Int J Clin Exp Pathol 2019;12(9):3408-3416
www.ijcep.com /ISSN:1936-2625/IJCEP0098531

Original Article

Relationship between esophageal cancer-related gene 2 polymorphism and esophageal squamous cell carcinomas in Kazakhs and Hans of Xinjiang

Limin Gao[1], Jun Li[2], Yi Zheng[3]

Departments of [1]Obstetrics and Gynecology, [2]Ultrasound, [3]Gastroenterology, The First Affiliated Hospital, Shihezi University School of Medicine, Shihezi, Xinjiang, China

Received June 16, 2019; Accepted August 26, 2019; Epub September 1, 2019; Published September 15, 2019

Abstract: In recent years, many studies have focused on the novel esophageal cancer-related gene 2 (ECRG2), which might be important in esophageal cancer development. The aim of this study is to investigate the relationship between ECRG2 short tandem repeat (STR) polymorphism and susceptibility to esophageal squamous cell carcinomas (ESCCs) in Kazakhs and Hans in Xinjiang. ECRG2 genotypes were detected by PCR-SSCP in 178 cases of esophageal carcinomas and 153 blood samples from the Kazakh and Han population. In Kazakhs and Hans, the frequencies of ECRG2 STR genotypes TCA3/TCA3, TCA4/TCA4, and TCA3/TCA4 were 47.8%/8.7%, 43.5%/67.9%, and 7.1%/25.0% in esophageal carcinomas with metastasis, respectively; and 14.1%/38%, 47.9%/14.3%, and 44.6%/41.1% in carcinomas without metastasis, respectively. A significant difference was observed between the groups with metastasis and without metastasis (Kazakh: χ^2=13.77, P<0.01; Han: χ^2=26.183, P<0.01). Compared with patients who carried the TCA4/TCA4 genotype, those who carried the TCA3/TCA3 genotype were at an increased risk of ESCC, with the adjusted odds ratios being 4.06 (95% confidence interval (CI), 1.69-9.74) in Kazakhs and 3.25 (95% CI, 1.25-8.45) in Hans. Our findings suggested that subjects who carried the TCA3/TCA3 genotype are at an increased risk of ESCC and metastasis compared with those who carried the TCA4/TCA4 genotype.

Keywords: Esophageal cancer, ECRG2, genetic polymorphism, STR, Kazakh population, Han

Introduction

Esophageal cancer is one of the most common malignant tumors in the world [1, 2]. It ranks eighth among the most common incident tumors and fifth in cancer-related death [3]. Epidemiological studies have found that the incidence of esophageal cancer in different areas varies [4], and its geographic distribution has obvious characteristics [5]. Esophageal cancer is a multifactor, multi-gene mutation accumulation and interaction complex [6, 7]. Epidemiological studies have revealed that the incidence of esophageal squamous cell carcinoma (ESCC) is related to many factors, such as N-nitrosamines [8], which have been shown to be involved in the etiology of ESCC in Linxian [9]. In addition, previous studies have shown many genetic factors, including amplification of C-myc [10], Int-2 [11] and Hst [12], mutation and/or deletion of p53 and Rb; and allelic deletion, in human ESCC and esophageal cancer cells [13, 14]. However, the factors that promote the development of esophageal cancer still need to be determined. Therefore, genetic factors may be important in ESCC incidence.

In recent years, many studies of esophageal cancer have focused on the novel esophageal cancer related genes (ECRG) 1-4, which might be important in its development [15-18]. ECRG2, which is located in chromosome 5q33.1, is closely related to esophageal cancer and has three short tandem repeat (STR) genotypes, TCA3/TCA3, TCA3/TCA4, and TCA4/TCA4 [19]. Bioinformatics analysis has shown that 97% of the amino acid sequences of ECRG2 are similar to a tumor-related KAZAL-type serine protease inhibitor, which may be important in the protection of esophageal mucosal cells [20]. These findings indicate that the ECRG2 STR is a genetic susceptibility factor for ESCC. Moreover, it

has significance for studying the genetic background and early screening of ESCC.

So far, the relationship between ECRG2 STR polymorphism and esophageal cancer risk in the Kazakh and Han populations of Xinjiang has not been studied. Furthermore, the distribution of ECRG2 STR polymorphisms between Kazakh and Han ESCC patients still needed to be investigated to determine significant differences. Therefore, in the present study, we used PCR-SSCP to examine the ECRG2 and 3 STR genotype polymorphisms and their distribution characteristics in 100 Kazakh and 87 Han esophageal cancer tissues and 103 Kazakh and 57 Han blood samples. We explored the relationships between ECRG2 STR polymorphism and esophageal cancer metastasis. Our findings suggested that genetic variants in ECRG2 may serve as candidate markers for Kazakh and Han ESCC susceptibility.

Material and methods

Study subjects

In Kazakhs, ECRG2 genotypes were detected by PCR-SSCP in 94 esophageal carcinomas and 100 blood samples for control. All patients received surgical treatment at the Department of Pathology of Yili Friendship Hospital and the First Affiliated Hospital of School of Medicine, Shihezi University. All cases were referred by two senior pathologists, with 52 being male and 42 being female. A total of 17 cases werewelldifferentiated squamous cell carcinomas, 68 were moderately differentiated, and 9 were poorly differentiated. Lymph node metastases were found in 23 cases. Moreover, we collected from a same aged group of Kazakh people without a tumor history through physical,blood samples from 100 cases, to be used as controls. In addition, 84 Han patients diagnosed with histologically confirmed ESCC and 53 normal cases were also randomly recruited for this study by multistage cluster sampling.

DNA extraction and identification of ECRG2 STR

Using standard procedures, DNA samples were extracted from blood or tissues of subjects. PCR-based SSCP analysis was used to scan the fourth exon of ECRG2 for selecting variations. Based on the exon 4 flanking DNA sequences, PCR primers were designed to amplify a 235 bp fragment, and they were 5'-CTTGTG CTA ATG AAT CTT GTG AAC TGT G-3' (forward) and 5'-AAA CTT TCT CCA TTC AGT CAA GAT TAC-3' (reverse). PCR was performed in a GeneAmp 2400 Thermal Cycler (Perkin-Elmer, Norwalk, CT) with a 25 μL reaction mixture containing about 100 ng DNA, 200 pmol primer, 200 pmol dNTP, 1.5 mM Mg^{2+}, 2 U Platinum Pfx DNA Polymerase, and 1× reaction buffer (Promega, Madison, WI). Thermal cycles were 95°C for 2 min, 35 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 1 min, followed by extension at 72°C for 7 min.

The PCR products were introduced into the mobile phase at an injection volume of 5 μL using the autosampler on a WAVE DNA Fragment Analysis System (Transgenomic, Omaha, NE) using a method described by Gross et al. [21]. Each PCR product was not denatured and kept 50°C. The variations determined by DHPLC were further confirmed by DNA sequencing. PCR product of each variation was cloned in T-vector (Promega) and sequenced on an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA) by using M13 primers.

The polymorphisms of the ECRG2 STR among cases and controls were detected by PCR-based SSCP analysis, as described above.

Statistical analysis

Statistical evaluation was performed using χ^2 tests. Statistical significance was considered $P<0.05$. The association between the ECRG2 TCA polymorphism and the risk of esophageal cancer were measured using odds ratios (ORs) and 95% confidence intervals (CIs), which were calculated using unconditional logistic regression. The ORs were adjusted for age and gender. Compared with the TCA4/TCA4 genotype, the ORs for TCA3/TCA4 and TCA3/TCA3 were computed. All analyses were done using the Statistical Analysis System.

Results

Distribution of age and gender among cases and controls in Kazakhs and Hans

A total of 278 subjects, including 94 Kazakh patients with ESCC, 100 normal controls, 84

Table 1. General demographic characteristics of cases and control

	Kazakh Population			Han Population		
	Case (n=94)	Controls (n=100)	*P**	Case (n=84)	Controls (n=53)	*P**
Gender, n (%)			0.605			0.721
Male	52 (55.3)	59 (59.0)		47 (56.0)	28 (37.7)	
Female	42 (44.7)	41.1		37 (44.0)	25 (62.3)	
Mean age ± SD (years)	55±9.63	50±12.78		61±7.53	47±10.90	
<50	23	47	0.011	4	30	<0.01
50-60	42	32		30	16	
60-70	22	14		41	6	
>70	7	7		9	1	

**P* for χ^2 test for comparison with controls.

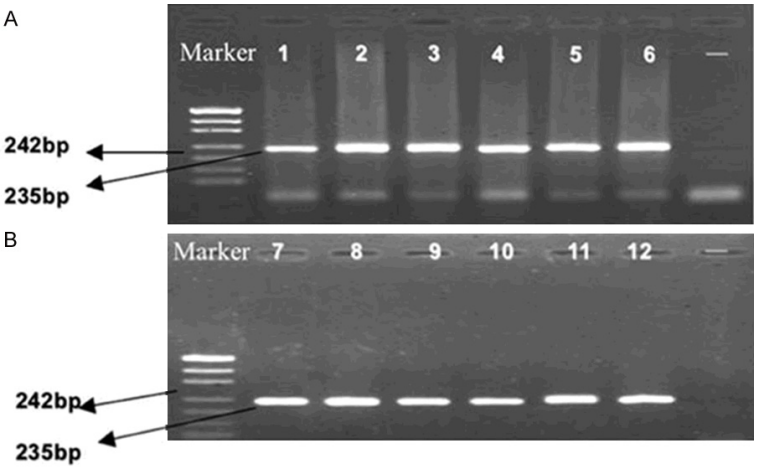

Figure 1. ECRG2 gene PCR products by agarose gel electrophoresis. Numbers 1, 2, and 3 are Han esophageal carcinoma samples. Numbers 4, 5, and 6 are Han blood samples (A). Numbers 7, 8, and 9 are Kazakh esophageal carcinoma samples. Numbers 10, 11, and 12 are Kazakh blood samples (B).

Han patients with ESCC, and 53 normal controls, were analyzed for STR in ECRG2 (**Table 1**). The demographic variables of the subjects are shown in **Table 1**. The distributions of gender among subjects were not statistically different (P>0.05), suggesting that frequency matching was adequate. However, the age distributions of cases and controls were different (P< 0.05) (**Table 1**). The average age of incidence of esophageal cancer in Kazakh patients is five years younger than Han patients (**Table 1**).

PCR amplification and genotyping

In our study, the ECRG2 gene PCR products of agarose gel electrophoresis were 235 bp DNA fragments (**Figure 1**). Three PCR products were obtained by acrylamide gel electrophoresis. The two front bands were 235 bp fragments and represent the genotype of the homozygous TCA3/TCA3. The bands toward the rear were 238 bp fragments that represented the homozygous genotype TCA4/TCA4. The TCA3/TCA4 is a heterozygous genotype that has two kinds of bands (**Figure 2**). This finding proved that ECRG2 has three STR genotypes, TCA3/TCA3, TC3/TCA4, and TCA4/TCA4.

Genotype frequency distribution of ECRG2 TCA STR among ESCC cases and controls

We found three STR genotypes that were identified in exon 4, namely, TCA3/TCA3, TCA3/TCA4, and TCA4/TCA4. To investigate the relationship between the ECRG2 TCA STR genotype and ESCC incidence, we compared the distribution and allele frequency of the ECRG2 TCA STR genotype between Kazakhs, Hans, and a the combined groups (**Table 2**). Among Kazakhs with ESCC, the distribution of ECRG2 STR genotypes (TCA4/TCA4, 56.0%; TCA3/TCA4, 34.0%; and TCA3/TCA3, 10.0%) and controls did not deviate from the Hardy-Weinberg equilibrium (P< 0.01). The frequencies of the three genotypes (30.9%, 46.8%, and 22.3%) among ESCC patients significantly differed from controls (P<0.01), with the TCA3/TCA3 and TCA3/TCA4 genotypes being more prevalent. Compared with the TCA3/TCA4 genotype, subjects that were homozygous for the TCA3/TCA3 genotype

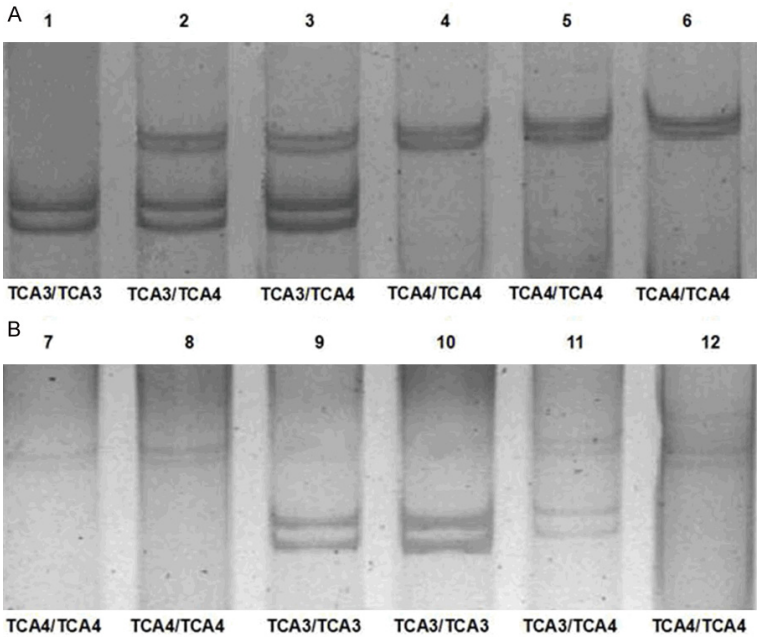

Figure 2. PCR assay for analyzing the ECRG polymorphism in ESCC tissue. Lanes 1, 9, and 10, TCA3/TCA3; lanes 2, 3, and 11, TCA3/TCA4; lanes 4, 5, 6, 7, 8, and 12, TCA4/TCA4.

were at an increased risk of developing ESCC: Kazakhs (adjusted OR, 4.06; 95% CI, 1.69-9.74), Hans (adjusted OR, 3.25; 95% CI, 1.25-8.45), and total samples (adjusted OR, 3.91; 95% CI, 2.06-7.40). By contrast, subjects that were heterozygous for the TCA4/TCA4 genotype did not have a significant association with risk of cancer.

ECRG2 gene sequence diagram

DNA samples were extracted from blood and tumor tissue of ESCC patients and exhibited single bands after PCR amplification. SSCP analysis and DNA sequencing revealed STR in the noncoding region of the exon 4 of ECRG2, and the genotypes of ECRG2 STR were readily discerned. Three size variations were observed: TCA3/TCA3, TCA3/TCA4, and TCA4/TCA4 (**Figure 3**). The genotypes of the ECRG2 STR in DNA samples from blood and tumor were the same for the same individuals.

Relationship between genotype distribution and metastasis of esophageal cancer

The frequencies of the three genotypes (TCA3/TCA3, 58.8%; TCA4/TCA4, 7.8%; and TCA3/TCA4, 33.3%) among ESCC patients with metastasis significantly differed from controls ($P<0.01$). The result suggested that subjects carrying the TCA3/TCA3 genotype are at an increased risk of metastasis compared with those carrying the TCA4/TCA4 genotype (**Table 3**).

Relationship between genotype frequency of ECRG2 TCA STR and differentiation of esophageal cancer

No statistical difference was observed between the frequencies of the three genotypes and differentiation in Kazakhs with esophageal cancer ($\chi^2=7.497$, $P=0.112$), Hans with esophageal cancer ($\chi^2=3.296$, $P=0.510$), and total samples ($\chi^2=1.739$, $P=0.784$) (**Table 4**).

Discussion

ECRG2 is expressed in many tissues, such as fetal liver, lung, brain, heart, stomach, spleen, colon, and kidney [22-26]. The study of Kaifi showed that ECRG2 STR polymorphism TCA3/TCA3 in exon 4 is the most prevalent polymorphism found in pancreatic adenocarcinoma and chronic pancreatitis [27]. However, its biologic significance has not been fully understood in Kazakhs in Xinjiang. Therefore, we studied the relationship between ECRG2 polymorphism and ESCC in Kazakhs and Hans in Xinjiang.

Research by Helena showed that the male-to-female incidence rate ratios in esophageal cancer vary considerably according to histology, age, and race. The highest M: Fratios were seen in 50-59 year old patients [28] However, our research data showed that the distributions of gender between ESCC and control subjects had no statistical difference. Nonetheless, the average age of incidence of esophageal cancer in Kazakhs is five years earlier than in Han patients; therefore, the hypothesis that environmental and genetic factors affect the pathogenesis of esophageal cancer of Kazakhs and Hans in Xinjiang is plausible.

According to Yue's study, exon 4 of ECRG2 has STRs, whose genotypes are TCA3/TCA3, TCA3/

Table 2. Genotype frequency of ECRG2 TCA STR among controls and cases with ESCC

Genotype	Kazak*			Han**			Total***		
	Cases n (%)	Controls n (%)	OR (95%)	Cases n (%)	Controls n (%)	OR (95%)	Cases n (%)	Controls n (%)	OR (95%)
TCA4/TCA4	29 (30.9)	56 (56.0)	1.00	27 (32.1)	26 (49.1)	1.00	56 (31.4)	82 (53.6)	1.00
TCA3/TCA4	44 (46.8)	34 (34.0)	2,50 (1.33-4.71)	30 (35.7)	19 (35.8)	1.52 (0.69-3.34)	74 (41.6)	53 (34.6)	2.05 (1.25-3.34)
TCA3/TCA3	21 (22.3)	10 (10.0)	4.06 (1.69-9.74)	27 (32.1)	8 (15.1)	3.25 (1.25-8.45)	48 (27.0)	18 (11.8)	3.91 (2.06-7.40)

*χ^2=13.589, *P*<0.01; **χ=6.100, *P*=0.047; ***χ^2=20.24, *P*<0.01.

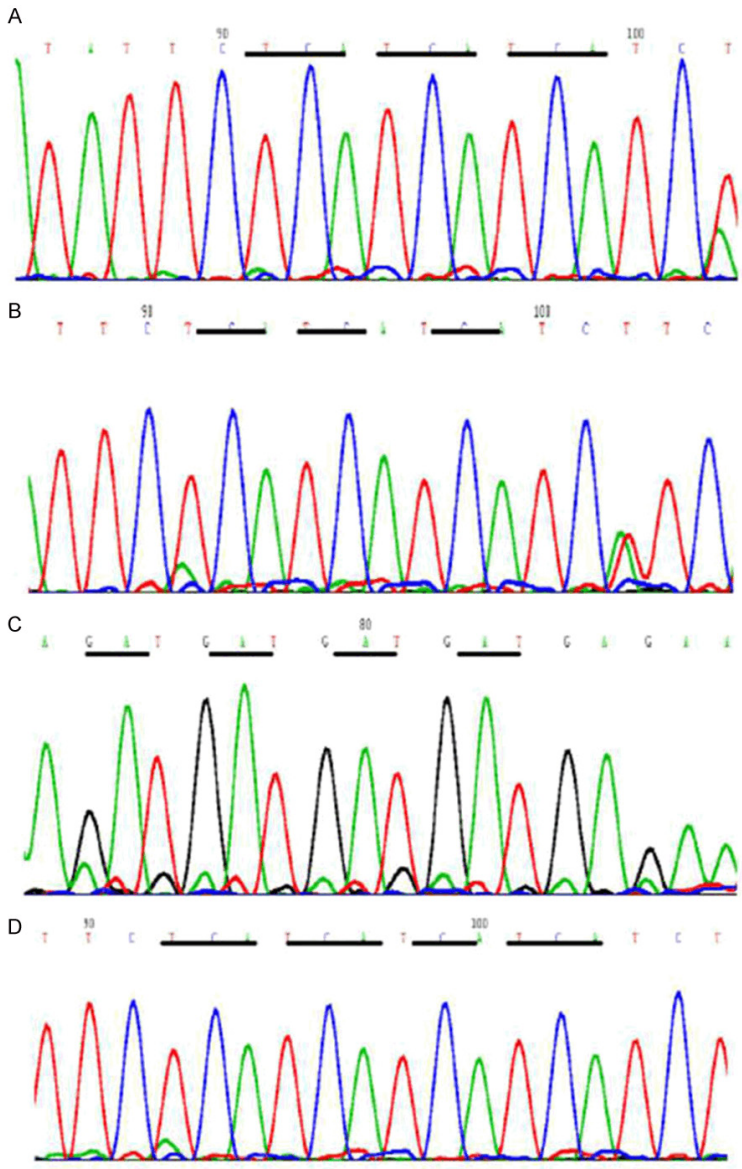

Figure 3. ECRG2 gene PCR products by acrylamide gel electrophoresis.

TCA4, and TCA4/TCA4. The data showed that the risk of carrying TCA3/TCA3 genotype in patients suffering from esophageal cancer was higher than for those with the TCA4/TCA4 genotype [26]. Our research explored the STRs in ECRG2 exon 4 in Han and Kazakh esophageal carcinoma tissues and normal blood samples through PCR-SSCP applications. The frequencies of the three genotypes among ESCC patients significantly differed from controls, with the TCA3/TCA3 and TCA3/TCA4 genotypes being more prevalent. Compared with the TCA3/TCA4 genotype, subjects that were homozygous for the TCA3/TCA3 genotype were at an increased risk of developing ESCC. Our results are consistent with Yue's study and further confirmed that the ECRG2 polymorphism is related to ESCCs [26].

Yue's study showed that the genotypes of the ECRG2 STR in DNA samples from blood, tumor, and normal tissues adjacent to the tumor were identical in the same individuals [26]. Moreover, we extracted DNA samples from blood and tumor tissue of ESCC patients and the genotypes of the ECRG2 STR in the DNA samples were the same for the same individuals. Our result further confirmed that ECRG2 polymorphism is related to ESCC. Huang et al. suggested that ECRG2 inhibits the aggressiveness of cancer cells, possibly through the down-regulation of uPA/plasmin activity [29]. Our study showed that subjects carrying the TCA3/TCA3 genotype are at an increased risk of metastasis compared to those carrying the TCA4/TCA4 genotype. This finding indicated that TCA3/TCA3 is a risk factor for metastasis. We hypothesized that aside from environmental factors, genetic factors may influence the activity of the ECRG2 gene.

The research of Song showed that ECRG2 is a significant inhibitor of cancer growth, as shown in in vivo experiments using intratumoral Ad-ECRG2 administration [30]. No evident toxicity was observed in an animal model during the study. This study concluded that ECRG2 is a potential molecular target in cancer treatment. However, further study needs to investigate ECRG2 gene expression in a larger number of esophageal tissue samples and test the inhibitory effect of the ECRG2 gene on migration and invasion in vitro and in vivo.

In conclusion, our study demonstrated for the first time, a significant association between

Table 3. Relationship between the genotype distribution and metastasis of EC

Genotype	Hazak*		Han**		Total***	
	Metastatic	Non-metastatic	Metastatic	Non-metastatic	Metastatic	Non-metastatic
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
TCA3/TCA3	11 (47.8)	10 (14.1)	19 (67.9)	8 (14.3)	30 (58.8)	18 (14.2)
TCA4/TCA4	2 (8.7)	27 (38.0)	2 (7.1)	25 (44.6)	4 (7.8)	52 (40.9)
TCA3/TCA4	10 (43.5)	34 (47.9)	7 (25.0)	23 (41.1)	17 (33.3)	57 (44.9)
Total	23 (100)	71 (100)	28 (100)	56 (100)	51 (100)	127 (100)

*χ^2=13.589, P<0.01; **χ^2=26.283, P<0.01; ***χ^2=40.74, P<0.01.

Table 4. Relationship between the genotype frequency of ECRG2 TCA STR and the differentiation of esophageal cancer (EC)

Genotype		TCA3/TCA3	TCA4/TCA4	TCA3/TCA4
Kazakh EC*	Well differentiated	4	1	12
	Intermediate differentiation	16	24	28
	Poorly differentiated	1	4	4
Han EC**	Well differentiated	6	9	6
	Intermediate differentiation	13	12	19
	Poorly differentiated	8	6	5
Total*** I	Well differentiated	10	10	18
	Intermediate differentiation	29	36	47
	Poorly differentiated	9	10	9

*χ^2=7.497, P=0.112; **χ^2=3.296, P=0.510; ***χ^2=1.739, P=0.784.

STR genetic polymorphisms and ESCC in Kazakh and Han populations in Xinjiang. Our findings raise the possibility that the influence of the ECRG2 gene polymorphism on the risk of esophageal cancer may be more pronounced in high-risk populations. Further studies from other regions would be helpful to confirm the role of ECRG2 as a high-risk allele in esophageal cancer.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 81360358).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yi Zheng, Department of Gastroenterology, The First Affiliated Hospital, Shihezi University School of Medicine, Shihezi, Xinjiang, China. Tel: +86- 13649993691; E-mail: zhengyi@shzu.edu.cn

References

[1] Cui Y, Bi M, Su T, Liu H and Lu SH. Molecular cloning and characterization of a novel esophageal cancer related gene. Int J Oncol 2010; 37: 1521-1528.

[2] Qin JM, Yang L, Chen B, Wang XM, Li F, Liao PH and He L. Interaction of methylenetetrahydrofolate reductase C677T, cytochrome P4502E1 polymorphism and environment factors in esophageal cancer in Kazakh population. World J Gastroenterol 2008; 14: 6986-6992.

[3] Parkin DM, Pisani P and Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. Int J Cancer 1993; 54: 594-606.

[4] Fan YJ, Song X, Li JL, Li XM, Liu B, Wang R, Fan ZM and Wang LD. Esophageal and gastric cardia cancers on 4238 Chinese patients residing in municipal and rural regions: a histopathological comparison during 24-year period. World J Surg 2008; 32: 1980-1988.

[5] Tang WR, Fang JY, Wu KS, Shi XJ, Luo JY and Lin K. Epidemiological characteristics and prediction of esophageal cancer mortality in China from 1991 to 2012. Asian Pac J Cancer Prev 2014; 15: 6929-6934.

[6] Ganesh B, Talole SD and Dikshit R. Tobacco, alcohol and tea drinking as risk factors for esophageal cancer: a case-control study from Mumbai, India. Cancer Epidemiol 2009; 33: 431-434.

[7] Zheng H, Wang Y, Tang C, Jones L, Ye H, Zhang G, Cao W, Li J, Liu L, Liu Z, Zhang C, Lou F, Liu

Z, Li Y, Shi Z, Zhang J, Zhang D, Sun H, Dong H, Dong Z, Guo B, Yan HE, Lu Q, Huang X and Chen SY. TP53, PIK3CA, FBXW7 and KRAS mutations in esophageal cancer identified by targeted sequencing. Cancer Genomics Proteomics 2016; 13: 231-238.

[8] Pillay V, Isaacson C, Mothobi P, Hale M, Tomar LK, Tyagi C, Altini M, Choonara YE and Kumar P. Carcinogenic nitrosamines in traditional beer as the cause of oesophageal squamous cell carcinoma in black South Africans. S Afr Med J 2015; 105: 656-658.

[9] Wu Y, Chen J, Ohshima H, Pignatelli B, Boreham J, Li J, Campbell TC, Peto R and Bartsch H. Geographic association between urinary excretion of N-nitroso compounds and oesophageal cancer mortality in China. Int J Cancer 1993; 54: 713-719.

[10] Huang XP, Rong TH, Wang JY, Tang YQ, Li BJ, Xu DR, Zhao MQ, Zhang LJ, Fang Y, Su XD and Liang QW. Negative implication of C-MYC as an amplification target in esophageal cancer. Cancer Genet Cytogenet 2006; 165: 20-24.

[11] Takeuchi H, Ozawa S, Ando N, Kitagawa Y, Ueda M and Kitajima M. Cell-cycle regulators and the Ki-67 labeling index can predict the response to chemoradiotherapy and the survival of patients with locally advanced squamous cell carcinoma of the esophagus. Ann Surg Oncol 2003; 10: 792-800.

[12] Chikuba K, Saito T, Uchino S, Sato K, Miyahara M, Tsuda H, Hirohashi S and Kobayashi M. High amplification of the hst-1 gene correlates with haematogenous recurrence after curative resection of oesophageal carcinoma. Br J Surg 1995; 82: 364-367.

[13] Liu P, Zhao HR, Li F, Zhang L, Zhang H, Wang WR, Mao R, Su WP, Zhang Y and Bao YX. Correlations of ALDH2 rs671 and C12 or f30 rs4767364 polymorphisms with increased risk and prognosis of esophageal squamous cell carcinoma in the Kazak and Han populations in Xinjiang province. J Clin Lab Anal 2018; 32:

[14] An JY, Fan ZM, Gao SS, Zhuang ZH, Qin YR, Li JL, He X, Tsao GS and Wang LD. Loss of heterozygosity in multistage carcinogenesis of esophageal carcinoma at high-incidence area in Henan Province, China. World J Gastroenterol 2005; 11: 2055-2060.

[15] Umar M, Upadhyay R, Kumar S, Ghoshal UC and Mittal B. Modification of risk, but not survival of esophageal cancer patients by esophageal cancer-related gene 1 Arg290Gln polymorphism: a case-control study and meta-analysis. J Gastroenterol Hepatol 2013; 28: 1717-1724.

[16] Matsuzaki J, Torigoe T, Hirohashi Y, Tamura Y, Asanuma H, Nakazawa E, Saka E, Yasuda K, Takahashi S and Sato N. Expression of ECRG4 is associated with lower proliferative potential of esophageal cancer cells. Pathol Int 2013; 63: 391-397.

[17] Li MN, Huang G, Guo LP and Lu SH. Inhibitory effects of esophageal cancer related gene 2 on proliferation of human esophageal cancer cell EC9706. Zhonghua Yi Xue Za Zhi 2005; 85: 2785-2788.

[18] Jain M, Kumar S, Ghoshal UC and Mittal B. Association of ECRG2 TCA short tandem repeat polymorphism with the risk of oesophageal cancer in a North Indian population. Clin Exp Med 2008; 8: 73-78.

[19] Lam AK. Molecular biology of esophageal squamous cell carcinoma. Crit Rev Oncol Hematol 2000; 33: 71-90.

[20] Marchbank T, Chinery R, Hanby AM, Poulsom R, Elia G and Playford RJ. Distribution and expression of pancreatic secretory trypsin inhibitor and its possible role in epithelial restitution. Am J Pathol 1996; 148: 715-722.

[21] Gross E, Arnold N, Goette J, Schwarz-Boeger U and Kiechle M. A comparison of BRCA1 mutation analysis by direct sequencing, SSCP and DHPLC. Hum Genet 1999; 105: 72-78.

[22] Cui Y, Wang J, Zhang X, Lang R, Bi M, Guo L and Lu SH. ECRG2, a novel candidate of tumor suppressor gene in the esophageal carcinoma, interacts directly with metallothionein 2A and links to apoptosis. Biochem Biophys Res Commun 2003; 302: 904-915.

[23] Cheng X, Lu SH and Cui Y. ECRG2 regulates ECM degradation and uPAR/FPRL1 pathway contributing cell invasion/migration. Cancer Lett 2010; 290: 87-95.

[24] Su T, Liu H and Lu S. Cloning and identification of cDNA fragments related to human esophageal cancer. Zhonghua Zhong Liu Za Zhi 1998; 20: 254-257.

[25] Huang G, Wang D, Guo L, Zhao N, Li Y and Lu SH. Monoclonal antibodies to esophageal cancer-related gene2 protein. Hybridoma (Larchmt) 2005; 24: 86-91.

[26] Yue CM, Bi MX, Tan W, Deng DJ, Zhang XY, Guo LP, Lin DX and Lu SH. Short tandem repeat polymorphism in a novel esophageal cancer-related gene (ECRG2) implicates susceptibility to esophageal cancer in Chinese population. Int J Cancer 2004; 108: 232-236.

[27] Kaifi JT, Cataldegirmen G, Wachowiak R, Schurr PG, Kleinhans H, Kosti G, Yekebas EF, Mann O, Kutup A, Kalinin V, Strate T and Izbicki JR. Short tandem repeat polymorphisms of exon 4 in Kazal-type gene ECRG2 in pancreatic carcinoma and chronic pancreatitis. Anticancer Res 2007; 27: 69-73.

[28] Nordenstedt H and El-Serag H. The influence of age, sex, and race on the incidence of esophageal cancer in the United States (1992-2006). Scand J Gastroenterol 2011; 46: 597-602.

[29] Huang G, Hu Z, Li M, Cui Y, Li Y, Guo L, Jiang W and Lu SH. ECRG2 inhibits cancer cell migration, invasion and metastasis through the down-regulation of uPA/plasmin activity. Carcinogenesis 2007; 28: 2274-2281.

[30] Song H, Song C, Wang H, Li C, Yang F, Lu SH, Lin C, Zhan Q, Wang X and Qian H. Suppression of hepatocarcinoma model in vitro and in vivo by ECRG2 delivery using adenoviral vector. Cancer Gene Ther 2012; 19: 875-879.