Functional STK15 Phe31Ile Polymorphism Is Associated with the Occurrence and Advanced Disease Status of Esophageal Squamous Cell Carcinoma

Xiaoping Miao, Tong Sun, Yonggang Wang, Xuemei Zhang, Wen Tan, and Dongxin Lin

Departments of 1 Etiology and Carcinogenesis and 2 Thoracic Surgery, Cancer Institute & Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People’s Republic of China

Abstract

STK15/BTAK/Aurora-A involved in regulating centrosomes and chromosome segregation is amplified and overexpressed in human cancers. A T91A polymorphism in STK15 causes Phe31Ile substitution, and the 31Ile variant has been shown to be preferentially amplified and associated with degree of aneuploidy in human tumors. We genotyped 656 patients with esophageal squamous cell carcinoma (ESCC) and 656 controls for the polymorphism to examine the hypothesis that the STK15 variation may affect individual susceptibility to the occurrence and aggression of ESCC. It was found that the Ile/Ile genotype was significantly associated with increased risk of ESCC occurrence (odds ratio (OR) = 1.97, 95% confidence interval (CI) = 1.36–2.85) compared with the Phe/Phe genotype. The 31Ile allele frequency significantly increased as ESCC stage increased (trend test, \( P = 0.006 \)). Patients with the Ile/Ile genotype had an increased risk for invasive disease (stage II–IV; OR = 2.13, 95% CI = 1.04–4.39) or metastatic disease (stage III and IV; OR = 2.31, 95% CI = 1.06–5.05) compared with those with the Phe/Phe genotype. A positive correlation between the Ile/Ile genotype and high ESCC grade was also observed. Our results demonstrate for the first time that the STK15 polymorphism is a genetic susceptibility factor for the occurrence and aggression of ESCC.

Introduction

Centrosomes are the microtubule-organizing centers of mammalian cells, which organize microtubule arrays throughout the cell cycle, thereby influencing both cell architecture and the accuracy of chromosome segregation (1, 2). Centrosome abnormalities, which may lead to chromosome instability, are common events in both cancer cell lines and primary human tumors (3). Chromosome instability and aberrations are thought to play a pivotal role in developing human tumors and in acquiring malignant phenotypes of the tumors (4, 5). However, it is not clear yet how centrosome aberrations occur and how they might contribute to chromosome instability in human cancers. Recently, a kinase STK15/BTAK/Aurora-A, which belongs to the Drosophila aurora and yeast Ipl1 kinase family and is essential for chromosome segregation and centrosome functions, has been identified, and it may thus elucidate this conundrum (6). Human STK15 maps to chromosome 20q13.2, a region that is often amplified in a number of cancer cell lines and primary tumor types (7–12). Previous studies have shown that ectopic expression of STK15 in mammalian cell lines causes centrosome amplification, chromosome instability, transformation in vitro, and tumorigenesis in nude mice, suggesting that STK15 may play a critical role in these oncogenic processes (6, 13).

Recently, a T91A transversion at the coding region of STK15 resulting in Phe31Ile amino acid substitution has been identified (14). Functional analysis revealed that the 31Ile variant is preferentially amplified and associated with a degree of aneuploidy in human tumors and thus has more potent transforming property compared with the 31Phe counterpart (14). In view of the role that STK15 plays in chromosome instability and tumorigenesis, we hypothesized that the functional STK15 polymorphism might act as a genetic modifier in individual susceptibility to certain cancers and their disease progressive status. On the basis of this hypothesis, we investigated the STK15 Phe31Ile polymorphism in a large case-control study of esophageal squamous cell carcinoma (ESCC), one of the most common malignancies in the world and particularly in the People’s Republic of China. We examined the contribution of STK15 genotype to the occurrence and invasiveness and metastasis of the disease.

Materials and Methods

Study Subjects. This study consisted of 656 ESCC patients and 656 controls, and all subjects were ethnic Han Chinese. The newly diagnosed and histologically confirmed ESCC patients were recruited consecutively from January 1997 to July 2002, at Cancer Hospital, Chinese Academy of Medical Sciences (Beijing). A portion of cases was enrolled in our previous molecular epidemiological studies of ESCC (15). In the present study, we extended the sample sizes of ESCC patients to 656. All patients underwent esophagectomy, which had detailed metastatic data. The pathological stage of ESCC at the time of diagnosis was classified into stage I (T1N0M0), stage IIa (T2–3N0M0), stage IIb (T1–2N1M0, stage III (T3–4N0 or T1N0M0), and stage IV (TnmM0) according to the Tumor-Node-Metastasis classification (16). Tumor grade was classified into low grade (well-differentiated), intermediate grade (moderately differentiated), and high grade (poorly differentiated) following the WHO grade classification (17). Population controls were cancer-free individuals living in the Beijing region, and they were selected from a community cancer screening program for early detection of cancer conducted during the same period that the cases were collected (18). These controls were randomly selected from a pool of 2800 individuals based on a physical examination, and the response rate was 96%. The inclusion criteria included no individual history of cancer and frequency matched to ESCC cases on the basis of sex and age (±5 years). At recruitment, informed consent was obtained from each subject, and each participant was then interviewed to collect detailed information on demographic characteristics and lifetime history of tobacco use. This study was approved by the Institutional Review Board of the Chinese Academy of Medical Sciences Cancer Institute.

STK15 Genotyping. The STK15 genotypes at the Phe31Ile site were analyzed by PCR-RFLP assays. The primers used were 5'-CTTTGATATGGCAAGATCTGGAGGAATTTGAAGGACA-3'. Amplification was accomplished with a 25-μL reaction mixture containing 20 ng DNA, 0.2 μM each primer, 0.2 mM each deoxynucleoside triphosphate, 1.5 mM MgCl2, and 1 unit Hot-StarTag DNA polymerase with 1× reaction buffer (Qiagen, Chatsworth, CA). The reaction was carried out in the following conditions: an initial melting step of 15 min at 95°C; followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, and a final elongation step of 7 min at 72°C. The 165-bp PCR products were digested with Fnu4H111 (New England Biolabs, Beverly, MA) and 1 unit of the enzyme PenI (New England Biolabs). The digested products were electrophoresed in a 2% agarose gel.
products were then digested with ApoI (New England BioLabs, Inc., Beverly, MA) and separated on a 2.5% agarose gel (Fig. 1A). The 31Phe allele had one ApoI restriction site that resulted in two bands (153 and 12 bp), whereas the 31Ile allele had two ApoI restriction sites and thus produced three bands (89, 64 and 12 bp). The Phe31Ile polymorphism revealed by PCR-RFLP analysis was further confirmed by DNA sequencing (Fig. 1B). Genotyping was performed without knowledge of case control status, and a 10% random sample of cases and controls was genotyped twice by different investigators; the reproducibility was 100%.

Statistical Analysis. \( \chi^2 \) test or unpaired \( t \) test was used to examine differences in demographic variables, smoking, and distributions of genotypes between cases and controls and between metastatic and nonmetastatic cases. The associations between STK15 genotype and risk of the occurrence and metastasis of ESCC were estimated by ORs and their 95% CIs, which were calculated by unconditional logistic regression models. The ORs were adjusted for age, sex, and pack-years smoked. All analysis was carried out with Statistical Analysis System software (Version 6.12; SAS Institute, Cary, NC).

**Results**

**Subjects’ Characteristics.** The male subjects among patients and controls were 70.1% and 67.5%, respectively. The mean age (±SD) was 58.3 ± 9.6 years for the patients and 57.5 ± 9.5 years for the controls. There were 57% smokers among patients compared with 56.1% among controls. No significant differences in the mean age (\( P = 0.110 \)), sex distribution (\( P = 0.311 \)), and smoking status (\( P = 0.738 \)) were found between the ESCC patients and controls, suggesting that the frequency matching was adequate. Of the 656 patients, 101 (15.4%) had stage I ESCC, 244 (37.2%) had stage II ESCC, and 295 (45.0%) had stage III ESCC, whereas only 16 (2.4%) patients had stage IV disease with hepatic metastasis. In total, 170 (25.9%) patients were classified into well-differentiated ESCC (low grade), 369 (56.3%) and 117 (17.8%) patients were classified into moderately differentiated (intermediate grade) or poorly differentiated (high grade) ESCC, respectively (Table 1).

**The Association between STK15 Phe31Ile Genotype and ESCC Risk.** Genotyping results showed that the allele frequencies for STK15 31Phe and 31Ile were 0.38 and 0.62 in controls compared with 0.31 and 0.69 in patients, respectively. The genotype frequencies among controls were 13.8% (Phe/Phe), 48.2% (Phe/Ile), and 38.0% (Ile/Ile), which did not deviate from those expected from the Hardy-Weinberg equilibrium (\( P = 0.934 \)). However, they were significantly different from those (Phe/Phe, 8.8%; Phe/Ile, 44.2% and Ile/Ile, 47.0%) among patients (\( \chi^2 = 14.67, P = 0.001 \)). Subjects having the STK15 Ile/Ile genotype were at a nearly 2-fold-increased risk for developing ESCC [adjusted odds ratio = 1.97, 95% confidence interval = 1.36–2.85; \( P = 0.0004 \)] compared with subjects having the STK15 Phe/Phe genotype. Furthermore, a borderline elevated risk of ESCC was also observed among subjects having the STK15 Phe/Ile genotype (adjusted odds ratio = 1.43, 95% confidence interval = 0.99–2.07, \( P = 0.051 \); Table 2). In the stratification analysis, age, sex, or smoking status had no effect on the risk of ESCC related to the STK15 polymorphism (data not shown).

**The Association between STK15 Phe31Ile Genotype and ESCC Disease Status.** The association between STK15 Phe31Ile genotype and ESCC stage or grade at the time of diagnosis was further evaluated. As shown in Table 1, the frequency of the variant 31Ile allele significantly increased as ESCC stage increased, with the frequencies being 0.614, 0.700, 0.728, 0.714, and 0.781 in patients with tumor stage I, stage IIa, stage IIb, stage III, and stage IV, respectively (trend test, \( P = 0.006 \)). We observed a significant difference in the genotype frequency between the stage I patients and patients with advanced disease, i.e., stage II-IV ESCC (\( \chi^2 = 4.84, P = 0.028 \)). Compared with the Phe/Phe genotype, patients with the Ile/Ile genotype had >2-fold-increased risk for developing advanced ESCC, although the heterozygous Phe/Ile genotype did not have such an effect (Table 2). Concerning the tumor grade, no significant differences in the allele and genotype frequencies were found among low, intermediate, and high-grade ESCC (trend test, \( P = 0.066 \); Table 1), and the association between the tumor grade and the Ile/Ile genotype against the Phe/Phe genotype was not significant. However, when the patients with Phe/Phe and Phe/Ile genotypes were combined as a reference group for analysis, those with the Ile/Ile genotype were at a significantly increased risk for being poorly differentiated ESCC, with the ORs of 1.99 (95% confidence interval = 1.23–3.22, \( P = 0.005 \)) for high grade against low grade and 1.81 (95% confidence interval = 1.20–2.71, \( P = 0.004 \)) for high grade against combined low and intermediate grades (Table 2).

![Fig. 1. Analysis of the STK15 Phe31Ile polymorphism. A. representative gel picture showing PCR-RFLP analysis of the STK15 genotypes in genomic DNAs of study subjects with the restriction enzyme ApoI. M, DNA size markers; subjects 1, 3, 4, Phe/Ile genotype; subjects 2 and 6–8, Ile/Ile genotype; subjects 5 and 9, Phe/Phe genotype. B. partial DNA sequence of three different allelic PCR products analyzed directly with an ABI PRISM 377 automatic sequencer showing a T-to-A transversion at the nucleotide location at which the arrow points.](image-url)
Discussion

The present study demonstrates an association between the functional STK15 Phe31Ile polymorphism and ESCC in a Chinese population. Subjects carrying the variant Ile/Ile genotype had about 2-fold-increased risk for developing the cancer. Furthermore, a significant correlation between the polymorphism and risk for advanced disease status among the ESCC patients was also observed. Patients with the Ile/Ile genotype were at >2-fold-increased risk for having invasive and metastatic ESCC at the time of diagnosis. These findings strongly support our hypothesis that STK15 may be involved in the occurrence and aggression of ESCC, and functional polymorphism in STK15 may have substantial impact on individual susceptibility. To the best of our knowledge, this is the first study with large sample size to investigate the association between STK15 polymorphism and risk of human cancer and the effect of the gene on the cancer.

The results in this molecular epidemiological study showing the association between STK15 genotype and risk of the occurrence of ESCC are biologically plausible because they are parallel in several ways to the laboratory and clinical findings. First, STK15 has been recognized as an oncogene (reviewed in Ref. 19), and numerous studies have shown that STK15 is amplified and overexpressed in many human cancer types, such as gastric cancer, bladder cancer, breast cancer, colon cancer, and pancreatic cancer (7–12). Although a published report on STK15 and esophageal cancer is currently unavailable in the literature, Tong et al. are able to show that STK15 is also overexpressed in human ESCC, and the overexpression is correlated with the advanced stage of the cancer. Overexpression of STK15 disrupts mitotic progression, which may result in cytokinesis failure and therefore produce aneuploidy and malignant transformation (6, 19). It has been shown that mouse NIH-3T3 cells transfected with STK15 give rise to tumors when injected into nude mice (6, 13). These findings clearly demonstrate that STK15 plays an important role in carcinogenesis. Secondly, the Phe31Ile polymorphism in STK15 has functional implication. Ewart-Toland et al. (14) have demonstrated that the STK15 31Ile allele is amplified more commonly than the 31Phe allele in heterozygous carriers of colon cancer patients. In a comparative genome hybridization array analysis of colon cancer, patients with the heterozygous Phe/Ile genotype were found to have more aneuploidy than patients with the homozygous Phe/Phe genotype, which is in agreement with the known role of STK15 in cancer progression (14). Moreover, it has been shown that compared with the STK15–31Phe counterpart, the STK15–31Ile is more able to induce cell growth and xenograft tumor formation in nude mice but lagging in binding to the E2-ubiquitin-conjugating enzyme UBE2N, suggesting that the Phe31Ile amino acid substitution might change STK15 function through modifying interactions with its binding partners in the cell (14). Having these findings in mind, one expects that individuals carrying the STK15 Ile/Ile genotype may be more susceptible to developing cancer.

We also found that the STK15 polymorphism was associated with advanced ESCC stage and grade. These results might demonstrate an important role of the polymorphism as a relevant genetic factor in the progression of ESCC. Cancer invasiveness and metastasis are the major cause of death in patients with the disease. Although it is not fully understood how primary tumors evolve to invasive and metastatic disease, accumulating evidence has shown that the overexpression of STK15 is correlated with these malignant phenotypes of many cancers (8, 9, 11). Overexpression of STK15 may lead to centrosome amplification and consequently cause chromosome instability (20), which may facilitate tumor cells to gain invasive and metastatic phenotypes. Taken together, these findings suggest that STK15 may play its role not only in the initiation but also in the progression of cancer. In view of these effects of STK15 on cancer and the functional relevance of the Phe31Ile polymorphism, it is biologically reasonable to observe a positive correlation between the STK15 Ile/Ile genotype and the risk of developing advanced ESCC.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Total subjects</th>
<th>Phe/Phe</th>
<th>Phe/Ile</th>
<th>Ile/Ile</th>
<th>31Ile allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>656 (100.0)</td>
<td>91 (13.8)</td>
<td>316 (48.2)</td>
<td>249 (38.0)</td>
<td>0.620</td>
</tr>
<tr>
<td>Patients</td>
<td>656 (100.0)</td>
<td>58 (8.8)</td>
<td>290 (44.2)</td>
<td>308 (47.0)</td>
<td>0.691</td>
</tr>
</tbody>
</table>

Stage I: T1aN0M0, stage IIa: T2aN0M0, stage IIb: T2bN0M0, stage III: T3N0M0 or T4aN0M0, stage IV: T4bNanyM0.

Table 2 The association of STK15 genotypes with the occurrence and disease status of ESCC

<table>
<thead>
<tr>
<th>OR (95% CI) according to STK15 genotype</th>
<th>Phe/Phe</th>
<th>Phe/Ile</th>
<th>Ile/Ile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients vs. controls</td>
<td>1.00</td>
<td>1.43 (0.99-2.07)</td>
<td>1.97 (1.36-2.85)</td>
</tr>
<tr>
<td>Tumor stage: Stage II + III + IV vs. stage I</td>
<td>1.00</td>
<td>1.37 (0.69-2.74)</td>
<td>2.13 (1.04-4.39)</td>
</tr>
<tr>
<td>Tumor grade: High vs. low</td>
<td>1.00</td>
<td>1.24 (0.59-2.62)</td>
<td>2.31 (1.06-5.05)</td>
</tr>
</tbody>
</table>

Adjusted for sex, age, and smoking.

Classification systems are the same as Table 1.

Adjusted OR = 1.99 (95% CI = 1.23-3.22, P = 0.005) when the Phe/Phe and Phe/Ile genotypes were combined as reference group for analysis.

Adjusted OR = 1.81 (95% CI = 1.20-2.71, P = 0.004) when the Phe/Phe and Phe/Ile genotypes were combined as reference group for analysis.
Although the patients with ESCC recruited in the present study were from only one hospital and thus may not be representative of the general Chinese population, the results of this study, which used incident cases and had a considerable large number of subjects, solid and reproducible genotyping techniques, and significantly increased odds ratios with small \( P \)s, are unlikely to be attributable to selection bias. The fact that genotype frequencies among our controls and cases fit the Hardy-Weinberg equilibrium further supports the randomness of subject selection. In addition, the observed effect of STK15 genotype was not influenced by other potential modifiers of ESCC risk such as age, sex, and tobacco smoking. Therefore, it is improbable that subject selection or unknown confounding factors could have biased our findings in this study.

In conclusion, our study demonstrates that functional polymorphism in the \( STK15 \) gene is a genetic susceptibility factor for the occurrence and aggression of ESCC. Because this is the first report demonstrating the contribution of \( STK15 \) polymorphism to ESCC and because \( STK15 \) is overexpressed in many cancer types, additional studies on ESCC and other types of common cancers would be warranted in different ethnic populations.

References

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