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PIK3CA Mutations in Resected Small Cell Lung Cancer*

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Despite advances in chemotherapy and radiotherapy in recent decades, the prognosis for small cell lung cancer (SCLC) patients is still poor. Targeted therapies in SCLC must be applied systemically to target not only the primary tumor but also metastases. The phosphatidylinositol 3-kinase (PI3K)/AKT pathways play a key regulatory function in the survival, proliferation, energy metabolism and cellular architecture advantages of malignant cells. The phosphatidylinositol 3-kinase catalytic α (PIK3CA) gene, which encodes the p110α catalytic subunit, plays a key role in the activation of AKT downstream signaling and mammary tumor progression. More than 75% of PIK3CA mutations are clustered in the helical (exon 9) and catalytic domains (exon 20). There have been very few studies reporting the PIK3CA mutations status of patients with SCLC who have undergone surgical treatment in mainland China.
Objectives. The aim of the study was to investigate the PIK3CA mutation in SCLC.
Material and Methods. Reverse transcription polymerase chain reaction (RT-PCR) and direct sequencing technology was used to detect the PIK3CA mutation in 14 cases of retrospectively collected SCLC patients who underwent surgical treatment at Zhejiang Cancer Hospital, Hangzhou, PRC, between 2002 and 2010.
Results. The research revealed no mutations in exons 9 and 20 of the PIK3CA gene. A nucleotide alteration of A1634C (E545A) of exon 9 turned out to be a pseudogene-positive, because the mutation disappeared when near--duplicate detection was employed.
Conclusions. The incidence of PIK3CA mutation is low in SCLC patients, and the pseudogene-positive alteration of A1634C is prone to occur in exon 9 of PIK3CA mutations in human cancers (**Adv Clin Exp Med 2016, 25, 3, 397–402**).

Key words: PIK3CA, gene mutation, SCLC.

Lung cancer is the major cause of cancer-related death worldwide. Although progress has been made in reducing its incidence and mortality rates and improving survival, cancer still accounts for more deaths than heart disease in people younger than 85 years old [1]. The proportion of small cell lung cancer (SCLC) among all lung cancer histological types decreased from 17.26% in 1986 to 12.95% in 2002 [2]. The stages of SCLC are usually categorized by the Veterans Administration Lung Study Group (VALSG) staging system [3], which classifies patients into limited-stage disease (LD) or extensive-stage disease (ED). The TNM staging system is also used for SCLC, especially for those

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patients who have received surgical treatment [4]. Chemotherapy is the foundation of treatment in SCLC. Although there have been modest improvements in recent years, the survival statistics for SCLC remain very poor.

Cancer gene therapy has a lower toxicity than conventional chemotherapy, but no targeted drugs have been approved for the treatment of SCLC [5]. There have only been a few case reports about epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) successfully being used in SCLC with the EGFR mutation [6–7]. A phase-2 trial of gefitinib in patients with chemosensitive and chemorefractory relapsed SCLC failed to show any benefit from the use of gefitinib [8]. The EGFR mutation is rare in SCLC and mainly occurs in combined SCLC or female non-smoker patients [9–13]. A phase-2 trial of imatinib (ST1571) in relapsed SCLC patients with expression of the c-kit gene reported negative results [13]. A pyrosequencing assay was used to detect mutations in c-kit exons 9 and 11 in 36 SCLC patients who underwent surgical treatment at the Zhejiang Cancer Hospital, Hangzhou, China, between 1998 and 2010; no mutations in c-kit exons 9 and 11 were detected [15]. More research on SCLC is needed to determine its molecular characteristics and to seek effective targeted therapies.

The PI3K/AKT pathways play a key regulatory function in the survival, proliferation, energy metabolism and cellular architecture advantages of malignant cells. By suppressing the phosphoinositide 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) pathway through its lipid phosphatase activity, the PTEN gene governs a plethora of cellular processes. As Hernandez et al. wrote: "The PIK3CA gene, which encodes the p110α catalytic subunit, plays a key role in the activation of AKT downstream signaling and mammary tumor progression" [16]. More than 75% of PIK3CA mutations are clustered in the helical (exon 9) and catalytic domains (exon 20) [17]. A study by Shibata et al. showed that phosphatidylinositol-3-kinase catalytic α PIK3CA-mutated SCLC cells are more sensitive to Tricribine than PIK3CA wild-type cells, and that a cisplatin-resistant subclone of PIK3CA--mutant SCLC cells was equally sensitive to Tricribine [18]. These data imply that PI3KCA seems to be an attractive therapeutic target for cancer treatment.

In order to investigate the PIK3CA mutation of SCLC, two hot spots of the PIK3CA mutation were detected by reverse-transcription polymerase chain reaction (RT-PCR) and direct sequencing technology for 14 SCLC patients who received surgical treatment at the Zhejiang Cancer Hospital, Hangzhou, China between 2002 and 2010 and were retrospectively studied.

Material and Methods

Patient Characteristics

A total of 14 patients with stage IA-IIIA SCLC who underwent surgical resection at Zhejiang Cancer Hospital between March 2002 and July 2010 were recruited for this study. The study was approved by the Ethics Review Committee of Zhejiang Cancer Hospital (Hangzhou, China). In each case a histologic diagnosis of SCLC was made by the standard criteria defined by WHO classifications. The median age of the study group was 56 years (range: 35–72); 13 (92.8 %) were male and 1 (7.1%) was female. The group included one non-smoker, three moderate smokers, and 10 heavy smokers. The stages involved, according to the seventh edition of the TNM classification for lung cancer, were IA: three cases; IB: one case; IIA: three cases; IIB: one case; and IIIA: six cases. All 14 patients underwent lobectomy and lymph node dissection or total lung resection. The patients' characteristics are presented in Table 1.

Table 1. Characteristics of the study participants (n = 14)

Clinical characteristics	Cases
Gender (female/male)	1/13
Left lung/right lung	9/5
Smoking history	
non-smoker	1
light smoker	0
moderate smoker	3
heavy smoker	10
Stage	
IA	3
IB	1
IIA	3
IIB	1
IIIA	6

DNA Purification and PCR Amplification

Six 10 μm paraffin sections were deparaffinized with xylene and then immersed in cell lysis solution. The lysis solution was incubated at 55°C overnight for digestion. The DNA was isolated with isopropanol after protein precipitation. The target DNA fragments containing mutations, including PIK3CA exons 9 and 20, were enriched by PCR amplification (Table 2). PCR amplifications were performed in 25-μL reaction system using FastStart Taq DNA polymerase (Roche Diag-

Table 2. PIK3CA primer sequence

Name	Site	Sequence
PIK9AF	PIK3CA Exon 9	TGAAAATAAAGTCTTGCAATGAAAA
PIK9AR	PIK3CA Exon 9	TTCCACAAATATCAATTTACAACCA
PIK9SF (Sequencing primer)	PIK3CA Exon 9	TTGAAAATGTATTTGCTTTTTCTGT
PIK20AF	PIK3CA Exon 20	GCTCCAAACTGACCAAACTG
PIK20AR	PIK3CA Exon 20	ATGCTGTTCATGGATTGTGC
PIK20SF (Sequencing primer)	PIK3CA Exon 20	TCAGGAGATGTGTTACAAGGCTTA

nostics GmbH, Mannheim, Germany). The PCR amplification conditions consisted of an initial denaturation step at 95°C for 6 min, followed by 35 cycles of 95°C for 40 sec, a primer annealing step at 55°C for 35 sec, an elongation step at 72°C for 45 sec, and a final extension step at 72°C for 7 min. Genomic DNA from the tumor tissue was purified with Gentra Puregene Tissue Kit (Qiagen Sciences Inc., Germantown, USA).

Direct Sequencing of the PCR Products

The PCR amplification products were identified through 1.5% agarose gel electrophoresis, and then the PCR products were purified using a clean-up kit (ThermoFisher Scientific Inc., Waltham, USA). The BigDye V3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) was used for all sequencing reactions. The sequencing reaction process was performed in a 3130xl genetic analyzer (Applied Biosystems) after purification (Table 2). Each PCR product was sequenced from two directions to identify mutations. If any mutations were found, the process was repeated to confirm the results.

Data Analysis

The statistical analysis was carried out using SPSS 13.0 software (SPSS Inc., Chicago, USA).

Results

Genetic Mutation Analysis

RT-PCR was used to detect the genetic mutation of PIK3CA in paraffin-embedded tissues from the 14 patients with stage IA-IIIA SCLC. Case 14 was found to have mutations in exon 9 of the PIK3CA gene. However, this nucleotide change A1634C (E545A) of exon 9 turned out to be a pseudogene-positive, because the mutation disappeared when near-duplicate detection was employed (Fig. 1). No mutations were detected in PIK3CA exon 20 (Fig. 2).

Case 14

The fourteenth patient was a 55-year-old male heavy smoker who reported a chronic cough and hemoptysis for three months, accompanied with chest tightness and pain for over one month. A chest CT revealed a tumor mass in the lower left space. The patient underwent left pneumonectomy. Immunohistochemical stains revealed positivity of CHG-A/CgA, Syn, CD56, NSE, CK, EMA, P53 and TopoII. The pathological diagnosis was pure SCLC (Fig. 3). The initial TNM stage was T3N2M0 (IIIA). The patient received four courses of chemotherapy with etoposide and cisplatin. Brain metastases appeared five months after surgery. The patient then received whole brain radiation therapy (WBRT) followed by three courses of docetaxel, and achieved progressive disease.

Discussion

SCLC, which accounts for approximately 15% of primary lung cancer, is a highly aggressive cancer in humans, with a high relapse rate even among patients who achieve a complete response [19–21]. Combined SCLC has been reported to occur in, at most, 1–3.2% of all SCLC cases. These tumors are combined with an additional component that consists of any of the histological types of non-small cell lung cancer (NSCLC), usually squamous cell carcinoma (Sq), adenocarcinoma (Ad) or large cell carcinoma. Despite advances in chemotherapy and radiotherapy in recent decades, the prognosis of SCLC patients is still poor, with a median survival under two years [22].

Surgical resection is rarely applied as the mainstay in SCLC; most patients with SCLC undergo

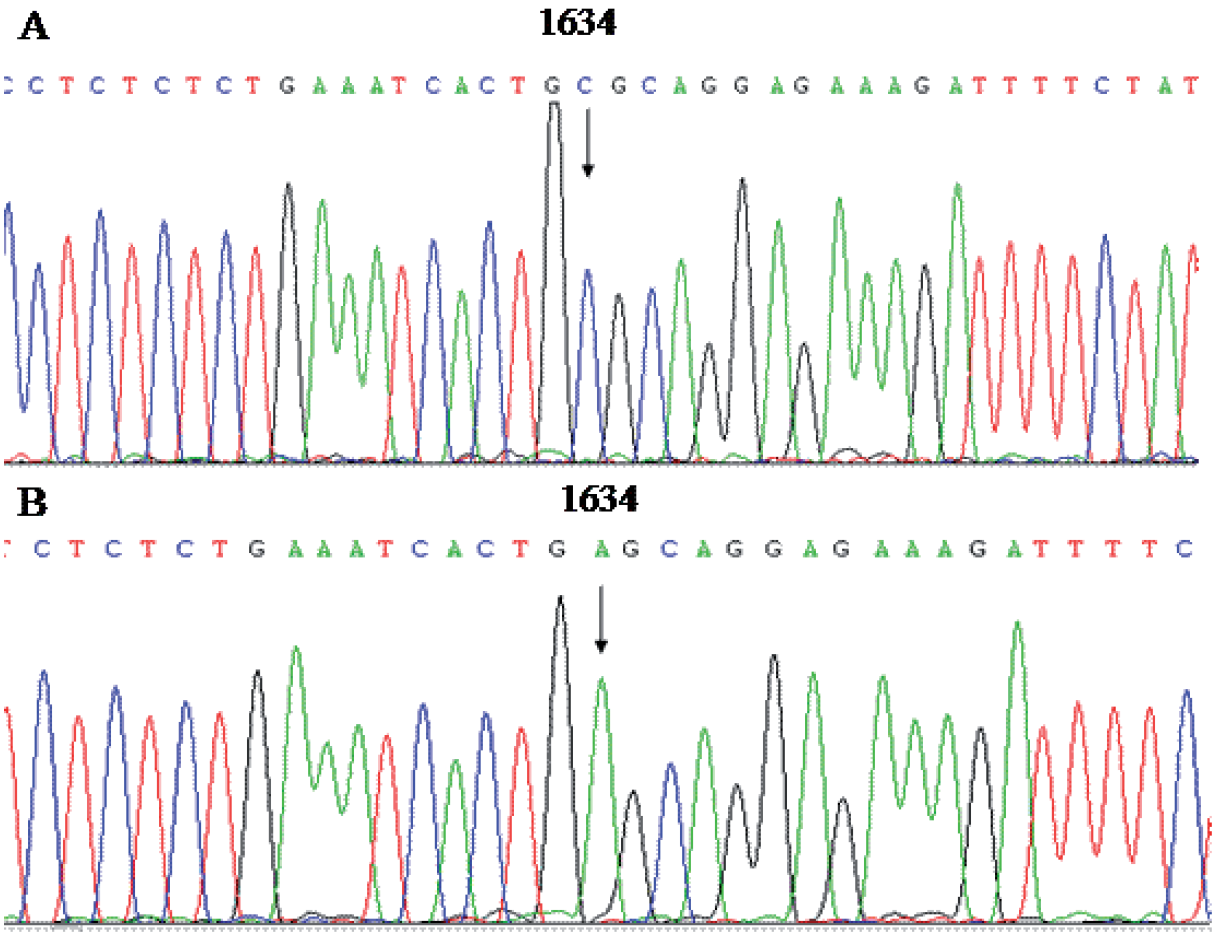

Fig. 1. Identification of sequences on exon 9 of PIK3CA. A: The false positive A1634C (E545A) "mutation" of exon 9 of the PIK3CA gene detected in Case 14. B: No mutation was detected in exon 9 of PIK3CA gene in near-duplicate detection

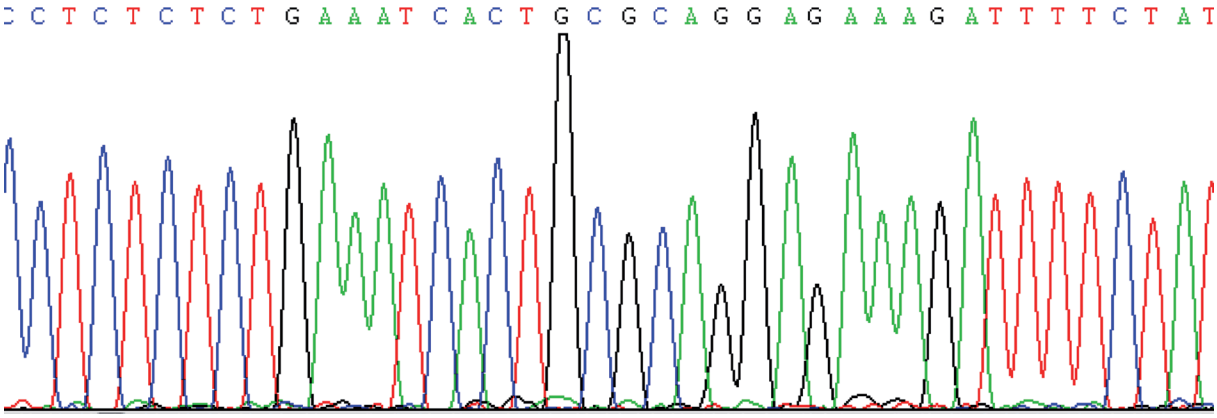

Fig. 2. No mutation was detected in exon 20 of PIK3CA gene in any of the cases

chemotherapy and radiotherapy instead. Less than 5% of all SCLCs are considered for surgical treatment in stage T1-2N0M0 patients [23]. In the present study, specimens obtained from surgery more accurately reflect the clinicopathologic features than small biopsies or cytology specimens. Since carcinogens involve multistep processes with alterations in signal transduction pathways, the potential gene therapies are many, and as the comprehension of cancer genetics improve, the number of novel targets increases. Targeted therapies in SCLC must be applied systemically to target not only the primary tumor but also metastases.

Previous studies have shown that tumors with PIK3CA mutations are predicted to be more sensitive to PI3K pathway inhibitors, which play an

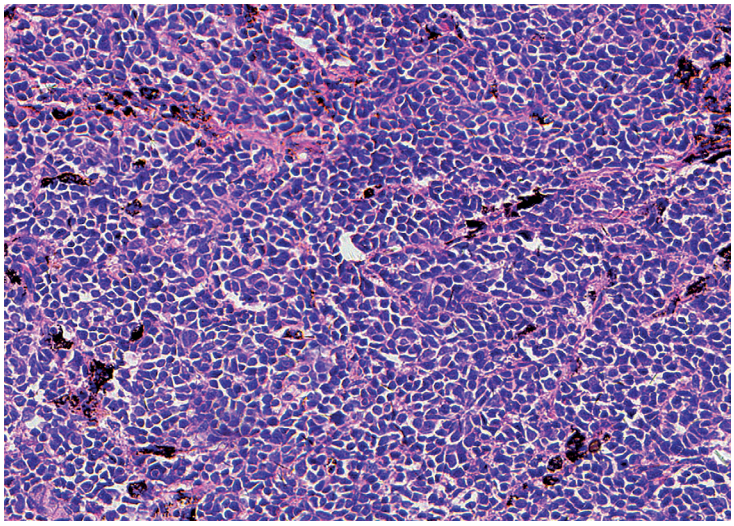

Fig. 3. The morphology of Case 14 (HE ×200). Small cancer cells linked closely to each other, with few cytoplasms

indispensable role in the activation of PIK3/AKT downstream signaling and mammary neoplastic progression in *in vitro* experiments [24, 25]. Samuels et al. reported that the two PIK3CA mutation hot spots – exons 9 and 20, which correspond to the helical and catalytic domains of p110 – have a low frequency (4%) in lung cancer [25]. Additionally, there are hardly any studies reporting the PIK3CA mutation status of patients with SCLC who have undergone surgical treatment in mainland China. To determine the PIK3CA mutation status and clinical features of SCLC, the authors of the current study retrospectively analyzed the clinical features of 14 patients who underwent surgical therapy at Zhejiang Cancer Hospital between 2002 and 2010.

Although an amazing nucleotide alteration was found at A1634C (E545A) of exon 9 in one case (Fig. 1A), this change was not verified by the sequencing of a second PCR product (Fig. 1B). The false positive result is consistent with previous studies reported by Qiu et al. [26]. They found that the exon 9 nucleotide sequence homology (97%) with its flanking intronic sequences and the so-called PIK3CA A1634C (E545A) "mutation" disappeared by moving the PCR primer sites, utilizing the primers published by Samuels et al. or increasing the stringency of the PCR conditions. The current authors found that the A1634C (E545A) "mutation" of exon 9 detected in this study was an artifact created by interference from the sequence homolog. Screening SCLC tissues for PIK3CA mutations yielded negative results in the present study; also, it remains unclear whether there is any significant correlation between PIK3CA gene mutations and the smoking histories, gender, age and stage of the patients.

The data presented by Qiu et al. [26] suggest that a mutant PIK3CA gene is likely to function as an oncogene in human head and neck cancers. At present, the authors cannot confirm the mutation of PIK3CA based on clinical factors in certain SCLC patients. Knowledge of PIK3CA's involvement in SCLC is important because selective small-molecule inhibitors could be considered as a future therapeutic regimen for SCLC patients with PIK3CA mutations. Therefore, further investigations are required to identify whether or not the exact functions of the domains of class PIK3CA can be used as a response prediction factor in targeted therapy.

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