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ORIGINAL RESEARCH

EGFR, *KRAS*, *BRAF*, *PTEN*, and *PIK3CA* mutation in plasma of small cell lung cancer patients

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Background: Small cell lung cancer (SCLC) is an aggressive and deadly neuroendocrine tumor derived from bronchial epithelial cells. Although it results in a 95% mortality rate, the development of targeted therapies for SCLCs has lagged behind. The aim of this study is to better research mutation characteristics of SCLC and identify potential biomarkers for target therapy.

Methods: We utilized high- resolution melting analysis to identify the mutations in epidermal growth factor receptor (*EGFR*), Kirsten rat sarcoma viral oncogene (*KRAS*), v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*), phosphatase and tensin homolog (*PTEN*), and phosphatidylinositol-3-kinase catalytic (*PIK3CA*) from the blood. A cohort of 99 SCLC patients including 44 limited-stage disease patients and 55 extensive-stage disease patients were prospectively collected.

Results: *EGFR* 18 (G719X) mutation was found in 5 patients, *EGFR* 19 (del) mutation in 2, *EGFR* 20 (T790M) in 3, *EGFR* 21 (L858R) in 2, *KRAS* 2 (G13D) in 5, *BRAF* 15 (V600E) in 1, *PIK3CA* 9 (E542K) in 1, and no mutations in *PTEN* 5 (R130G), *PTEN* 6 (R173C), *PTEN* 8 (T319fs*1), and *PIK3CA* 20 (H1047R) were identified. Among these patients, two harbored *EGFR* double mutation, one patient with *EGFR* double mutation and *KRAS* 2 (G13D) mutation.

Conclusion: The mutation form of *EGFR* may differ from lung adenocarcinoma, and mutations of *KRAS*, *BRAF*, and *PIK3CA* were rare in SCLC. These results aided us in comprehensively analyzing genetic features and laid the foundation for exploring the possibility of target therapy.

Keywords: epidermal growth factor receptor, small cell lung cancer, plasma, high-resolution melting

Introduction

Small cell lung cancer (SCLC), which accounts for 15% of all the lung cancers, is an extremely aggressive disease with particularly poor survival rates.[1] Most often, the SCLC patients are categorized into the limited-stage disease (LD) or extensive-stage disease (ED) according to the Veterans Administration Lung Study Group (VALSG) staging system. Furthermore, rapid doubling time and early onset dissemination are the main characteristics of SCLC. SCLC is also characterized by initial chemosensitivity, and the first-line treatment of choice is the platinum-based regimen, usually cisplatin and etoposide.[2,3] However, despite initial high response rates to chemotherapy, SCLC inevitably relapses. The follow-up treatment for relapsed SCLC is still a challenge. Currently, target therapies and immunotherapeutic agents are under clinical investigation.[4,5] However, most of them have limited efficacy, and till now, there is no efficient drug approved for SCLC.[6] Thus, discovering novel predictive biomarkers to differentiate subtypes of SCLC for personalized treatments is the urgent need.

Epidermal growth factor receptor (*EGFR*) is a transmembrane protein that is a receptor for members of the EGF family of extracellular protein ligands. When endogenous ligands, such as EGF, bind to *EGFR*, receptor homodimerization or

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heterodimerization occurs at the cell surface and eventually leads to internalization of the dimerized receptor.[7] After receptor dimerization, the autophosphorylation of the intracytoplasmic *EGFR* tyrosine kinase domain will happen to stimulate the intracellular signal transduction cascade through several downstream pathways.[8] The mutations in *EGFR* exons 18, 19, and 21 in NSCLC are correlated to the response of tumors to tyrosine kinase inhibitors (TKIs). More concretely, short in-frame deletions in exon 19 and a specific point mutation in exon 21 at codon 858 are the most common *EGFR* mutations in NSCLC patients and correlated with the sensitive response of small-molecule *EGFR*-TKI.[3,4] Whereas the T790M gatekeeper point mutation at exon 20, accounting for ~50% of all cases, is reported to be the most frequently observed resistance mechanism.[9] Kirsten rat sarcoma viral oncogene (*KRAS*) is a GTP-binding protein, involved in G-protein-coupled receptor signaling. The mutation status of the *KRAS* gene predicts the therapeutic efficacy of *EGFR*-TKIs in NSCLC. The patients with the wild-type *KRAS* gene may benefit from the TKI therapy.[10] v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) kinase links the RAS GTPase to the downstream signaling pathway of *KRAS* to phosphorylate the MEK protein directly. The mutations in the *BRAF* gene increase the kinase activity and, in turn, the constitutive stimulation of MAPK2 and MAPK3. The rate of mutation in the *BRAF* gene in NSCLC is relatively low. *BRAF* mutations could predict the effect of MEK inhibitors on NSCLC cells.[11] Phosphatase and tensin homolog (*PTEN*) regulates several physiological processes by inhibiting the phosphoinositide 3-kinase-v-akt murine thymoma viral oncogene homolog-mammalian target of rapamycin pathway (PI3K/AKT/mTOR pathway) through its lipid phosphatase activity. Shibata et al showed that the mutation in phosphatidylinositol-3-kinase catalytic α (*PIK3CA*) in SCLC cells renders them more sensitive to tricribine than the cells with the wild-type gene. Moreover, the SCLC cells harboring a cisplatin-resistant subclone of *PIK3CA* mutant were sensitive to tricribine similar to those without the resistance.[12,13] Another study reported one adenocarcinoma patient with an L858R mutation in *EGFR* who displayed a robust response to erlotinib (first line of drug). The largest nodule progressed after 1 year. The core biopsy of this lesion revealed a histological transformation to SCLC that carried the L858R mutation in *EGFR* and acquired a *PIK3CA* mutation that disappeared after 6 months. Subsequently, the patient with the histological transformation to adenocarcinoma responded to a repeat course of erlotinib as the second-line course treatment.[14] Le et al revealed a lack of response to *EGFR* TKIs in *EGFR*-mutated de novo SCLC and large cell neuroendocrine carcinomas.[15] Therefore, elucidating the mutations in *EGFR*, *KRAS*, *BRAF*, *PTEN*, and *PIK3CA* in SCLC is imperative.

Two prospective studies from Taiwan and Japan reported 2.6% and 4% *EGFR* mutations, respectively.[16,17] Surgery can be used in SCLC patients with T1-2N0M0, which is <5% of all the SCLC patients. Thus, obtaining tumors for the detection of *EGFR* mutation in SCLC patients is rather challenging.[18] High-resolution melting (HRM) is a precise method, with 92% sensitivity and 100% specificity, for detecting the *EGFR* mutation using formalin-fixed tissues. This approach is also useful in predicting the clinical outcomes of NSCLC patients treated with gefitinib.[19] Thus, the simplicity, promptness, high sensitivity, and low rate of false-positive mutation in the HRM analysis renders it to be an optimal approach for detecting the *EGFR* mutations in circulating DNA of lung cancer patients.[20,21] In order to distinguish the mutation status of *EGFR*, *KRAS*, *BRAF*, *PTEN*, and *PIK3CA* in SCLC patients, we used the HRM technology to assess the genes in the plasma samples.

Materials and methods

Patients

From August 2012 to December 2015, 99 SCLC patients (10 females and 89 males) were prospectively assimilated in this study from the Zhejiang Cancer Hospital, People's Republic of China. The pathological diagnosis comprising of 98 conventional SCLC and 1 combined SCLC was based on the standard criteria defined by World Health Organization Classification. The mean age of patients was 60 years (range, 24–79 years). The stages as per the VALSG were as follows: LD in 44 patients and ED in 55 patients. These encompassed 14 patients who were nonsmokers, 1 patient was light, 3 were moderate, and 81 were heavy smokers. The mean pack-years was 46 (range, 0–150). The characteristics of 99 SCLC patients are summarized in Table 1. The study was approved by the Medical Ethical Committee of Zhejiang Cancer Hospital, and the patients signed the informed consent.

Detection of gene mutation using HRM

A total of 99 blood samples were included in this study for assessing *EGFR*, *KRAS*, *BRAF*, *PTEN*, *PIK3CA* mutations. Five milliliters of vascular blood samples was withdrawn in EDTA-anticoagulation tubes. The plasma was isolated from blood samples by centrifugation at 4,000 rpm for 10 min. Then, DNA was extracted from the plasma samples using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's protocol. Human

Table 1 Patient characteristics

Characteristic	Number of patients
Total	99
Gender	
Male	89
Female	10
Smoking status	
Never	14
Light	1
Moderate	3
Heavy	81
Stage	
LD	44
ED	55

Notes: Smoking status was evaluated on the PY, and PY were calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. Never represents patients who never smoked light represents PY≤10; moderate represents PY>10 and <20; heavy represents PY≥20.
Abbreviations: ED, extensive-stage disease; LD, limited-stage disease; PY, pack years.

genomic DNA obtained from Suzhou MicroDiag Biomedical Co., Ltd (Suzhou, People's Republic of China) was utilized as the control sample.

Eleven primer pairs were designed (Suzhou MicroDiag) to span codons 18 (G719X), 19 (del), 20 (T790M), and 21 (L858R) of the *EGFR* gene, codon 2 (G13D) of the *KRAS* gene, codon 15 (V600E) of the *BRAF* gene, codons 5 (p.R130G), 6 (p.R173C), and 8 (p.T319fs*1) of the *PTEN* gene, and codons 9 (p.E542K) and 20 (p.H1047R) of the *PIK3CA* gene (Table 2).

EVA-Green (Biotium, Fremont, CA, USA) was used as the intercalating dye. The reaction mixture consisted of 5 ng plasma DNA, 10× PCR buffer, 2.5 mM $MgCl_2$, 0.5 μM of each primer, 200 μM dNTP, 1U of FastStart Taq polymerase (Roche), and PCR grade water in a volume of 20 μL.

PCR cycling and HRM analysis were performed on the LightCycler® 480 Real-Time PCR System (Roche

Table 2 Primer sequences for amplifying target genes

Gene	Exon	Site	Primer sequences	
EGFR	18	G719A	**F**:TAAGTGTCTGGAAGTCAACTGGGGA	**R**:ACATTGTTTCACACTTATTATCTCT
		G719S	**F**:ATTGCCCTCAACACAGTGGAGCGAA	**R**:TTGAGGGCAATGAGGACATAACCAG
		G719C	**F**:TCCCCGCCTCGCCGCCAACGCCACA	**R**:CCACCTCACAGTTATTGAACATCCT
	19	E746_ A750del(1)	**F**:GCCTTGTGCTCCCCCGAGGGCTGCT	**R**:CCAGCAGCCCTCGGGGGAGCACAAG
		E746_ A750del(2)	**F**:GAAAACAACACCCTGGTCTGGAAGT	**R**:ACTTCCAGACCAGGGTGTTGTTTTC
		L747_ P753>S	**F**:GAAGCAACATCTCCGAAAGCCAACA	**R**:ATTTCCTTGTTGGCTTTCGGAGATG
		E746_ T751>I	**F**:AGCAACATCTCCGAAAGCCAACAAG	**R**:TCCTTGTTGGCTTTCGGAGATGTTG
		E746_ T751del	**F**:CTCACTCTCCATAAATGCTACGAAT	**R**:CCAGAGGAGGAGTATGTGTGAAGGA
		E746_ T751>A	**F**:GAACTGCCAGAAACTGACCAAAATC	**R**:GGCAGACCAGGCAGTCGCTCTCCCG
		E746_ S752>A	**F**:CCCTTCGGCTGCCTCCTGGACTATG	**R**:ACATAGTCCAGGAGGCAGCCGAAGG
		E746_ S752>V	**F**:TGGGCAACCCCGAGTATCTCAACAC	**R**:CAGGTGGGCTGGACAGTGTTGAGAT
		E746_ S752>D	**F**:AGCCATCACCCCAACCCCCCAAAAT	**R**:AGAAAACTATCTTCCATAAGTAACA
		L747_ A750>P	**F**:TGGGCAACCCCGAGTATCTCAACAC	**R**:ACAGGTGGGCTGGACAGTGTTGAGA
		L747_ T751>Q	**F**:ATTTTCAGCCTACAGTTATGTTCAG	**R**:GTGACTGAACATAACTGTAGGCTGA
		L747_ E749del	**F**:TCAGCCTACAGTTATGTTCAGTCAC	**R**:TGTGTGACTGAACATAACTGTAGGC
		L747_ T751del	**F**:CTTGACTGAGGACAGCATAGACGAC	**R**:TTTTGGGAACGGACTGGTTTATGTA
		L747_ S752del	**F**:AGCAGAGACCCACACTACCAGGACC	**R**:TGTGGGGGTCCTGGTAGTGTGGGTC
		L747_ A750>P	**F**:TCCTAGGTTCAAATCTGGAAGTGGT	**R**:TTCCAGATTTGAACCTAGGACCTCC
		L747_ P753>Q	**F**:TTATTACTCAACCTTCAACCCAGAC	**R**:GTCTGGGTTGAAGGTTGAGTAATAA
		L747_ T751>S	**F**:ATTGGTACAGATGAGACTTCAGAAA	**R**:TTTTCTGAAGTCTCATCTGTACCAA
		L747_ T751del	**F**:CATGGCATAGGGTTCGAATATCTGA	**R**:GAACCCTATGCCATGGGGATAAGTG
		L747_ T751>P	**F**:ATTGGTACAGATGAGACTTCAGAAA	**R**:CTTTTCTGAAGTCTCATCTGTACCA
	20	T790M	**F**:CTGCCCCCAAACCCCCTCCTTACGC	**R**:TGTGTGACAAAGCGTAAGGAGGGGGG
	21	L858R	**F**:CAAAGAGTATATGTTCCCTCCAGGT	**R**:AAGCGTAAGGAGGGGGTTTGGGGGC
KRAS	2	G13D	**F**:CAATCCACCAGCTAACCGCCTGAAA	**R**:ATGCACATGTTTAAGTCTATTTCAG
BRAF	15	V600E	**F**:ATAAACCTGAGATAATGGCATGGCT	**R**:CCTCCAGCCATGCCATTATCTCAGG
PTEN	5	R130G	**F**:AGCAACCAGGACCGAGACCCACACT	**R**:TCCTCGGAGTTGTTGGCTTATGTTG
	6	R173C	**F**:ATCTGGAAGTCCTAGGTTCAATGGT	**R**:CCAGTATGTGTAGAGGAGGGAAGGA
	8	T319fs*1	**F**:TACCTCAACCTTTATTACAACCCAG	**R**:GGCCAGTCGCTAGACCAGGCTCCCG
PIK3CA	9	E542K	**F**:ATAGACTTCAGAAATGGTACAGATG	**R**:ACATGCAGCAGTCCAGGAGCGAAGG
	20	H1047R	**F**:CTATCGGTTCGAATGAATGGCATAG	**R**:CAGGCAGTGTTGATGGGCTGGAGAT

Abbreviations: *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma viral oncogene; *PIK3CA*, phosphatidylinositol-3-kinase catalytic α; *PTEN*, phosphatase and tensin homolog.

Diagnostics). The amplification was carried out according to the following conditions: 1 cycle of 95°C for 5 min; 50 cycles of 95°C for 10 s, 60°C for 15 s, 72°C for 25 s. Before the HRM step, the products were heated to 95°C for 1 min and cooled to 40°C for 1 min. HRM was carried out from 65°C to 95°C, rising at 0.02°C/s with 25 acquisitions per degree.

Results

By HRM, blood samples from 99 SCLC patients were sequenced to detect the genetic mutation of target genes (*EGFR*, *KRAS*, *BRAF*, *PIK3CA*, and *PTEN*). Consequently, target-site mutation was detected in 15 patients. Among them, these mutations of 12 patients were mutually exclusive, and the remaining 3 patients carried multiple mutations. As shown in Table 3, the number of patients who carried *EGFR*/*KRAS*/*BRAF*/*PIK3CA*/*PTEN* mutation was 9/5/1/1/0, respectively. In detail, *EGFR* 18 (G719X) mutation in 5 (5.05%) patients, *EGFR* 19 (del) mutation in 2 (2.02%) patients, *EGFR* 20 (T790M) mutation in 3 (3.03%) patients, *EGFR* 21 (L858R) mutation in 2 (2.02%) patients, *KRAS* 2 (G13D) mutation in 5 (5.05%) patients, *BRAF* 15 (V600E) mutation in 1 (1.01%) patient (Figure 1), *PIK3CA* 9 (E542K) mutation in 1 (1.01%) patient (Figure 2), and no mutations of *PTEN* 5 (R130G), *PTEN* 6 (R173C), *PTEN* 8 (T319fs*1), and *PIK3CA* 20 (H1047R) were found. Three patients with multiple mutations, one patient harboring *EGFR* 18 (G719X) and *EGFR* 19 (del) mutations (Figure 3), one patient harboring *EGFR* 20 (T790M) and *EGFR* 21 (L858R) (Figure 4), and another patient harboring *EGFR* 18 (G719X), *EGFR* 20 (T790M), and *KRAS* 2 (G13D) mutations (Figure 5) were identified. The nine patients (one female and eight males) harboring *EGFR* mutations expressed a mean age of 59 years (range, 48–71 years). The stages were as follows: LD in four patients and ED in five patients. One patient was a nonsmoker, and eight were heavy smokers.

Discussion

In the present study, the percentage of *EGFR* mutations in SCLC plasma is higher than from the tumor tissue in previous reports.[16–18,22] The *EGFR* mutation from tumor tissue can only reflect the mutation status of the tissue that is evaluated; heterogeneity occurs widely in different parts of the tissue in the same patient.[23] The *EGFR* mutation in plasma may originate from all the tumor tissues of the whole body. The patients with *EGFR*-mutated SCLC tended to be females, nonsmokers, and in SCLC including "conventional SCLC and combined SCLC". Combined SCLC is SCLC containing discrete areas of non-mall cell morphologic components.[25–27] A study revealed 28% of the SCLC patients who underwent surgical resection exhibited combined SCLC,[28] while other reports demonstrated that combined SCLC accounted for 1%–3.2% of all the SCLC cases who received no surgery.[29,30] Surgery can be considered only in T1-2N0M0 SCLC patients;

Table 3 Results of patients with mutated genes by HRM

Patient number	Stage	Gender	Age	Smoking history	Mutation	Melting temperature (°C)
1	LD	Female	71	No	*EGFR*-E19	83
6	ED	Male	55	Heavy	*EGFR*-E18	87.3
					EGFR-E19	83.8
8	ED	Male	50	Moderate	*KRAS*-E2	83.4
14	ED	Male	59	Heavy	*EGFR*-E20	90.5
					EGFR-E21	88.9
20	LD	Male	46	Heavy	*KRAS*-E2	83.2
21	ED	Male	79	No	*KRAS*-E2	83.9
30	ED	Male	66	Heavy	*EGFR*-E18	88
32	ED	Male	51	Heavy	*EGFR*-E18	87.6
					EGFR-E20	90.8
					KRAS-E2	83.8
42	ED	Male	62	Heavy	*BRAF*-E15	82.3
43	LD	Female	67	No	*KRAS*-E2	83
48	LD	Male	62	Heavy	*EGFR*-E18	86.9
51	ED	Male	59	Heavy	*EGFR*-E18	86.4
54	LD	Male	57	Heavy	*EGFR*-E20	90.3
80	LD	Male	48	Heavy	*EGFR*-E21	83.8
83	ED	Male	52	Heavy	*PIK3CA*-E9	80.8

Notes: Smoking status was evaluated on the PY, and PY were calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. Never represents never smoked patient; light represents PY≤10; moderate represents PY>10 and <20; heavy represents PY≥20.

Abbreviations: ED, extensive-stage disease; *EGFR*, epidermal growth factor receptor; HRM, high-resolution melting; *KRAS*, Kirsten rat sarcoma viral oncogene; LD, limited-stage disease; *PIK3CA*, phosphatidylinositol-3-kinase catalytic α; PY, pack years.

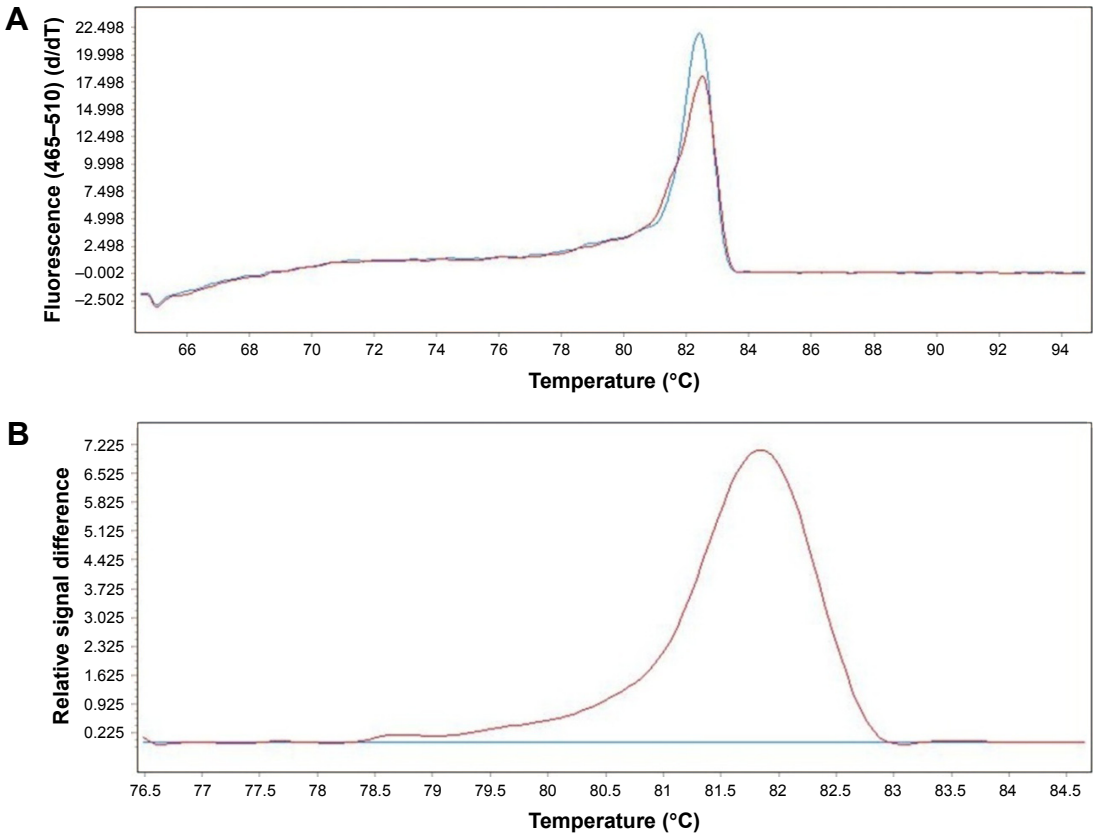

Figure 1 The patient was a 62-year-old male ED-SCLC with heavy smoking. Result of *BRAF* (V600E) mutation. (**A**) Melting peaks. The blue line represents the melting curve of standard product, and the red line represents the melting curve of genome DNA from SCLC patients. All the red lines and blue lines of the figures below represent the same meaning. (**B**) Normalized and temperature-shifted difference plot.
Abbreviations: ED, extensive-stage disease; SCLC, small cell lung cancer.

however, these cases have been reported to account for <5% of all the SCLC patients.[18] None of our SCLC patients underwent surgery. The diagnosis of combined SCLC using biopsy or needle puncture is extremely arduous. Twenty to thirty percent of our SCLC patients may have combined SCLC, and we could not diagnose combined SCLC through small specimens obtained from biopsy or needle puncture. Heterogeneity can also explain the double mutation of *EGFR* in plasma of the same patient. SCLC developing in association with adenocarcinoma, either synchronously or metachronously, seemed to correlate with the *EGFR* mutation, irrespective of TKI usage.[31] The *EGFR* mutation may easily occur in SCLC combined adenocarcinoma than in SCLC combined with other NSCLC complements.[25] Because of the specific involvement of *EGFR* mutations in adenocarcinoma, it is suggested that the SCLCs may have developed from preexisting adenocarcinomas.[17] As we all know, *EGFR* activating mutations, including exon 19 deletions or an exon 21 Leu858Arg mutation, present favorable outcome after treating with *EGFR* TKI.[32,33] In SCLC, several studies have also performed a similar research. A 72-year-old woman with *EGFR* exon 19 mutation (del E746-A750) utilized gefitinib as the treatment method.[34] After 3 weeks, CT was performed, which revealed marked regression of both the primary lung tumor and the metastatic liver tumor. The histological examination of this patient was confirmed as SCLC. Another clinical report also revealed a pronounced positive effect of gefitinib in a patient with metastatic SCLC.[35] Second-generation inhibitors, including afatinib, dacomitinib, and neratinib, have been verified markedly with high efficacy in NSCLC patients with the G719X mutation.[36,37] Based on our research, *EGFR* 18 (G719X) is the most common *EGFR* mutation of SCLC in the current report, which is different from lung adenocarcinoma with the main mutations *EGFR* 19 (del) and *EGFR* 21 (L858R).[38,39] Thus, we speculated that G719X might be a promising mutation for treatment. Bordi et al reported that all the SCLC cases harbored wild-type *BRAF*, *KRAS*, platelet-derived growth factor receptor alpha, and c-KIT (data available for 82 patients) genes.[24] Other studies reveal ~2%–5% *KRAS* mutations in patients with SCLC.[40,41] Our study also confirmed that fewer mutations of *KRAS* and *BRAF* in SCLC patients might be correlated with

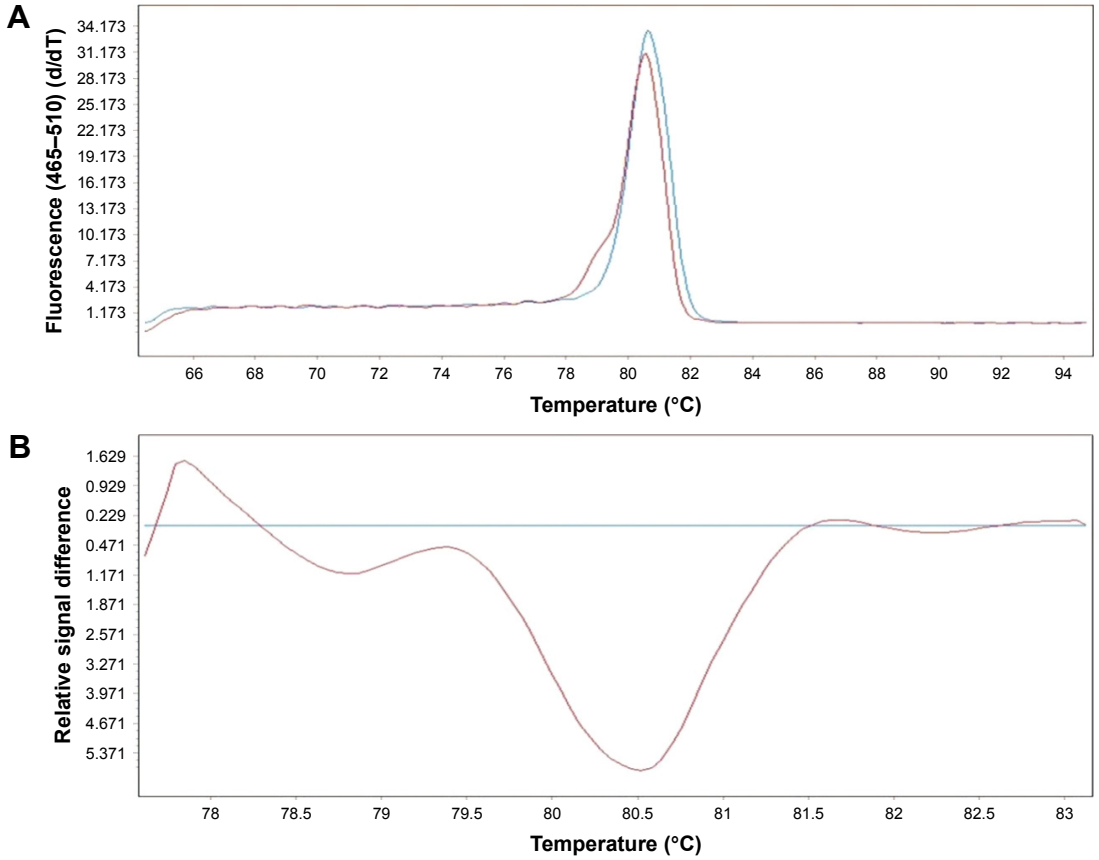

Figure 2 The patient was a 62-year-old male ED-SCLC with heavy smoking. Result of *PIK3CA* 9 (E542K) mutation. (**A**) Melting peaks. (**B**) Normalized and temperature-shifted difference plot. The blue line represents the melting curve of standard product, and the red line represents the melting curve of genome DNA from SCLC patients. The meaning of red line and blue line on each figure is the same.
Abbreviations: ED, extensive-stage disease; *PIK3CA*, phosphatidylinositol-3-kinase catalytic α; SCLC, small cell lung cancer.

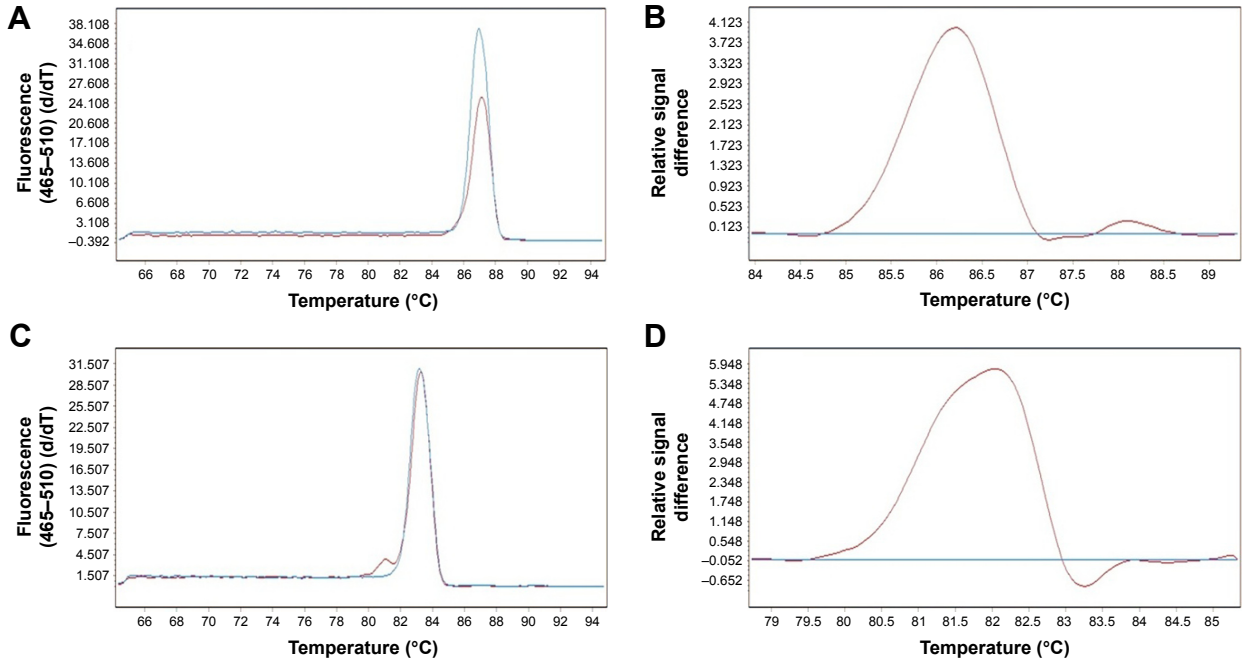

Figure 3 The patient was a 55-year-old male ED-SCLC with heavy smoking. (**A**) Melting peaks of *EGFR* 18 (G719X) mutation. (**B**) Normalized and temperature-shifted difference plot of *EGFR* 18 (G719X) mutation. (**C**) Melting peaks of *EGFR* 19 (del) mutation. (**D**) Normalized and temperature-shifted difference plot of *EGFR* 19 (del) mutation.
Abbreviations: ED, extensive-stage disease; *EGFR*, epidermal growth factor receptor; SCLC, small cell lung cancer.

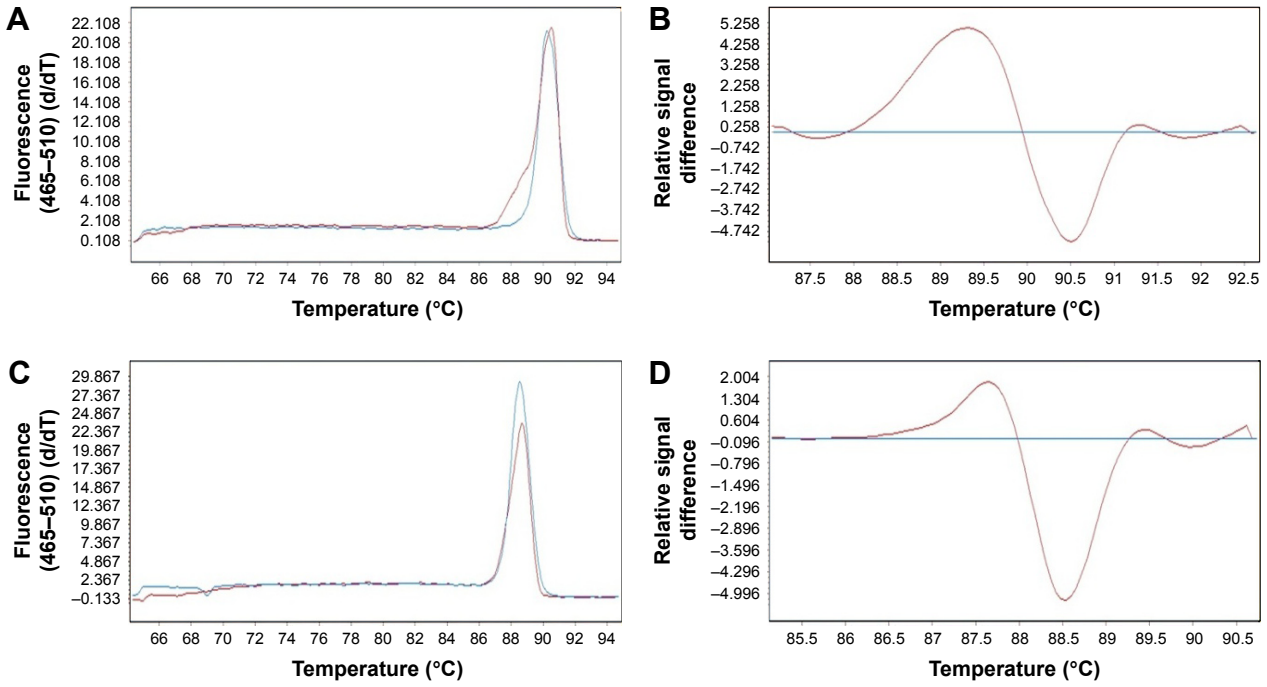

Figure 4 The patient was a 59-year-old male ED-SCLC with heavy smoking. (**A**) Melting peaks of *EGFR* 20 (T790M) mutation. (**B**) Normalized and temperature-shifted difference plot of *EGFR* 20 (T790M) mutation. (**C**) Melting peaks of *EGFR* 21 (L858R) mutation. (**D**) Normalized and temperature-shifted difference plot of *EGFR* 21 (L858R) mutation.

Abbreviations: ED, extensive stage disease; *EGFR*, epidermal growth factor receptor; SCLC, small cell lung cancer.

the resistance to TKI in NSCLC in the background of these mutations.[10,42–45] In our study, *EGFR* and *KRAS* mutations are detected in one SCLC patient. Le et al demonstrated the lack of response to *EGFR* TKIs in one *EGFR*-mutated de novo SCLC patient, which might be attributed to the *KRAS* or *BRAF* mutation; however, the underlying mechanism is yet to be elucidated.[15] Selumetinib is a tight-binding, non-competitive inhibitor of MEK 1 and 2 that acts downstream of *KRAS* and is administered orally. Recently, a randomized, Phase II trial involving selumetinib plus docetaxel improved the progression-free survival when compared with docetaxel alone in patients who were treated previously for advanced NSCLC harboring the *KRAS* mutant.[46] Furthermore, the role of *BRAF* mutation in NSCLC and the optimal treatment are yet to be elucidated. The regression of intracranial disease by Robinson et al indicated that vemurafenib traversed the blood–brain barrier and efficaciously treated the brain metastasis in lung cancer patients with a V600E mutation in *BRAF* gene.[47] Thus, selumetinib and vemurafenib in SCLC with *KRAS* or *BRAF* mutation can be explored prospectively.

The PI3K/AKT/mTOR pathway plays a unique role with respect to genomic alterations in SCLC.[48] The inactivated *PTEN* advances the SCLC in a genetic mouse model, thereby suggesting that a subset of patients with SCLC can be treated by targeting the *PTEN* pathway.[49] Li et al indicated that adenovirus-mediated *PTEN* together with cisplatin could be an efficient novel therapeutic modality for the treatment of patients with SCLC.[50] Tricribine is a small molecule inhibitor of AKT signaling. It is localized downstream of *PIK3CA* and inhibited the growth and colony formation of "*PIK3CA*-addicted" cells significantly. Moreover, the SCLC cells harboring the mutated *PIK3CA* are more sensitive to tricribine than the wild-type cells. On the other hand, the cisplatin-resistant subclones of *PIK3CA*-mutant SCLC cells exhibited sensitivity to tricribine similar to those with *PIK3CA*. The anti-AKT molecular therapy was found to be efficient for a subgroup of SCLC with *PIK3CA* mutation.[51] PF-4989216 is a potential cancer therapeutic candidate for the treatment of SCLC patients with *PIK3CA* mutation and without *PTEN* loss.[52] Reverse transcription-polymerase chain reaction and direct sequencing technology were employed for the detection of *PIK3CA* mutation in 14 cases of patients with SCLC, assimilated retrospectively, who underwent surgical treatment at the Zhejiang Cancer Hospital from 2002 to 2010. The analysis did not reveal any mutations in exons 9 and 20 of the *PIK3CA* gene in the tumor tissue of patients with SCLC.[53] In the current study, one patient presented the *PIK3CA* mutation. The percentage of *PIK3CA* mutations is similar to *PTEN*: ~5%.[27,40,48] Sasaki et al and Schmid et al[43,54] established the synergistic effects of the combination therapy

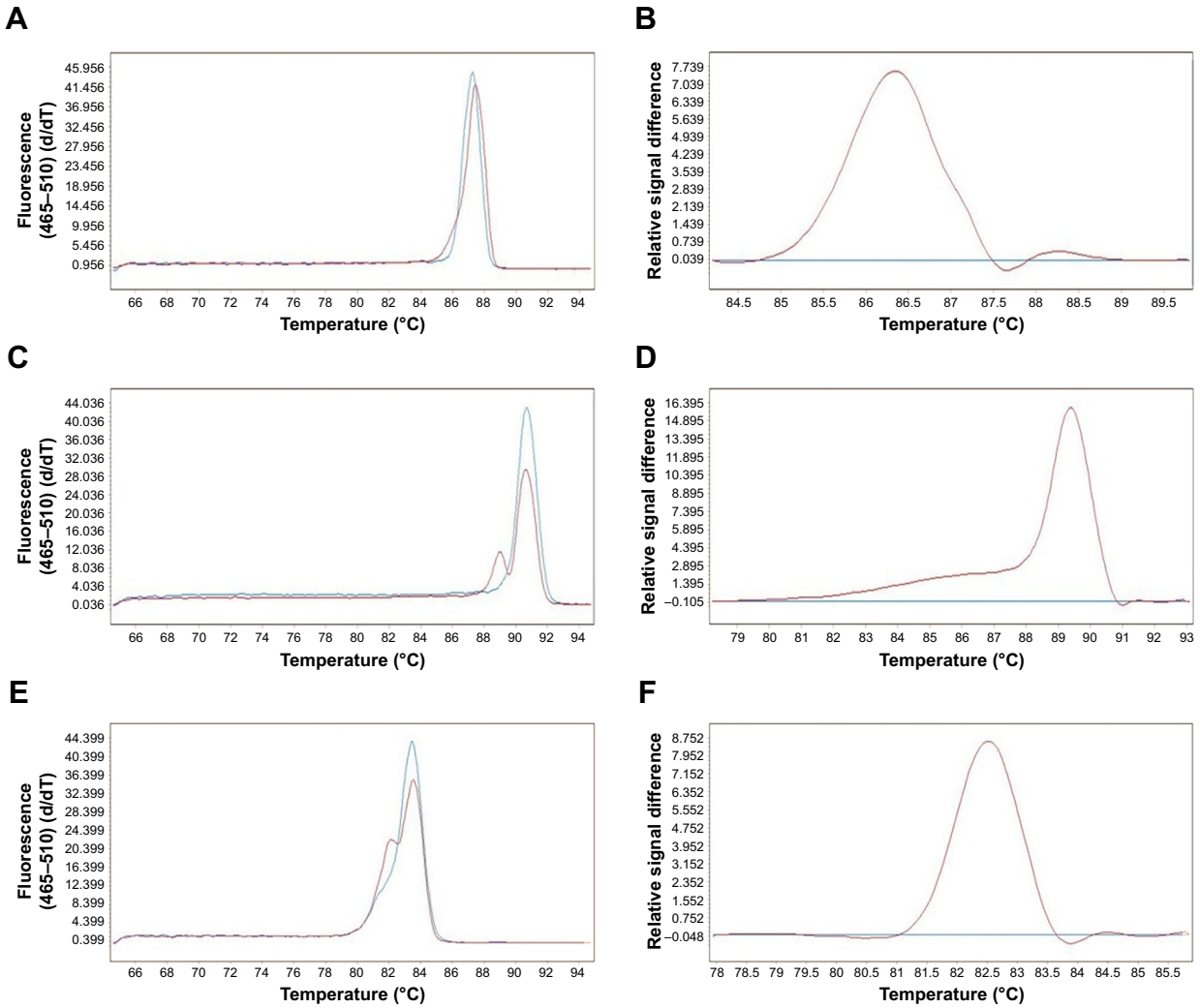

Figure 5 The patient was a 51-year-old male ED-SCLC with heavy smoking. (**A**) Melting peaks of *EGFR* 18 (G719X) mutation. (**B**) Normalized and temperature-shifted difference plot of *EGFR* 18 (G719X). (**C**) Melting peaks of *EGFR* 20 (T790M) mutation. (**D**) Normalized and temperature-shifted difference plot of *EGFR* 20 (T790M) mutation. (**E**) Melting peaks of *KRAS* 2 (G13D) mutation. (**F**) Normalized and temperature-shifted difference plot of *KRAS* 2 (G13D) mutation.
Abbreviations: ED, extensive-stage disease; *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma viral oncogene; SCLC, small cell lung cancer.

of Erlotinib and RAD001 with respect to cell viability, proliferation, and autophagy.

Conclusion

Our study revealed the mutation of the targeted genes from SCLC. The mutations in *KRAS*, *BRAF*, and *PIK3CA* are rare in patients with SCLC, and the mutation in *EGFR* might differ from that in lung adenocarcinoma. The experimental results laid the foundation for better understanding of the mutation characteristics of SCLC and the following research on the application of targeted drugs.

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Disclosure

The authors report no conflicts of interest in this work.

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