

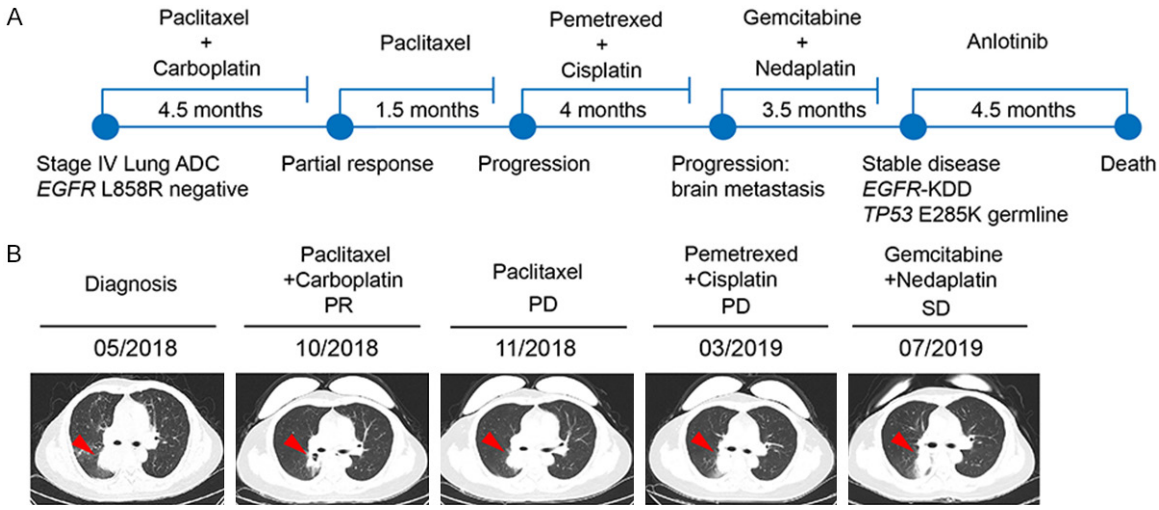

Figure 1. Representative clinical images obtained during the course of treatment. A. The disease timeline shows the treatments received by the patient and her clinical response. ADC: adenocarcinoma. B. Chest-CT scans show the disease progression following treatment. Tumor lesions are indicated by red arrows. PR: partial response; PD: progressive disease; SD: stable disease.

With the advance of genetic testing techniques, rare *EGFR* alterations, such as *EGFR* kinase domain duplication (KDD), are increasingly observed [6]. *EGFR*-KDD typically consists of an intragenic alteration in *EGFR,* which leads to the tandem duplication of EGFR exons 18-25 and constitutively activated *EGFR* signaling [7]. One study reviewed several lung ADC cases with *EGFR*-KDD alterations and identified significant antitumor responses to EGFR TKI therapies [7]. However, the efficacy of EGFR TKIs for LFS patients with *EGFR*-KDD was unclear.

Case presentation

A 39-year-old female non-smoker with a diagnosis of breast cancer at the age of 28 years, was diagnosed with stage IV (cT4N2M1b) lung ADC with multiple lymph node metastases in May 2018 (**Figure 1**). Immunohistochemistry (IHC) staining of a lung tumor biopsy and mammary gland/axillary lymph nodes was positive for the pulmonary ADC markers, TTF-1 and napsin A, but negative for the breast cancer markers, lactoglobulin and GATA-3; thus, confirming that they were lung ADC samples and not recurrent breast cancer samples. Due to a negative result of a PCR-based genetic test for *EGFR*-L858R, the patient was subsequently administered six cycles of TP therapy (paclitaxel, 30 mg d1 and carboplatin, 750 mg d1) and achieved a partial response (PR) (**Figure 1**). Paclitaxel monotherapy was used for an additional two cycles for maintenance. However, the patient developed progressive disease (PD) with an enlarged primary tumor in November 2018 (**Figure 1B**). Therefore, the patient was transitioned to AP treatment (pemetrexed, 1 g d1 and cisplatin, 40 mg d1-3) for four cycles. In March 2019, a CT scan and cerebrospinal fluid (CSF) cytology revealed a progression of the pulmonary lesions and newly-occurring meningeal metastasis, respectively. The patient received combination therapy of gemcitabine (1.6 g d1 and d8) plus nedaplatin (130 mg d2). After four cycles, the patient achieved a stable disease (SD) in July 2019 (**Figure 1B**), but the meningeal metastasis progression continued. The total survival benefit of multi-line chemotherapy was 13.5 months.

To seek a more effective treatment strategy, tissues of lung, mammary gland and axillary lymph nodes, which were collected before chemotherapy, accompanied with newly collected CSF and blood biopsies, were subjected to NGS of 425 cancer-relevant-genes in July 2019. Genomic profiling identified an extremely rare *EGFR*-KDD mutation and a *TP53* E285K (c.853G>A) germline mutation in all samples (Supplementary Table 1).

To determine whether the patient was a case of LFS, we investigated her family history and tested for the *TP53* E285K germline mutation in blood biopsies provided by six relatives in

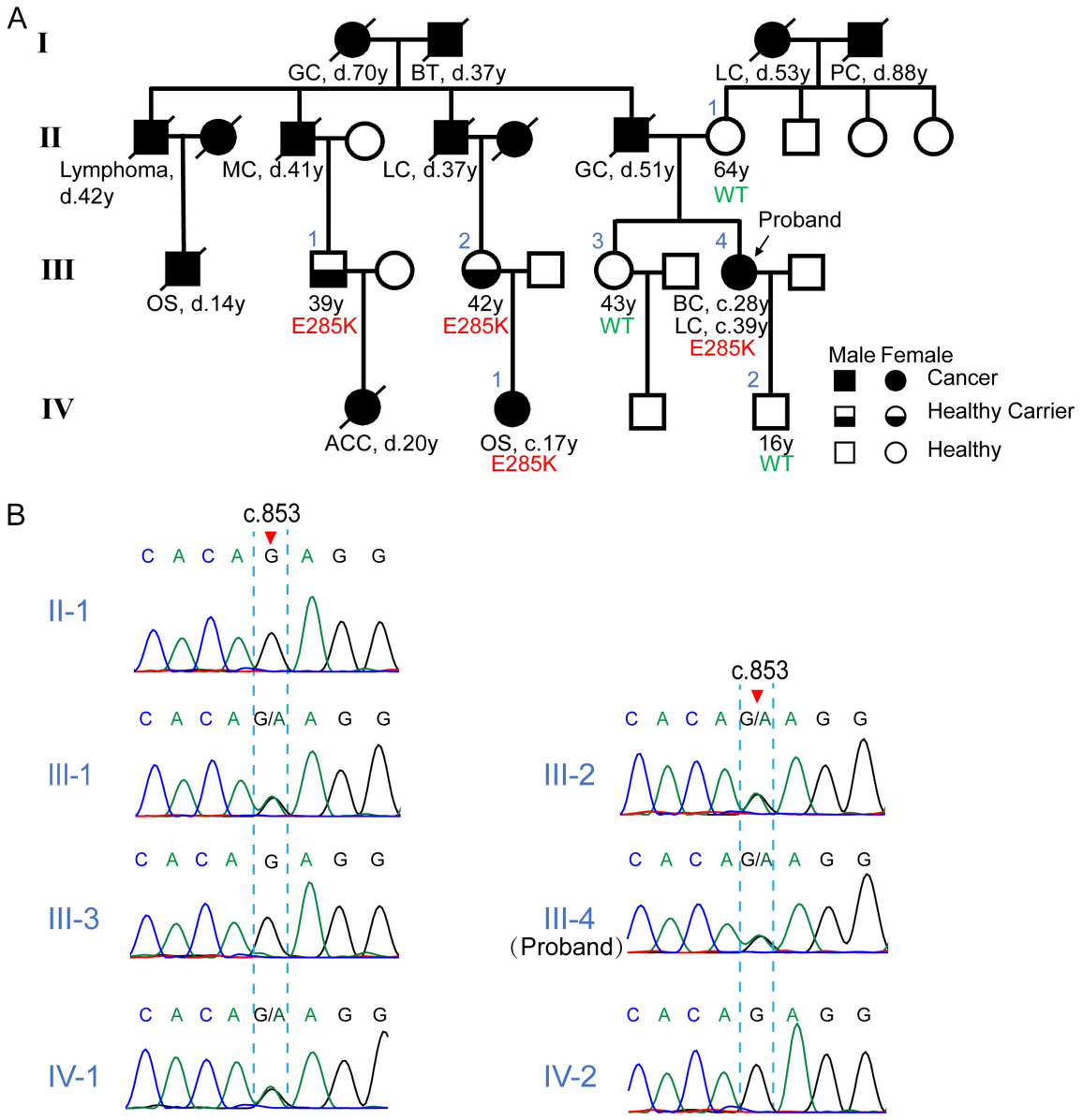

Figure 2. Pedigree of the proband's family with LFS and *TP53* E285K germline mutations. (A) The pedigree of the proband's family with LFS. Patients labeled with numbers were screened for *TP53* E285K mutations. The status of each patient is indicated as wild-type (WT) or as harboring the E285K mutation (E285K). The proband is indicated by an arrow. The numbers below each symbol indicate the following: age of death (d.70y), age at diagnosis of cancers (c.28y) or current age (39y). GC: gastric cancer; BT: brain tumor; LC: lung cancer; PC: pancreatic cancer; MC: mediastinal cancer; BC: breast cancer, ACC: adrenocortical carcinoma; OS: osteosarcoma. (B) The chromatograms of the DNA sequences of the mutated locus in family members who were labeled as II-1 to IV-2, based on to the pedigree in (A). The wild-type sequence was observed in II-1, III-3 and IV-2, while a c. G853A germline transversion was observed in III-1, III-2, III-4 and IV-1.

her family, five of whom had no symptoms of cancer up to date, and one was diagnosed with osteosarcoma at 17 years of age. Family history revealed that the patient's father died of gastric cancer at the age of 51 years, her three uncles died of lung cancer (age 37 years), mediastinal cancer (age 41 years) and lymphoma (age 42 years), respectively, and her grandfather died of a brain tumor at age 37 years (**Figure 2A**). *TP53* E285K germline mutation screening revealed another three carriers aside from the proband; her younger male cousin (age 39 years), elder female cousin (age 42 years) and her niece with osteosarcoma (**Figure 2A**, **2B**). As an *EGFR*-KDD alteration was detected by NGS, the patient then received

anlotinib, a multi-target TKI, once per day (12 mg) on days 1-14 of a 21-day cycle, but died 4.5 months post-treatment. The patient achieved an overall survival (OS) of 18 months.

Discussion

In this report, we described a patient with LFS harboring an *EGFR*-KDD and *TP53* E285K germline mutation who received multi-line chemotherapy, followed by TKI-treatment and achieved an OS of 18 months. *EGFR*-KDD is an extremely rare oncogenic alteration that occurred in approximately 0.12% of all participants, and 0.24% of *EGFR* mutation-positive participants in a study of 10,759 NSCLC patients, and could not be detected via traditional PCR-based approaches [7]. Therefore, NGS provided a more precise option for detecting rare gene alterations.

Studies have shown that EGFR-targeted therapies for *EGFR*-KDD patients achieved significant antitumor responses [7]. Anlotinib, a multi-target TKI, has been reported to significantly improve the OS of patients with refractory NSCLC and benefitted patients with brain metastases [8, 9]. Therefore, anlotinib was the choice for our patient, but only achieved 4.5 months of PFS. This may partially due to our patient having a *TP53* E285K germline mutation as non-disruptive *TP53* mutations are associated with shorter survival in advanced NSCLC treated with TKI [10]. As a strictly temperature-dependent loss of function mutation, E285K is located in the DNA binding domain of *TP53* and pathogenic in a cell-based study and an LFS-linked family [11]. A preclinical study suggested that *TP53* mutations could reduce gefitinib-induced cell apoptosis via regulation of the Fas (factor-associated suicide) signaling pathway in an NSCLC cell line [12]. However, the underlying mechanisms of the effect of *TP53* E285K mutations on TKI treatment needs to be further explored.

The clinical definition of classic LFS includes a proband with a sarcoma at <45 years of age, a first-degree relative with cancer at <45 years of age, and a first- or second-degree relative with any cancer at <45 years of age or a sarcoma at any age [2]. Our patient's family did not entirely fulfill those criteria, but was defined as LFS-like [2]. For the two *TP53* E285K carriers who did not show any symptoms, comprehensive cancer screening and routine medical examinations should be performed.

A recent publication showed that most *EGFR*-mutant lung cancer carriers with *TP53*-mutated LFS were female never-smokers, with medical histories of early-onset breast cancer. Such observations are similar to our patient who was diagnosed with breast cancer at the age of 28 years [13]. Despite the paucity of specific recommendations for tumor therapies in LFS patients, minimizing radiation therapy to prevent treatment-related carcinogenesis is recommended. In a report of two lung cancer patients with LFS harboring targetable common *EGFR* mutations, the disease progressed rapidly during TKI treatment in one case, which indicated that targeted therapy is not suitable for all *EGFR*-mutated LFS patients [13]. In our case, the patient was treated with multi-line chemotherapy and achieved a total survival benefit of 14 months; findings that provided valuable insight into potential treatment options for LFS patients.

Conclusions

In summary, this study reported the first case of a *TP53* germline-mutated LFS patient with confirmed family history of advanced lung ADC harboring *EGFR*-KDD. The patient received multi-line chemotherapy, followed by TKI-treatment and achieved an OS of 18 months. This report highlights the importance of performing comprehensive genomic profiling in discovering rare gene mutations, and providing valuable insights into treatment-decision making for LFS patients with *EGFR*-KDD alterations. The search for effective therapeutic strategies for such patients with LFS harboring *EGFR* mutations must also be continued in the future.

Acknowledgements

The authors thank the patient for providing written informed consent for the publication of the clinical details. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure of conflict of interest

Xue Han and Sisi Liu are employees of Nanjing Geneseeq Technology Inc., China; Xue Wu is an

employee of Geneseeq Technology Inc., Canada. The remaining authors declare no conflicts of interest.

Address correspondence to: Dr. Zhaoxia Dai, The Second Department of Thoracic Medical Oncology, The Second Hospital of Dalian Medical University, No. 467, Zhongshan Road, Shahekou District, Dalian, Liaoning, China. E-mail: ZhaoxiaDai0331@163.com

References

[1] Li FP and Fraumeni JF Jr. Rhabdomyosarcoma in children: epidemiologic study and identification of a familial caneer syndrome. J Natl Cancer Inst 1969; 43: 1365-1373.

[2] Kamihara J, Rana HQ and Garber JE. Germline TP53 mutations and the changing landscape of Li-Fraumeni syndrome. Hum Mutat 2014; 35: 654-662.

[3] Caron O, Frebourg T, Benusiglio PR, Foulon S and Brugières L. Lung adenocarcinoma as part of the Li-Fraumeni syndrome spectrum: preliminary data of the LIFSCREEN randomized clinical trial. JAMA Oncol 2017; 3: 1736-1737.

[4] Shu Y, Wu X, Tong X, Wang X, Chang Z, Mao Y, Chen X, Sun J, Wang Z and Hong Z. Circulating tumor DNA mutation profiling by targeted next generation sequencing provides guidance for personalized treatments in multiple cancer types. Sci Rep 2017; 7: 583.

[5] Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, Dechaphunkul A, Imamura F, Nogami N and Kurata T. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med 2018; 378: 113-125.

[6] Gallant JN, Sheehan JH, Shaver TM, Bailey M, Lipson D, Chandramohan R, Brewer MR, York SJ, Kris MG and Pietenpol JA. EGFR kinase domain duplication (EGFR-KDD) is a novel oncogenic driver in lung cancer that is clinically responsive to afatinib. Cancer Discov 2015; 5: 1155-1163.

[7] Wang J, Li X, Xue X, Ou Q, Wu X, Liang Y, Wang X, You M, Shao YW and Zhang Z. Clinical outcomes of EGFR kinase domain duplication to targeted therapies in NSCLC. Int J Cancer 2019; 144: 2677-2682.

[8] Liang W, Zhao Y, Zhang Y, Han X, Yang X, He J, Li K and Han B. P2.01-01 the impact of anlotinib on brain metastases of NSCLC: post-hoc analysis of a phase III randomized control trial (ALTER0303). J Thorac Oncol 2018; 13: S665.

[9] Li K, Han B, Wang Q, Zhang L, Shi J, Wang Z, Cheng Y, He J, Shi Y, Chen W, Wang X, Luo Y, Nan K, Jin F, Li B and Wang J. OS outcomes to anlotinib in patients (pts) with refractory NSCLC of both wild-type (WT) and mutant EGFR. J Clin Oncol 2018; 36: e21013.

[10] Molina-Vila MA, Bertran-Alamillo J, Gascó A, Mayo-de-las-Casas C, Sánchez-Ronco M, Pujantell-Pastor L, Bonanno L, Favaretto AG, Cardona AF and Vergnenègre A. Nondisruptive p53 mutations are associated with shorter survival in patients with advanced non-small cell lung cancer. Clin Cancer Res 2014; 20: 4647-4659.

[11] Lee DS, Yoon SY, Looi LM, Kang P, Kang IN, Sivanandan K, Ariffin H, Thong MK, Chin KF, Mohd Taib NA, Yip CH and Teo SH. Comparable frequency of BRCA1, BRCA2 and TP53 germline mutations in a multi-ethnic Asian cohort suggests TP53 screening should be offered together with BRCA1/2 screening to early-onset breast cancer patients. Breast Cancer Res 2012; 14: R66.

[12] Rho JK, Choi YJ, Ryoo BY, Na II, Yang SH, Kim CH and Lee JC. p53 enhances gefitinib-induced growth inhibition and apoptosis by regulation of Fas in non-small cell lung cancer. Cancer Res 2007; 67: 1163-1169.

[13] Ricordel C, Labalette-Tiercin M, Lespagnol A, Kerjouan M, Dugast C, Mosser J, Desrues B and Léna H. EFGR-mutant lung adenocarcinoma and Li-Fraumeni syndrome: report of two cases and review of the literature. Lung Cancer 2015; 87: 80-84.

Supplementary Table 1. Genomic alterations detected in different sample types during the course of disease

Gene	Alterations	Nucleotide change	Before chemotherapy			After multi-line chemotherapy	
			Lung	MGN	ALN	Plasma	CSF
TP53	p. E285K	c.853G>A				Germline	
EGFR	*EGFR*-KDD	c.2284-1880_2284-1879ins (27926)	24.7%	10.1%	12.2%	10.0%	19.8%
ALK	p. T538K	c.1613C>A	19.3%	3.0%	2.5%	0.7%	36.3%
PRSS1	p. K170N	c.510G>C	13.7%	3.8%	4.9%	2.8%	15.1%
CCNE1	amplification	NA	6.93	2.68	2.48	2.16	6.72
APC	p. S1970R	c.5910T>G	-	2.2%	4.0%	4.1%	17.3%
AKT3	p. M145I	c.435G>A	6.2%	-	-	-	-
BMPR1A	p. R406C	c.1216C>T	8.8%	-	-	-	-
DDR2	amplification	NA	2.88	-	-	-	-
IL7R	amplification	NA	2.69	-	-	-	-
MAP3K1	p. G1423S	c.4267G>A	7.3%	-	-	-	-
MCL1	amplification	NA	2.51	-	-	-	-
NTRK1	amplification	NA	2.39	-	-	-	-
RICTOR	amplification	NA	2.72	-	-	-	-
EPHA3	p. G353C	c.1057G>T	-	-	2.2%	-	-
RAD50	p. R184K	c.551G>A	-	-	2.5%	-	-

MGN: Mammary gland lymph nodes; ALN: Axillary lymph nodes; CSF: cerebrospinal fluid; NA: not applicable; -: not detected. Each mutation is shown as the mutant allele frequency. Gene amplification is shown as the relative fold change compared to the normal control sample.