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ORIGINAL ARTICLE

Association of genetic polymorphisms in MDM2, PTEN and P53 with risk of esophageal squamous cell carcinoma

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Genetic variations in *MDM2*, *PTEN* and *P53* might be involved in cancer susceptibility. To assess the contribution of polymorphisms in these three genes to the risk of esophageal squamous cell carcinoma (ESCC) in a Chinese population, we genotyped MDM2 T309G, Del1518, PTEN rs701848, rs2735343 and P53 Arg72Pro polymorphisms using PCR-restriction fragment length polymorphism analysis in 226 ESCC cases and 226 cancer-free controls. Here we showed that the risk of ESCC was elevated in subjects with any of the variant genotypes of PTEN rs2735343 and P53 Arg72Pro polymorphisms, but not any genotype of MDM2 or PTEN rs701848. Moreover, multiplicative interactions were observed between PTEN rs2735343 and P53 Arg72Pro or smoking status on risk of ESCC. Our study firstly indicated that PTEN rs2735343 might be a susceptibility factor for ESCC and reaffirmed the role of P53 Arg72Pro in ESCC in this Chinese population, but did not replicate the positive association between MDM2 T309G and ESCC found previously.
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Keywords: esophageal squamous cell carcinoma; MDM2; P53; polymorphism; PTEN

INTRODUCTION

MDM2 is a crucial negative regulator of P53. Besides directly inhibiting the transcriptional activity of P53, MDM2 also stimulates its nuclear export and degradation as an E3 ubiquitin ligase.[1,2] PTEN tumour-suppressor gene is second only to P53 in mutation frequency in human cancers.[3] It controls cellular growth mainly by inhibiting phosphoinositide 3-kinase activation, and this restricts MDM2 to the cytoplasm and thus protects P53 from degradation.[4]

MDM2-PTEN-P53 tumor suppressor–oncoprotein network regulates cell growth and viability.[5] Genetic variations in these genes might lead to the disturbance of cell cycle and foster cancer development. One polymorphism in the promoter region of MDM2, T309G (rs2279744), has been reported and cells carrying the GG genotype show high-level expression of MDM2 protein and significant attenuation of the P53 pathway.[6] This polymorphism is supposed to be associated with early onset of breast cancer in Li-Fraumeni patients and with risk for gastric carcinoma.[7,8] The Arg72 variant of P53 Arg72Pro (rs1042522) induces apoptosis more effectively than the Pro72 variant.[9]

Esophageal squamous cell carcinoma (ESCC) is the sixth leading cause of cancer death worldwide. This disease shows a considerable geographic variation globally, with an exceptionally high incidence in certain areas of China, South Africa and Iran.[10] Such an uneven distribution of ESCC suggests a dominant role of environmental factors in its etiology,[10] while individual susceptibility to ESCC may be associated with specific genetic variations.[11,12] Till now, the P53 Arg72Pro polymorphism has been well studied in ESCC in Chinese populations.[13–16] But little is known about the association between MDM2 or PTEN polymorphisms and ESCC susceptibility.[16,17]

Here we genotyped MDM2 T309G, Del1518 (rs150550023), PTEN rs701848 and rs2735343 and investigated the associations between these polymorphisms and ESCC risk in a case-control study conducted in a high-risk population of China. Though well illustrated, we also included P53 Arg72Pro in our study to replicate previous findings.

MATERIALS AND METHODS

Study subjects

This study included 226 ESCC patients and 226 healthy population controls. Patients were recruited in 2005 at Anyang Cancer Hospital, Henan Province, China, all confirmed by histopathological diagnosis. Patients that received chemotherapy or radiotherapy before surgery were excluded from this study. Healthy controls were randomly selected from a population screening program for risk factors of ESCC in the same regions and 1:1 matched to cases on the basis of age (± 2 years) and sex. At recruitment, informed consent was obtained from each subject and detailed personal information on demographic characteristics, smoking and drinking status were collected by interview.

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Genotyping

Genomic DNA was isolated from 5 ml peripheral blood using Qiagen DNA Isolation Kit (Qiagen, Dusseldorf, Germany). Genotypes were determined by PCR-restriction fragment length polymorphism assay. Detailed information about primers, restriction enzymes, length of PCR and digest products were summarized in Supplementary Table. Every PCR amplification was performed in a 25-μl reaction mixture containing ~100 ng template DNA, 0.2 mM dNTP, 0.2 mM each primer, 2.5U of Easy Taq DNA polymerase (5 U μl^{-1}) with 10× PCR buffer. Reaction was carried out with an initial melting step of 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at indicated annealing temperature and 30 s at 72 °C, and a final elongation step of 10 min at 72 °C. PCR products were then digested by indicated restriction enzymes at 37 °C for 8 h. Genotypes were identified by separating digested fragments on 3% agarose gel, whereas MDM2 Del1518 was identified by direct separation of PCR products on 2% agarose gel. Genotypes of 10% randomly selected samples obtained by PCR-restriction fragment length polymorphism were further confirmed by direct sequencing of PCR products.

Statistical analysis

The SPSS statistical software package ver.18.0 was used for statistical analysis (IBM, New York, NY, USA). Demographic variables between the study groups were compared by conditional univariate logistic regression. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by conditional multivariate logistic regression as a measure of association with ESCC, adjusted for age, smoking and drinking status. A *P*-value <0.05 was considered significant and all tests were two-sided. Haplotype frequencies were estimated by Phase2.1.[18]

RESULTS

Demographic characteristics of the study subjects

Table 1 depicts the distributions of selected demographic characteristics of the study subjects. Though cases and controls were 1:1 matched on the basis of age (±2 years) and sex, there was still a slight difference in age distribution (P=0.045). Smoking and drinking rates in cases were significantly higher than those in controls (41.6% vs 28.8% and 28.3% vs 17.3%, respectively; $P<0.01$).

Gene polymorphisms and risk of ESCC

Table 2 shows the distributions of the genotypes and ESCC risk. The genotype distribution for each polymorphism was in Hardy–Weinberg equilibrium in controls.

After adjusted for age, smoking, drinking status and other genotypes where appropriate, no association was observed between MDM2 T309G, Del1518 or PTEN rs701848 and ESCC risk. Both variant genotypes (CG and GG) of PTEN rs2735343 were associated with a significantly increased risk of ESCC (OR=2.76 and 4.30, respectively, $P<0.005$). The frequencies of P53 Arg/Pro and Pro/Pro genotypes among cases were moderately different from those among controls (P=0.032 and 0.012, respectively). Compared with the Arg/Arg genotype, both the Arg/Pro and Pro/Pro genotypes inferred an about two-fold increased risk of ESCC (OR=1.74, 95%CI 1.05–2.89 and OR=2.06, 95%CI 1.17–3.62, respectively).

Haplotype and interaction analyses on risk of ESCC

A total of four PTEN haplotypes were constructed using PHASE software, CC, CT, GC and GT. Each individual had a combination of two most possible haplotypes. Table 3 showed that individuals carrying the homozygote (CT/CT) of haplotype CT had a lower risk of ESCC compared with those without haplotype CT (OR=0.21, 95%CI 0.11–0.42, $P<0.001$), whereas the heterozygote (- -/GC) of haplotype GC increased the risk of cancer development (OR=2.44, 95%CI 1.52–3.91, $P<0.001$).

Interaction analyses between genes or gene-smoking on risk of ESCC are shown in Table 4. Given the relative small sample size, detailed stratified analyses were not performed in the present study. The combination of G allele of PTEN rs2735343 and Pro allele of P53 Arg72Pro significantly increased the risk of ESCC (OR=3.21, 95%CI 1.79–5.78, $P<0.001$). Compared with nonsmokers carrying at least one G allele of rs2735343, smokers with at least one G allele had an OR of 2.25(95%CI 1.16–4.34) for ESCC risk.

Table 1 Demographic characteristics of the study subjects

Variables	*Cases,* n *(%)*	*Controls,* n *(%)*	P[a]
Age(mean ± s.d.)	59.73 ± 8.47	59.63 ± 8.46	0.045
Gender			
Male	126 (55.8)	126 (55.8)	—
Female	100 (44.2)	100 (44.2)	
Smoking Status			
No	132 (58.4)	161 (71.2)	0.000
Yes	94 (41.6)	65 (28.8)	
Drinking Status			
No	162 (71.7)	187 (82.7)	0.003
Yes	64 (28.3)	39 (17.3)	

[a]Conditional univariate logistic regression.

Table 2 Genotype frequencies and adjusted ORs for ESCC risk

Genotype	*Cases,* n *(%)*	*Controls,* n *(%)*	P[a]	*OR (95% CI)*[a]
MDM2 T309G				
TT	49 (21.7)	50 (22.1)	—	—
TG	119 (52.6)	118 (52.2)	0.610	1.14 (0.69–1.88)
GG	58 (25.7)	58 (25.7)	0.837	1.06 (0.61–1.86)
G allele	235 (52.0)	234 (51.8)		
MDM2 DEL1518				
–/–	15 (6.6)	16 (7.1)	—	—
+/–	91 (40.3)	92 (40.7)	0.641	1.21 (0.55–2.67)
+/+	120 (53.1)	118 (52.2)	0.747	1.14 (0.52–2.47)
+ allele	331 (73.2)	328 (72.6)		
PTEN rs701848				
TT	70 (31.0)	103 (45.6)	—	—
TC	121 (53.5)	90 (39.8)	0.160	1.47 (0.86–2.51)
CC	35 (15.5)	33 (14.6)	0.250	0.62 (0.27–1.40)
C allele	191 (42.3)	156 (34.5)		
PTEN rs2735343				
CC	38 (16.8)	81 (35.8)	—	—
CG	117 (51.8)	100 (44.2)	0.001	2.76 (1.58–4.82)
GG	71 (31.4)	45 (20.0)	0.000	4.30 (2.27–8.13)
G allele	259 (57.3)	190 (42.0)		
P53 Arg72Pro				
Arg/Arg	40 (17.7)	62 (27.4)	—	—
Arg/Pro	121 (53.5)	110 (48.7)	0.032	1.74 (1.05–2.89)
Pro/Pro	65 (28.8)	54 (23.9)	0.012	2.06 (1.17–3.62)
Pro allele	251 (55.5)	218 (48.2)		

Abbreviations: CI, confidence interval; ESCC, esophageal squamous cell carcinoma; OR, odds ratio.
[a]Adjusted for age, smoking, drinking status and other genotypes where appropriate.

Table 3 Correlation of PTEN haplotypes with ESCC risk

Haplotype combination	*Case,* n *(%)*	*Control,* n *(%)*	P[a]	*OR (95% CI)*[a]
CC				
--/--[b]	217 (96.0)	216 (95.6)		—
--/CC	8 (3.6)	9 (4.0)	0.775	0.86 (0.31–2.42)
CC/CC	1 (0.4)	1 (0.4)	—	—
CT				
--/--	72 (31.9)	47 (20.8)		—
--/CT	123 (54.4)	107 (47.3)	0.081	0.64 (0.39–1.06)
CT/CT	31 (13.7)	72 (31.9)	0.000	0.21 (0.11–0.42)
GC				
--/--	78 (34.5)	113 (50.0)		—
--/GC	116 (51.3)	82 (36.3)	0.000	2.44 (1.52–3.91)
GC/GC	32 (14.2)	31 (13.7)	0.065	1.81 (0.97–3.34)
GT				
--/--	159 (70.4)	184 (81.4)		—
--/GT	57 (25.2)	38 (16.8)	0.054	1.68 (0.99–2.77)
GT/GT	10 (4.4)	4 (1.8)	—	—

Abbreviations: CI, confidence interval; ESCC, esophageal squamous cell carcinoma; OR, odds ratio.
[a]Adjusted for age, smoking and drinking status.
[b]-- Denotes any other haplotype, for example, --/CC is a combination of CC haplotype with any of the other three haplotypes.

Table 4 Interaction analysis between genes or gene-smoking on ESCC risk

	Case, n *(%)*	*Control,* n *(%)*	P[a]	*OR (95% CI)*[a]
rs2735343/Arg72Pro				
CC; Pro/Pro,Arg/Pro	33 (14.6)	62 (27.4)	—	—
CC; Arg/Arg	5 (2.2)	19 (8.4)	0.026	0.21 (0.54–0.83)
CG, GG; Arg/Arg	35 (15.5)	43 (19.0)	0.150	1.67 (0.83–3.34)
CG, GG; Pro/Pro,Arg/Pro	153 (67.7)	102 (45.1)	0.000	3.21 (1.79–5.78)
rs2735343/smoking				
CG, GG; No	107 (47.3)	102 (45.1)	—	—
CC; Yes	13 (5.8)	22 (9.7)	0.284	0.59 (0.23–1.54)
CC; No	25 (11.1)	59 (26.1)	0.000	0.33 (0.18–0.60)
CG, GG; Yes	81 (35.8)	43 (19.1)	0.016	2.25 (1.16–4.34)

Abbreviations: CI, confidence interval; ESCC, esophageal squamous cell carcinoma; OR, odds ratio.
[a]Adjusted for age, smoking or dringking status.

DISCUSSION

In this study, we investigated the associations of genetic polymorphisms in MDM2, PTEN and P53 with ESCC susceptibility in a Chinese population.

Since the identification of MDM2, a variety of case-control studies have been published that investigate the possible association between MDM2 T309G and cancer risk, but the results remain contradictory. A recent report conducted by Hong *et al.*[16] demonstrated that the GG genotype of MDM2 T309G led to a 1.5-fold increased risk of ESCC compared with the TT genotype. The GG genotype frequency was 26.7% and 20.5% in cases and controls, respectively. Here we did not find this association, with a similar GG genotype frequency (25.7%) in cases but much higher in controls (25.7%). We inferred that difference in sample sources between cases and controls might account for this inconsistency. In Hong's study, ESCC patients were enrolled from the Cancer Hospital, Chinese Academy of Medical Sciences, and controls were selected from a community population in Beijing. As far as we know, these patients mostly came from other regions of China. Given that carcinogenesis in diverse genetic backgrounds might be different based on different levels of environmental exposure and there was considerable geographical variation in the incidence of ESCC, this matching mode might lead to poor comparability between cases and controls, and even to a result that varies from the real condition.[19] Conversely, cases and controls in our study were mostly from the same region with a high incidence of ESCC. Thus, all the participants were relatively homogeneous with regard to genetic background and environmental risk factors. Previous studies conducted in various tumour types in China showed that the GG genotype frequency ranged from 18 to 30% in controls,[16,20–24] which indicated that genotype frequency varied in different populations even in the same country. Two of these studies also included MDM2 Del1518 and no association was observed between this polymorphism and cancer risk.[20,21]

In spite of the above discrepancy, we observed a similar association between P53 Arg72Pro and ESCC risk compared with Hong's and other previous studies (OR ~2 for Pro/Pro genotype compared with Arg/Arg genotype, adjusted for age, sex, smoking or drinking status), though sample size, matching mode and study population were quite different from each other.[13–16] We confirm that P53 Arg72Pro polymorphism is a common susceptibility factor for ESCC in Chinese populations.

Because Mitsuo *et al.*[25] reported that nuclear PTEN expression might be a biologic marker of ESCC and Chang *et al.*[26] found that PTEN might have an important role in carcinogenesis and progression of ESCC, two polymorphisms, −9C/G and IVS4 (−/+) (a 5′-ACTAA-3′ deletion/insertion polymorphism), have been genotyped in a Chinese population with a high incidence of ESCC.[17] The authors found that only IVS4+/+ genotype was associated with a decreased risk of ESCC. Here we evaluated the possible roles of the rs701848 and rs2735343 polymorphisms in ESCC susceptibility for the first time. These two polymorphisms have been studied in endometriosis, breast and prostate cancer, colon cancer and hepatocellular carcinoma but no association is observed.[27–30] We found that the variant genotypes of rs2735343 were associated with a significantly increased risk of ESCC. The conflicting results could be attributable to the different ethnicities and tumorigenesis of different cancers.

It should be noted that several limitations existed in our study. First, our small sample size or inadequate adjustment for confounding factors could also cause the inconsistent results. Second, cases and controls recruited in this study were from a high-risk area of ESCC and thus might not be representative of the general Chinese populations.

In conclusion, our data firstly indicate that PTEN rs2735343 might be a susceptibility factor for ESCC and reaffirm the role of P53 Arg72Pro in ESCC risk in this Chinese population, but do not replicate the positive association between MDM2 T309G and ESCC found previously. Further investigations would be warranted to confirm these results in larger and different ethnic populations, and the functional analysis of PTEN rs2735343 should also be addressed.

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