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ORIGINAL ARTICLE

Clinical Trials Study

PIK3CA gene mutations in Northwest Chinese esophageal squamous cell carcinoma

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Abstract

AIM

To evaluate *PIK3CA* gene mutational status in Northwest Chinese esophageal squamous cell carcinoma (ESCC) patients, and examine the associations of *PIK3CA* gene mutations with clinicopathological characteristics and clinical outcome.

METHODS

A total of 210 patients with ESCC who underwent curative resection were enrolled in this study. Pyrosequencing was applied to investigate mutations in exons 9 and 20 of *PIK3CA* gene in 210 Northwest Chinese ESCCs. The associations of *PIK3CA* gene mutations with clinicopathological characteristics and clinical outcome were examined.

RESULTS

PIK3CA gene mutations in exon 9 were detected in 48 cases (22.9%) of a non-biased database of 210 curatively resected Northwest Chinese ESCCs. *PIK3CA* gene mutations were not associated with sex, tobacco

use, alcohol use, tumor location, stage, or local recurrence. When compared with wild-type *PIK3CA* gene cases, patients with *PIK3CA* gene mutations in exons 9 experienced significantly better disease-free survival and overall survival rates.

CONCLUSION

The results of this study suggest that *PIK3CA* gene mutations could act as a prognostic biomarker in Northwest Chinese ESCC patients.

Key words: *PIK3CA* gene mutations; Esophageal squamous cell carcinoma; Northwest Chinese; Prognostic significance

Core tip: *PIK3CA* gene mutations have been associated with various prognoses in patients with different cancers. However, no large-scale study has examined the prognostic impact of *PIK3CA* gene mutations in Northwest Chinese esophageal squamous cell carcinoma (ESCC). In this study, we quantified *PIK3CA* gene mutations *via* pyrosequencing technology using a non-biased database of 210 curatively resected ESCCs. It was found that *PIK3CA* gene mutations in Northwest Chinese ESCC are associated with favorable prognoses. It has been suggested that *PIK3CA* gene mutational status can have a potential role as a prognostic biomarker for ESCC.

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INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) is a major histologic type of esophageal cancer that is one of the most aggressive malignant tumors worldwide, especially in East Asian countries, and accounts for most esophageal malignancies in China and Japan[1,2]. As one of the most commonly diagnosed cancers among men in China, the estimated number of new cases of esophageal cancer was 291238 in 2011, while the numbers of deaths was 218957 in the same year[3]; by 2015, these numbers had increased to 477900 and 375000, respectively[4]. Both the incidence and mortality rates were higher in rural areas than in urban areas. Despite the continuing development of cancer multimodality therapies, including surgery, radiotherapy, and chemotherapy, the prognosis of ESCC patients remains poor, even for those who undergo complete resection of their carcinomas[5].

Phosphatidylinositol 3-kinases (PI3Ks) are expressed as heterodimers of p110 catalytic subunits and p85 regulatory subunits that interact with phosphatidylinositol-3-phosphate at the membrane and catalyze the phosphorylation of protein kinase B (PKB, also known as AKT), which activates the downstream signaling pathway[6]. Activation of the PI3K/AKT signaling pathway plays an important role in the development of a variety of human carcinomas[7]. The catalytic subunits of PI3K are encoded by three genes (α, β, γ), with p110α subunit (*PIK3CA*) amplification being reported in a number of different tumor types. The mutant *PIK3CA* gene stimulates the AKT pathway and promotes cell growth and invasion in various types of human cancer[8,9] (Samuels, 2004 #620; Samuels, 2005 #638), including lung, breast, gastric, and colon[10-17].

PIK3CA gene mutations have also been detected in Japanese and Korean ESCCs[18,19]. Although independently associated with a poor prognosis in Chinese breast cancer patients[13], it was found to be associated with improved outcome in breast cancer patients in the United States[20]; this seeming contradiction requires an intensive study of this gene in future research. In addition, *PIK3CA* gene mutations and their prognostic role in Chinese ESCC patients have been rarely reported. We therefore quantified *PIK3CA* gene mutations in 210 samples of curatively resected ESCCs using pyrosequencing, and examined the prognostic significance of *PIK3CA* gene mutations in Northwest Chinese ESCC patients.

MATERIALS AND METHODS

Study subjects

A total of 210 patients with ESCC who underwent curative resection at the Second Affiliated Hospital of Xi'an Jiaotong University between 2009 and 2015 were enrolled in this study. Patients were observed at 1 to 3 mo intervals until either death or December 30, 2015. Tumor staging was carried out according to the 7th American Joint Committee Cancer Staging Manual[21]. Disease-free survival was defined as the length of time after surgical treatment of the cancer during which the patient survived with no sign of cancer recurrence. Cancer-specific survival was defined as the time between the date of operation and the date of death, which was confirmed to be attributable to ESCC. Overall survival was defined as the time between the date of the operation and the date of death. Written consent was obtained from each subject and the study procedures were approved by the ethical committees of the Second Affiliated Hospital of Xi'an Jiaotong University.

Genomic DNA extraction, polymerase chain reaction, and pyrosequencing of PIK3CA exon 9 and exon 20

Genomic DNA was extracted from 210 paraffin-embedded tissue specimens of surgically resected

Table 1 Two sets of primers of exon 9 and 20 of *PIK3CA* gene for polymerase chain reaction

Exon		Primers
Exon 9	Forward	5′CAAAGCAATTTCTACACGAGATCC 3′
	Reverse	5′GTAAAAACATGCTGAGATCAGCCACAT 3′
Exon 20	Forward	5′TGGAATGCCAGAACTACAATCTTT 3′
	Reverse	5′GGTCTTTGCCTGCTGAGAGTT 3′

esophageal cancers using the QIAamp DNA Mini kit (Qiagen, Hilgen, Germany) according to the manufacturer's instructions.

Polymerase chain reaction (PCR) amplifications targeting the *PIK3CA* gene (exon 9 and 20) were performed. Two sets of primers (Table 1) were used for the detection of any mutation points in exons 9 and 20 of the *PIK3CA* gene. PCR was carried out in a total volume of 20 μL. The mixture included 1x HotStarTaq buffer, 2.0 mmol/L Mg^{2+}, 0.2 mmol/L dNTP, 0.2 μmol/L of each primer, 1U HotStarTaq polymerase (Qiagen, Hilgen, Germany), and 1 μL template DNA. The cycling program for exon 9 was initial denaturation at 95 ℃ for 15 min, followed by 11 cycles at 94 ℃ for 20 s, 62 ℃-0.5 ℃ per cycle for 40 s, and 72 ℃ for 1 min. The cycling program for exon 20 was initial denaturation at 95 ℃ for 15 min, followed by 27 cycles at 94 ℃ for 20 s, 56 ℃ for 30 s, and 72 ℃ for 1 min. The PCR products were electrophoresed on agarose gels to confirm successful amplification of the 81 (exon 9) and 74 bp (exon 20) products.

PIK3CA pyrosequencing was carried out using the Pyro-Mark Q24 System (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Primers of *PIK3CA* gene (exon 9 and exon 20) for pyrosequencing are shown in Table 2.

Statistical analysis

For the statistical analysis, we used GraphPad Prism 5 software (GraphPad Software, La Jolla, CA). The association between *PIK3CA* gene mutations and clinicopathological variables were performed using the χ^2-test or Fisher's exact probability test. All *P* values were two-tailed, with a *P*-value less than 0.01 being considered significant. Estimation of overall survival was calculated using the Kaplan-Meier method, with statistical differences analyzed *via* the log-rank test.

RESULTS

PIK3CA gene mutational status in ESCC

For 210 patients who had undergone curative resection of stage Ⅰ to Ⅲ ESCC, we examined *PIK3CA* gene mutations (exon 9 and exon 20) by pyrosequencing technology. In this study, *PIK3CA* gene mutations were only observed in exon 9 in 48 (22.9%) of 210 Northwest Chinese ESCC samples. The most common mutation of *PIK3CA* exon 9 was the c.1634A>C (p.E545A) mutation, which was present in 35 tumors, followed by c.1633G>A (p.E545K) in 13 tumors.

PIK3CA gene mutations and ESCC patient characteristics

We examined whether the influence of *PIK3CA* gene mutations on cancer-specific survival was modified by any of the evaluated clinical, pathologic, or epidemiologic variables of the ESCCs. As a result, we found that *PIK3CA* gene mutations were not significantly associated with any of the evaluated characteristics of ESCCs, namely sex (male *vs* female), tobacco use (yes *vs* no), alcohol use (yes *vs* no), tumor location (upper, middle *vs* lower thoracic), preoperative treatment (yes *vs* no), lymph node metastasis (yes *vs* no), or local recurrence (yes *vs* no) (all $P > 0.01$; Table 3).

PIK3CA gene mutations and patient survival

We assessed the influence of *PIK3CA* gene mutations on clinical outcome in Northwest Chinese patients with curatively resected ESCC. During the follow-up of the 210 patients, there were a total of 46 deaths confirmed to be attributable to esophageal cancer. The median follow-up time for censored patients was 36.5 mo. In the Kaplan-Meier analysis, patients with *PIK3CA* gene mutations experienced significantly longer disease-free survival (log rank $P = 0.0094$), cancer-specific survival (log rank $P = 0.0059$), and overall survival (log rank $P = 0.0066$) rates than those with the wild-type *PIK3CA* gene (Figure 1).

DISCUSSION

Numerous genetic and functional studies have clearly established a fundamental role for the PI3K signaling pathway in the development of neoplasia. As an oncogene in various human cancers, *PIK3CA* is one of the most genetically mutated genes in human cancers (including colorectal, brain, and gastric cancers)[22], having been displayed as mutated in various tumors, thereby making it a possible therapeutic marker. *PIK3CA* gene mutations and the subsequent activation of the PI3K/AKT pathway are considered to play a crucial role in cancer cell signaling pathways downstream of growth factors, cytokines, and other cellular stimuli in human neoplasm[6,23]. We therefore conducted this study to examine the prognostic impact of *PIK3CA* gene mutations among 210 Northwest Chinese patients with curatively resected ESCC.

In this study, we identified *PIK3CA* gene mutations in 48 out of 210 (22.9%) Northwest Chinese patients with curatively resected ESCC, which is a rate similar to that previously observed in ESCC (21%)[24], colorectal cancer (32%)[9], and breast cancer (25%-40%)[25,26], but slightly higher than that for gastric cancers (4.3%)[27] and brain tumors (5%)[28]. Additionally, we also found that c.1634A>C (p.E545A) was the dominant mutation type, which was consistent with a previous study in China[29]. The *PIK3CA* gene mutation

Table 2 Primers of *PIK3CA* gene for pyrosequencing

Exon		Primers
Exon 9 RS1	Nucleotide dispensation order	5′ CCATAGAAAATCTTTCTCCT 3′ 5′ ATCGACTACACTGACTGACTGACTGACTGACTG 3′
Exon 9 RS2	Nucleotide dispensation order	5′ TTCTCCTTGCTTCAGTGATTT 3′ 5′ ATACACATGTCAGTCAGACTAGCTAGCTAGCTAG 3′
Exon 9 RS3	Nucleotide dispensation order	5′ TAGAAAATCTTTCTCCTGCT 3′ 5′ ATAGCACTGACTGACTGACTACTGACTGACTGACTG 3′
Exon 20 RS	Nucleotide dispensation order	5′TGGAATGCCAGAACTACAATCTTT 3′ 5′GGTCTTTGCCTGCTGAGAGTT 3′

Table 3 *PIK3CA* mutations and clinicopathological characteristics in Northwest Chinese esophageal squamous cell carcinoma patients *n* (%)

Clinical, epidemiologic, or pathologic feature	Total, *n*	PIK3CA		*P* value
		Mutant	Wild-type	
All cases	210	48	162	
Sex				0.4756
Male	137 (65.3)	34 (70.8)	123 (75.9)	
Female	73 (34.7)	14 (29.2)	39 (24.1)	
Tobacco use				0.2684
Yes	149 (71.0)	31 (64.6)	118 (72.9)	
No	61 (29.0)	17 (35.4)	44 (28.1)	
Alcohol use				0.3778
Yes	175 (83.3)	38 (79.2)	137 (84.6)	
No	35 (16.7)	10 (20.8)	25 (15.4)	
Preoperative treatment				0.8467
Yes	28 (13.3)	6 (12.5)	22 (13.6)	
No	182 (86.7)	42 (87.5)	140 (86.4)	
Tumor location				0.9651
Upper thoracic	20 (9.5)	5 (10.4)	15 (9.2)	
Middle thoracic	109 (51.9)	25 (52.1)	84 (51.9)	
Lower thoracic	81 (38.6)	18 (37.5)	63 (38.9)	
Stage				0.1641
Ⅰ A	16 (7.6)	3 (6.3)	13 (8.0)	
Ⅰ B	20 (9.5)	5 (10.4)	15 (9.3)	
Ⅱ A	28 (13.3)	11 (22.9)	17 (10.5)	
Ⅱ B	44 (21.0)	10 (20.8)	34 (21.0)	
Ⅲ A	49 (23.3)	12 (25.0)	37 (22.8)	
Ⅲ B	23 (11.0)	1 (2.1)	22 (13.6)	
Ⅲ C	30 (14.3)	6 (12.5)	24 (14.8)	
Lymph node metastasis				0.2663
Yes	121 (57.6)	31 (64.6)	90 (55.6)	
No	89 (42.4)	17 (35.4)	72 (44.4)	
Local recurrence				0.7368
Yes	43 (20.5)	9 (18.8)	34 (21.0)	
No	167 (79.5)	39 (81.2)	128 (79.0)	
Prognosis				0.0885
Dead	88 (41.9)	15 (31.3)	73 (45.1)	
Survived	122 (58.1)	33 (68.7)	89 (54.9)	

frequency of ESCC in this study is slightly high when compared with those of previous studies; we believe this may be due to a difference in the patient cohorts, sample sizes, or methods used to assess *PIK3CA* gene mutation. When identifying *PIK3CA* gene mutations, other researchers typically use direct sequencing rather than the pyrosequencing used in the current study, which is a reliable high-throughput method that could be used as an alternative method for genotyping mutation studies[30]. There is also a non-electrophoretic nucleotide extension sequencing technology that can be used for mutation detection in tumors. Additionally, pyrosequencing has been shown to be more sensitive than regular sequencing in detecting EGFR and KRAS mutations in lung cancer patients[31,32]. *PIK3CA* gene mutational status was not identified as being associated with any clinicopathological characteristics of Northwest Chinese ESCC patients in our study, which is consistent with two other studies in Korea and China[19,33].

Identifying prognostic factors or biomarkers plays a crucial role in cancer research and clinical treatment[34-36]. Previous studies examining the relationship between *PIK3CA* gene mutations and prognosis in human cancers have yielded variable results and showed that *PIK3CA* gene mutational status is not associated with ESCC patient survival, although it does denote a better prognosis in breast cancer and ovarian cancer[37,38]. This discrepancy might be due to differences in tumor histologic type. We conducted this study to explore the prognostic impact of *PIK3CA* gene mutations among 210 Northwest Chinese patients with curatively resected ESCC. It was revealed that *PIK3CA* gene mutations were associated with a favorable prognosis among patients with curatively resected ESCC, suggesting *PIK3CA* gene mutational status may be a prognostic biomarker for Northwest Chinese ESCC patients that can be used to identify the clinical outcome of patients with curatively resected ESCC, which is consistent with its roles in Japanese ESCC patients[24]. Nonetheless, our findings regarding the correlation between *PIK3CA* mutations and favorable prognosis in esophageal cancer requires further confirmation by future independent studies using much larger non-biased cohorts of ESCCs.

In summary, this study suggests that *PIK3CA* gene mutations are associated with a favorable clinical outcome in operational resected ESCC, which supports the *PIK3CA* gene's role as a prognostic biomarker for ESCC. Our data correlates with that of previous studies suggesting that the acquisition of *PIK3CA* gene mutations may be an important molecular event in the etiology of a wide range of tumor types and highlights the potential broad applicability that the *PIK3CA* gene may have in the clinical outcome of human cancers. Future studies are needed to confirm this association and clarify the exact molecular mechanisms by which *PIK3CA* gene mutations affects human cancer behavior.

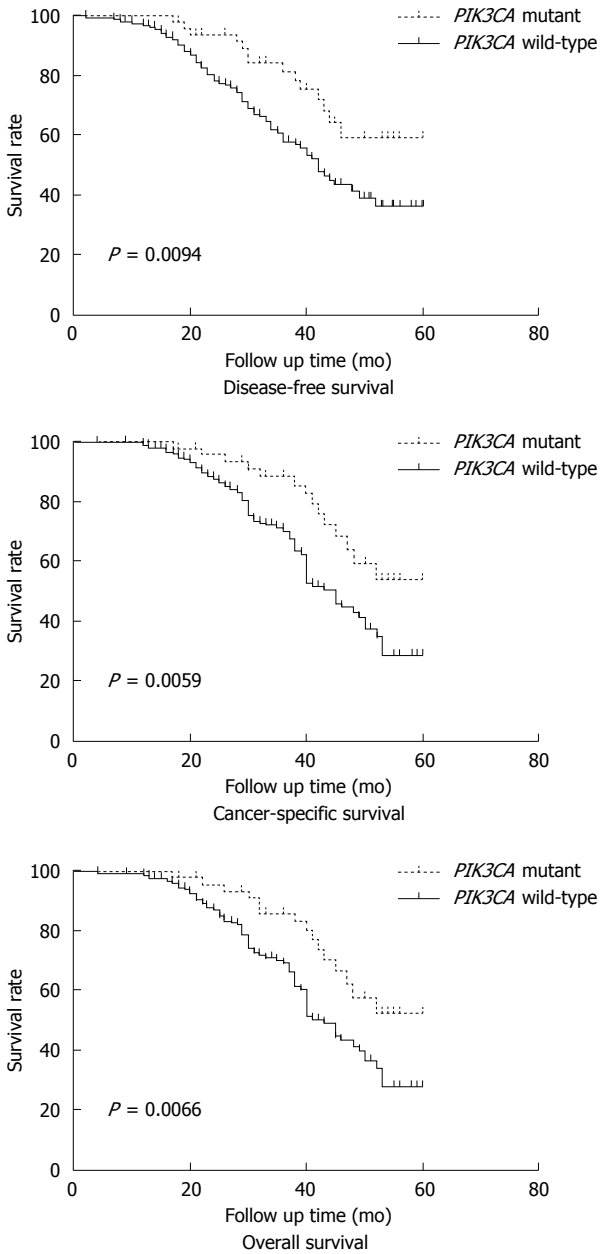

Figure 1 Kaplan-Meier curves for disease-free survival, cancer-specific survival, and overall survival in esophageal squamous cell carcinoma according to *PIK3CA* gene mutational status.

COMMENTS

Background

Esophageal squamous cell carcinoma (ESCC) is the predominant histological subtype of esophageal cancer in East Asian countries, where it accounts for more than 90% of total esophageal cancer cases. Despite the development of multimodality therapies, the prognosis of ESCC patients remains poor, even for those who undergo complete resection of their carcinomas. The 5-year survival rates of ESCC are between 11.1% and 56.5%, depending on the clinical stage at the time of diagnosis. With the development of high-throughput genome sequencing and screening technologies, an increasing number of cancer-associated genes have been identified to serve as potential therapeutic targets or prognostic indicators. High frequencies of somatic mutations conferring oncogenic potential have been found in the *PIK3CA* gene, which is associated with poor prognosis in patients with colorectal or lung cancer. In contrast, a relationship between *PIK3CA* gene mutations and favorable prognoses has been shown in breast cancer. However, no large-scale study has examined the prognostic impact of *PIK3CA* gene mutations in Northwest Chinese ESCC patients.

Research frontiers

The frequency of *PIK3CA* gene mutation in ESCC varied from 0% to 21%, which could likely introduce some bias in the statistical analyses of their clinical significance. More than 80% of *PIK3CA* gene mutations detected were localized in exons 9 and 20 (helical and kinase domain), with three "hot-spot" mutations: E542K, E545K, and H1047R. A recent report correlated with previous studies suggesting that the acquisition of *PIK3CA* mutations may be an important molecular event in the etiology of ESCC, and that mutations are associated with their clinical outcome.

Innovations and breakthroughs

This is, by far, one of the largest studies on the prognostic role of *PIK3CA* gene mutations in Northwest Chinese ESCC to date, and it shows that *PIK3CA* gene mutations in ESCC are associated with a favorable prognoses. It has been suggested that *PIK3CA* gene mutational status can have a potential role as a prognostic biomarker for ESCC patients.

Applications

PIK3CA gene mutations are associated with a favorable clinical outcome in operational resected Northwest Chinese ESCC patients, thereby suggesting that the acquisition of *PIK3CA* gene mutations may be an important molecular event in the etiology of a wide range of tumor types and highlighting the potential broad applicability that *PIK3CA* gene may have in the clinical outcome of human cancers.

Terminology

The *PIK3CA* gene is located on the 3q26.3 chromosome and encodes the catalytic p110 alpha subunit of phosphoinositide 3-kinase (PI3K). The PI3K signaling pathway is deregulated in many types of cancer, with only the *PIK3CA* gene being reported as mutated and amplified.

Peer-review

The authors examined the associations of *PIK3CA* gene mutations with clinicopathological characteristics and clinical outcome in esophageal squamous cell carcinoma patients in Northwest China. The authors exploited the most recent literature concerning the subject. The study suggests that *PIK3CA* gene mutations are associated with a favorable clinical outcome in esophageal squamous cell cancer and that in the future the evaluation of *PIK3CA* gene mutations may be potentially applied as a prognostic marker. The manuscript is worth sharing with other researchers. It is concise, clear, comprehensive, and convincing.

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