RESEARCH ARTICLE

Upregulation of STK15 in Esophageal Squamous Cell Carcinomas in a Mongolian Population

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Abstract

Background: The STK15 gene located on chromosome 20q13.2 encodes a centrosome-associated kinase critical for regulated chromosome segregation and cytokinesis. Recent studies have demonstrated STK15 to be significantly associated with many tumors, with aberrant expression obseved in many human malignancies. The purpose of this study was to investigate expression of STK15 in esophageal squamous cell carcinomas (ESCCs) in a Mongolian population. Methods: Two non-synonymous single nucleotide polymorphisms in the coding region of STK15, rs2273535 (Phe31Ile) and rs1047972 (Val57Ile) were assessed in 380 ESCC patients and 380 healthy controls. We also detected STK15 mRNA expression in 39 esophageal squamous cell carcinomas and corresponding adjacent tissues by real time PCR. Results: rs2273535 showed a significant association with ESCC in our Mongolian population (rs227353, P allele = 0.0447, OR (95%CI) = 1.259 (1.005~1.578)). Real time PCR analysis of ESCC tissues showed that expression of STK15 mRNA in cancer tissues was higher than in normal tissues (p = 0.013). Conclusions: Our study showed that functional SNPs in the STK15 gene are associated with ESCC in a Mongolian population and up-regulation of STK15 mRNAoccurs in ESCC tumors compared adjacent normal tissues. STK15 may thus have an important role in the prognosis of ESCC and be a potential therapeutic target.

Keywords: STK15 - association study - expression - esophageal SCC - Mongolian population

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Introduction

Esophageal squamous cell carcinoma (ESCC) is the seventh leading cause of cancer deaths worldwide, while ESCC ranks the fourth cause of cancer-related death and kills ~250 000 people per year (Lin et al., 2010). Previous studies revealed some demographic and clinicopathological characteristics, including dietary habits, tobacco-smoking and alcohol drinking, was responsible for the ESCC of poor prognosis and high mortality rate (Wu et al., 2011b; Thrift et al., 2012). However, these lifestyle factors can only explain part of the etiology and the ultimate biological cause of this cancer still remains unclear. Many Studies have suggested that genetic variant can provide important prognostic information for ESCC (Cheung et al., 2009).

Human STK15 is located on chromosome 20q13.2, which is frequently amplified in a number of cancer cell lines and primary tumor types (Zhou et al., 1998). Aurora-A encoded by STK15 involve in regulating centrosomes and chromosome segregation (Katayama et al., 2001). Several studies conducted the case-control study between STK15 and ESCC. Miao et al. (2004) genotyped Phe31Ile (rs2273535) polymorphism in the 656 ESCC patients and 656 controls, which reported the allele frequencies was significantly associated with ESCC occurrence. Kimura et al., (2005) performed association of 169G>A (rs1047972) and 91T>A (rs2273535) in STK15 with Japanese population. Their results indicated one risk Haplotype 91A-169A (rs2273535-rs1047972) of STK15 was statistically more frequent in cancer cases (odds ratio, 3.1452; 95% confidence interval, 1.0258-9.6435).

ESCC of Mongolia population has one of the highest incidences among many ethnics in China. The signs and symptoms of ESCC are found in the final stage, so mortality is very high. Meanwhile, nearly 40 percent of patients with ESCC significant was found the phenomenon familial aggregation (Zhang et al., 2012). In this study, to clarify the role of STK15 in ESCC of Mongolian population, we performed association studies between 2 nonsynonymous in the coding region of STK15, Phe31Ile (dBSNP reference rs2273535 T/A) and Val57Ile (dBSNP

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reference rs1047972 G/A) and ESCC. The 2 markers' position and genomic structure of STK15 gene are shown in Figure 1. We examined the expression of STK15 mRNA in ESCC tissues and corresponding normal tissues.

Materials and Methods

Subject

In total, 380 ESCC cancer patients (268 males and 112 females, age: 61.23±14.03 years) and 380 healthy controls (245 males and 235 females, age: 43.53±7.94 years) were recruited for the case-control study. All the ESCC patients had undergone curative resection between 1999 and 2007 at the surgical department of the Tongliao cancer center, Inner Mongolia, China. Controls were patients without cancer and recruited from the above hospital during the same time period. All subjects gave informed consent for the genetic analysis, which was reviewed and approved by the ethics committee.

Genotyping

DNA were extracted from peripheral whole blood of each subject using Tiangen DNA extraction kit (Biotech, Beijing, China). Two SNPs were genotyped on the ABI 7900 DNA detection system (Applied Biosystems, Foster City, California) using TaqMan® technology. All probes were designed by the Applied Biosystems service. The standard 5 μl PCR reaction was carried out using TaqMan® Universal PCR Master Mix reagent kits under the guidelines provided.

Quantitative Analysis of Gene Expression

Total RNA was purified from frozen stored tissues of ESCC patients by the TRIzol reagent (Invitrogen, Carlsbad, CA) method. 2 μg of each RNA sample was converted into first strand cDNA using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Ontario, Canada). Real time PCR was performed using SYBR Premix Ex Taq (TakaRa, Japan), following the manufacturer's instructions. The procedure for PCR was as following: 95 °C for 15 min, followed by 45 cycles of 94°C for 15s, 58°C for 30s, 70°C for 30s. The PCR primers used for STK15 were 5'-GAGGTCCAAAACGTGTTCTCG -3' for the forward primer and 5'- ACAGGATGAGGTACACTGGTTG -3' for the reverse primer. For GAPDH (glyceraldehyde-3-phosphate dehydrogenase), the forward primer used was 5'-TGGTATCGTGGAAGGACTCATGAC-3' and the reverse primer used was 5'-ATGCCAGTGAGCTTCCCGTTCAGC-3'. All reactions were performed in triplicates and the mean value of Ct was used for subsequent analysis. Relative gene expression quantification was performed using the 2 -ΔΔCt method. Comparisons of differences in STK15 mRNA expression between cancer tissues and corresponding controls were analyzed by the Student t-test (2-tailed) with SPSS16.0 for Windows.

Statistical Analysis

All the parameter calculations, including allele and genotype frequencies, Hardy-Weinberg equilibrium analysis were carried out online by SHEsis platform (Yong et al., 2005). The values are usually considered significant if they are below 0.05.

Results

Genetic Association of STK15 Variants with ESCC in Mongolia Population

A total of 380 ESCC patients and 380 normal controls were studied in our experiment. Genotype distributions for the 2 SNPs were in Hardy-Weinberg equilibrium in either ESCC or controls. The allele and genotype frequencies of the SNPs are showed in Table 1. ESCC patients and controls showed statistically significant differences for rs2273535 in the allelic distribution (rs2273535, p=0.0447 for allele, OR (95%CI) = 1.259 (1.005~1.578). However, rs1047972 showed no significant association with ESCC (rs1047972, p=0.0744 for allele, OR (95%CI) = 0.775 (0.586~1.026).

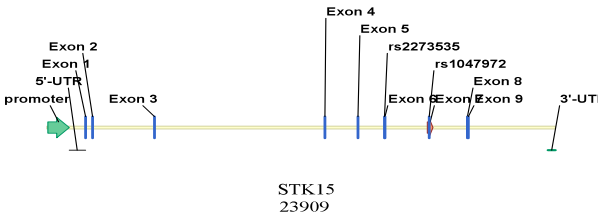

Figure 1. The Distribution of The Two SNPs in The STK15 Gene

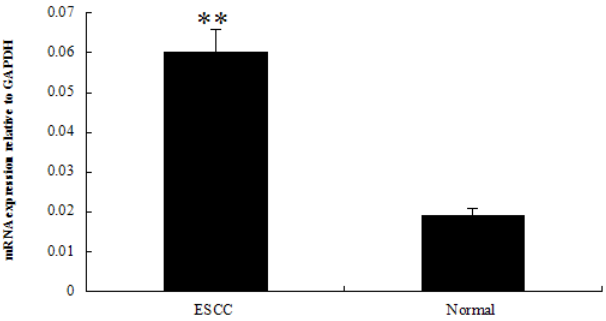

Figure 2. Levels of STK15 mRNA in ESCC Tumors (T) and their Adjacent Normal Tissues (N)

Table 1. Allele and Genotype Analysis of The ESCC Groups and The Healthy Control Group

SNP	Position	allele	sample	Allele frequency		OR (95%CI)	*P*-value	Genotype frequency			*P*-value
rs2273535	56386485	T/A		T (Phe)	A (Ile)			TT	TA	AA	
		(Phe/Ile)	ESCC	0.718	0.282	1.259 (1.005~1.578)	0.0447	0.499	0.438	0.063	0.0837
			Control	0.669	0.331						
rs1047972	56386226	G/A		G (Val)	A (Ile)			GG	GA	AA	
		(Val/Ile)	ESCC	0.827	0.173	0.775 (0.586~1.026)	0.0744	0.681	0.293	0.026	0.194
			Control	0.861	0.139			0.740	0.241	0.019	

STK15 mRNA expression in ESCC tumor specimens

Since rs2273535 showed significant association with ESCC, and it could influence STK15 expression. We conducted Real time PCR analysis of STK15 mRNA expression in the 39 cancer samples and their corresponding normal tissues. The results showed that STK15 expression was significantly higher in ESCC tumors than in their adjacent normal tissues in 32 of 39 (82.1%) individual cases, with the mean expression level (± s.e.m.) (0.06±0.0057 versus 0.019±0.002; p=0.013) (Fig. 2).

Discussion

Esophageal squamous cell carcinoma (ESCC), a highly lethal disease with poor prognosis, is one of the most common cancer in East Asian. Although more effective diagnostic and therapeutic approaches for ESCC was rapidly developed and used in the clinical research, the etiology of ESCC and effects of new treatment remain unclear (Cheng et al., 2010). Feng et al., (2014) established the prevalence and distribution profile of ESCC in north China and also found the prevalence of ESCC is higher in male and rural area patients compared with in female patients and urban areas. The previous studies suggested that socioeconomic level and unhealthy lifestyle , including intake of pickled or salted vegetables, reference for a high salt diet and prevalence of Helicobacter pylori infection contributed risk of the ESCC (Xibib et al., 2003; Lin et al., 2011; Song et al., 2013). Recently, it was reported that new risks, including smoking and high alcohol consumption, played a important role in the pathogenesis of ESCC (Lagergren et al., 2010; Amani et al., 2013). Many studies were conducted on a role of various gene mutations, and polymorphisms on esophageal mucosal cancers. Liu et al., (2014) conducted a case-control study to examine the association of PTGS2 and PLA2G2 with ESCC in 269 case and 269 controls. Their results suggest PTGS2 and PLA2G2 gene modify the risk of ESCC development. Meanwhile, genetic variant in RAD51 was investigated susceptibility of ESCC. the haplotype of three SNPs in RAD51 increased the ESCC risks (Zhang et al., 2014). PLEC1 on 10q23, C20orf54 on chromosome 20p13, SLC39A6 on chromosome 18q22 was identified by GWAS to be significantly associated with susceptibility to ESCC in Chinese population (Abnet et al., 2010; Wang et al., 2010; Wu et al., 2011a). In conclusion, ESCC develops as a result of complex epigenetic, multiple susceptibility loci and environmental interactions.

STK15 involved in tumor development and progression in several human cancers. Many studies reported that STK15 overexpression was responsible for centrosome dysfunction, chromosome instability, aneuploidy, malignant cell transformation and abrogation of DNA damage-induced G2-M checkpoint, indicating that STK15 might play important role in human carcinogenesis (Li et al., 2003). Comparative genomic hybridization demonstrated chromosomal rearrangements and copy number changes, where STK15 gene is located on the chromosome region, was observed in 29 ESCC cell lines (Tanaka et al., 2005). Chromosomal abnormalities within STK15 may be relevant for development and/or progression of ESCC (Reiter et al., 2006). Recently, Tanaka et al. (2007) reported the suppression of STK15 expression through RNAi caused an accumulation of the cells in the G2-M phase in vitro and the inhibition of proliferation of ESCC cell lines. Evidence suggest that suppression of STK15 expression might be a potential therapeutic target for ESCC.

Both rs2273535 and rs1047972 are non-synonymous single nucleotide polymorphisms and fallen in the NH-2 terminal region of the Aurora-A protein. Rs2273535 causes an amino acid change at codon 31 from phenylalanine to an isoleucine, rs1047972 causes an amino acid change from Valine to an Isoleucine. Miao et al. (2004) found Ile/Ile (AA) genotype in the Chinese population was significantly associated with increased risk of ESCC occurrence (OR=1.97) and positively correlated with high ESCC grade. Pan et al. (2012) also investigated the association between the two SNPs with clinical outcome of ESCC treated with preoperative chemoradiation. In our study, we found the frequency of allele A in the ESCC group was statistically higher than in healthy control. And subjects with rs2273535 TT genotype had an increased risk of ESCC as compared with compared with AA genotype (OR=1.259, 95% CI: 1.005-1.578), The results are in accordance with the previous study by Miao et al. (2004) showed rs1047972, which reduces STK15 kinase activity patients with low kinase activity of STK15, could occur with greater frequency in the high-risk category than in the low-risk population. However our data suggest that rs1047972 showed no significant association with ESCC, the presence of the A allele enhances the risk of ESCC (Kimura et al. 2005). mRNA expression analysis further suggests STK15 play an important role in the ESCC tumorigenesis.

Taken together, this is the first study to investigate the association between STK15 and ESCC in Mongolia Population. The two major findings of this study are that: 1) the heterozygous variant allele of the rs2273535 is associated with significantly higher risk of ESCC; 2) the higher expression of STK15 mRNA was correlated with ESCC in Mongolia population. Our present studies on the overexpression and association of STK15 in ESCC of Mongolia population have provided indicative clues to revealing the mechanism of ESCC and to find regimens to improve survival of ESCC patients.

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