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***Clinical Trials Study***

***PIK3CA* gene mutations in Northwest Chinese esophageal squamous cell carcinoma**

Liu SY *et al.* *PIK3CA* gene mutations in esophageal cancer

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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 as well as 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 rate.

***CONCLUSION***

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

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

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**Core tip:** *PIK3CA* gene mutations have been associated with different prognosis in patients with different cancer. However, no large-scale study examined the prognostic impact of *PIK3CA* gene mutations in Northwest Chinese esophageal squamous cell carcinoma (ESCC). In this study, we quantified the *PIK3CA* gene mutations by pyrosequencing technology using a non-biased database of 210 curatively resected ESCCs. And found that *PIK3CA* gene mutations in Northwest Chinese ESCC are associated with favorable prognoses. It’s suggest 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), the major histologic type of esophageal cancer, is one of the most aggressive malignant tumor in the world; especially in East Asian countries[[1](#_ENREF_1)]. It accounts for most esophageal malignancies in China and Japan[[2](#_ENREF_2)]. As one of the most commonly diagnosed cancers among men in China; the estimated number of new cases and deaths of esophageal cancer in 2011 were 291,238 and 218957 respectively[[3](#_ENREF_3)], while 477900 and 375000 respectively in 2015[[4](#_ENREF_4)]. Both the incidence and mortality rates were higher in rural areas than in urban areas. Although the 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](#_ENREF_5)].

Phosphatidylinositol 3-kinases (PI3Ks) are expressed as heterodimers of p110 catalytic subunits and p85 regulatory subunits interact with phosphatidylinositol-3-phosphate at the membrane and catalyze the phosphorylation of [protein kinase B](https://en.wikipedia.org/wiki/Protein_kinase_B) (PKB, also known as AKT), which activates the downstream signaling pathway[[6](#_ENREF_6)]. Activation of PI3K/AKT signal pathway plays an important role in the development of a variety of human carcinomas[[7](#_ENREF_7)]. The catalytic subunits of PI3K are encoded by three genes (α,β,γ), of which the p110α subunit (*PIK3CA*) amplification has been reported in a number of different tumor types. Mutant *PIK3CA* gene stimulates the AKT pathway and promotes cell growth and invasion in various types of human cancer[[8](#_ENREF_8),[9](#_ENREF_9)][Samuels, 2004 #620;Samuels, 2005 #638], including lung, breast, gastric and colon[[10-17](#_ENREF_10)].

*PIK3CA* gene mutations has also been detected in Japanese and Korean ESCCs[[18](#_ENREF_18),[19](#_ENREF_19)]. It is independently associated with a poor prognosis in Chinese breast cancer patients[[13](#_ENREF_13)] but associated with improved outcome of breast cancer patients in the United States[[20](#_ENREF_20)]. Such a mind boggling conclusion calls for an intensive study of this gene in the future research. In addition, *PIK3CA* gene mutations and its prognostic role in Chinese ESCC patients have been rarely reported. Therefore, we quantified the *PIK3CA* gene mutations in 210 samples of curatively resected ESCCs using pyrosequencing and examined the prognostic significance of *PIK3CA* gene mutations in the 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 interval until death or 30 December 2015, whichever came first. Tumor staging was carried out by the 7th American Joint Committee on Cancer Staging Manual[[21](#_ENREF_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 (FFPE) tissue specimens of surgically resected esophageal cancers using the QIAamp DNA Mini kit (Qiagen, Hilgen, Germany) according to manufacturer’s instructions.

The polymerase chain reaction (PCR) amplifications targeting *PIK3CA* gene (exon 9 and 20) were performed. Two sets of primers (Table1) were used for the detection of any mutation points in exons 9 and 20 of *PIK3CA* gene. The PCR was carried out in a total volume of 20 μL. The mixture included 1x HotStarTaq buffer, 2.0 mM Mg2+, 0.2 mM dNTP, 0.2 μM 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 °C for 15 min, followed by 11 cycles at 94°C for 20 s, 62 °C–0.5 °C per cycle for 40 s, 72 °C for 1 min. Where as cycling program for exon 20 was initial denaturation at 95 °C for 15 min, followed by 27 cycles at 94 °C for 20 s, 56 °C for 30 s, 72 °C 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 and primers of *PIK3CA* gene (exon 9 and exon 20) for pyrosequencing are shown in Table 2.

***Statistical analysis***

For the statistical analysis, we used the GraphPad Prism5 software (GraphPad Software, La Jolla, CA). The association between *PIK3CA* gene mutations and the clinicopathological variables were performed using the χ2-test or Fisher’s exact probability test. All *P* values were two-tailed and a *P*-value of 0.01 was considered significant. Estimation of overall survival was calculated using the Kaplan-Meier method and the statistical differences were analyzed using the log-rank test.

**RESULTS**

***PIK3CA gene mutational status in ESCC***

Among 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 patients characteristics***

We also examined whether the influence of *PIK3CA* gene mutations on cancer specific survival was modified by any of the ESCCs clinical, pathologic, and epidemiologic variables evaluated. As a result, we found that *PIK3CA* gene mutations were not significantly associated with any of the evaluated above mentioned characteristics of ESCCs. For instance, 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) and 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) than those with wild-type *PIK3CA* gene (Figure 1).

**DISCUSSION**

Numerous genetic and functional studies have clearly established a fundamental role for the PI3K signal 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](#_ENREF_22)], and has been shown to be mutated in various tumors and recognized as a possible therapeutic marker. *PIK3CA* gene mutations and 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](#_ENREF_6),[23](#_ENREF_23)]. Therefore, we conducted this study to examine the prognostic impact of *PIK3CA* gene mutations among 210 Northwest Chinese patients with curatively resected ESCC.

Interestingly, in this study we identified *PIK3CA* gene mutations in 48 out of 210 (22.9%) Northwest Chinese patients with curatively resected ESCC, a rate that is similar to that reported previously observed in ESCC (21%)[[24](#_ENREF_24)], colorectal cancer (32%)[[9](#_ENREF_9)] and breast cancer (25%–40%)[[25](#_ENREF_25),[26](#_ENREF_26)], but slightly higher than that for gastric cancers (4.3%)[[27](#_ENREF_27)] and brain tumors (5%)[[28](#_ENREF_28)]. And also found out that c.1634A>C (p.E545A) was the dominant mutation type, this is consistent with previous study in China[[29](#_ENREF_29)]. The *PIK3CA* gene mutations frequency of ESCC in this study is slightly high when compared with those of previous studies; we think the possible reasons for these different results might be due to a difference in the patient cohorts, sample sizes or the methods used to assess *PIK3CA* gene mutation. In the current study, when identifying *PIK3CA* gene mutation other researchers used direct sequencing while we used pyrosequencing, which is a reliable high-throughput method that could be used as an alternative method for genotyping mutations studies[[30](#_ENREF_30)]. Also, there is 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](#_ENREF_31),[32](#_ENREF_32)]. Moreover, *PIK3CA* gene mutational status was not identified to be 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](#_ENREF_19),[33](#_ENREF_33)].

Identifying prognostic factors or biomarkers that play crucial role in cancer research and clinical treatment[[34-36](#_ENREF_34)]. Previous studies examed 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 but denotes a better prognosis in breast cancer and ovarian cancer[[37](#_ENREF_37),[38](#_ENREF_38)]. This discrepancy might be due to differences in the 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. As it turned out, the *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, and can be used to identify clinical outcome of these patients with curatively resected ESCC, which is consistent with it’s roles in Japanese ESCC patients[[24](#_ENREF_24)]. Nonetheless, our finding of the correlation between *PIK3CA* mutations and favorable prognosis in esophageal cancer needs to be confirmed by independent studies in a much larger non-biased cohort of ESCCs in the future.

In summary, this study suggests that *PIK3CA* gene mutations are associated with a favorable clinical outcome in operational resected ESCC, this supports *PIK3CA* gene mutational status role as a prognostic biomarker for ESCC. Our data extends 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 highlighting the potential broad applicability that *PIK3CA* gene may have in the clinical outcome of human cancers. Future studies are needed to confirm above association and to clarify the exact molecular mechanisms by which *PIK3CA* gene mutations affects human cancer behavior.

**COMMENTS**

***Background***

Esophageal squamous cell carcinoma (ESCC) is the predominant histological subtype of esophageal cancer in East Asian countries, accounting for more than 90% of the total esophageal cancer cases. Despite the development of multimodality therapies, the prognosis of patients remains poor, even for those who undergo complete resection of their carcinomas. The 5-year survival rates of ESCC were 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, more and more 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, it have been associated with poor prognosis in patients with colorectal or lung cancer. In contrast, the 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 ignificance. More than 80% of the *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 extends previous studies suggesting that the acquisition of *PIK3CA* mutations may be an important molecular event in the etiology of ESCC and the mutations are associated with their clinical outcome.

***Innovations and breakthroughs***

This is, by far, one of the largest study 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 favorable prognoses. It’s suggest 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, this 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***

*PIK3CA* gene is located on 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 and only *PIK3CA* gene has been reported to be mutated and amplified.

***Peer-review***

The authors examined the associations of *PIK3CA* gene mutations with clinicopathological characteristics and the clinical outcome in esophageal squamous cell carcinoma patients in the population of Northwest Chinese. 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 in 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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**Table 1 Two sets of primers of exon 9 and 20 of *PIK3CA* gene for polymerase chain reaction**

|  |  |
| --- | --- |
| **Exon** | **Primers** |
| Exon9  | Forward | 5’CAAAGCAATTTCTACACGAGATCC 3’ |
|  | Reverse | 5’GTAAAAACATGCTGAGATCAGCCACAT 3’ |
| Exon20 | Forward | 5’TGGAATGCCAGAACTACAATCTTT 3’ |
|  | Reverse | 5’GGTCTTTGCCTGCTGAGAGTT 3’ |

**Table 2 Primers of *PIK3CA* gene for pyrosequencing**

|  |  |
| --- | --- |
| **Exon** | **Primers** |
| Exon9 RS1  | Nucleotide dispensation order | 5’ CCATAGAAAATCTTTCTCCT 3’5’ ATCGACTACACTGACTGACTGACTGACTGACTGACTG 3’ |
| Exon9 RS2Exon9 RS3 | Nucleotide dispensation orderNucleotide dispensation order | 5’ TTCTCCTTGCTTCAGTGATTT 3’5’ ATACACATGTCAGTCAGACTAGCTAGCTAGCTAG 3’5’ TAGAAAATCTTTCTCCTGCT 3’5’ ATAGCACTGACTGACTGACTACTGACTGACTGACTG 3’ |
| Exon20 |  | 5’TGGAATGCCAGAACTACAATCTTT 3’ |
| RS | Nucleotide dispensation order | 5’GGTCTTTGCCTGCTGAGAGTT 3’ |

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

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | ***PIK3CA*** |  |
| **Clinical, epidemiologic,****or pathologic feature** | **Total, *n*** | **Mutant** | **Wild-type** | ***P* value** |
| 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 |
| ⅠA | 16 (7.6) | 3 (6.3) | 13 (8.0) |  |
| ⅠB | 20 (9.5) | 5 (10.4) | 15 (9.3) |  |
| ⅡA | 28 (13.3) | 11 (22.9) | 17 (10.5) |  |
| ⅡB | 44 (21.0) | 10 (20.8) | 34 (21.0) |  |
| ⅢA | 49 (23.3) | 12 (25.0) | 37 (22.8) |  |
| ⅢB | 23 (11.0) | 1 (2.1) | 22 (13.6) |  |
| ⅢC | 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) |  |
|  Survival | 122 (58.1) | 33 (68.7) | 89 (54.9) |  |



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