Meta-analysis of *TP73* polymorphism and cervical cancer

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ABSTRACT. The aim of this study was to investigate the tumor protein p73 (*TP73*) G4C14-A4T14 polymorphism and to perform a meta-analysis to assess *TP73* expression in cervical cancer and precancerous tissue. Articles containing data regarding *TP73* status in cervical cancer patients and healthy controls were retrieved from PubMed, EMBASE, Cochrane, Chinese Biomedical Literature, and China National Knowledge Infrastructure databases. Then, the quality of the studies was evaluated according to inclusion and exclusion criteria. Odds ratios between the case and control groups were used as an effect evaluation index and the RevMan 5.0 software was employed to carry out the meta-analysis. Three independent investigations including 8452 cases of cervical cancer and 8326 healthy controls were included in our study following the application of inclusion and exclusion criteria. PCR genotyping revealed that the *TP73* GC/GC genotype produced a 193-bp product, while the AT/AT genotype produced a 270-bp fragment, and GC/AT genotype samples produced two fragments of 193 and 270 bp. Meta-analysis showed that *TP73* expression in cervical cancer was

significantly higher than that in normal cervical squamous epithelium ($P < 0.05$). This elevated expression may play an important role in the occurrence and development of cervical cancer. Therefore, *TP73* testing might be useful in the screening and diagnosis of cervical cancer and precancerous lesions.

Key words: Cervical cancer; *TP73*; Gene polymorphism; Meta-analysis

INTRODUCTION

Cervical cancer is a frequently observed malignant tumor in gynecology. Worldwide, it is the second most common cancer affecting women, after breast cancer. Annually, more than 500,000 people are diagnosed with cervical cancer across the world, and it causes the death of 275,000 patients (Chen and Yang, 2013; Nekulova et al., 2013). Tumor protein p73 (*TP73*, also known as *p73*) was reported as the first member of the cancer-suppressor *TP53* gene family. Kaghad et al. (1997) first described its discovery during an insulin receptor substrate 1 hybridization screen of a COS cell complementary DNA library. Owing to the similarity between its activity and that of TP53 (also known as p53), including roles in growth retardation and apoptosis, it was given the name "p73" (Kaghad et al., 1997). The aim of the present study was to investigate *TP73* expression and its significance in cervical cancer and precancerous tissues by conducting a meta-analysis of the literature.

MATERIAL AND METHODS

Inclusion and exclusion criteria

To be included in the meta-analysis, articles had to meet the following criteria: i) cervical cancer diagnoses must have been confirmed by cytopathology or histopathology, and patients must not have received chemotherapy or biological therapy; ii) cases and controls must not have been restricted by factors such as age and race; and iii) healthy controls must have been enrolled at the same period as cervical cancer patients.

The following were excluded from the analysis: i) studies involving patients with liver, kidney, or heart dysfunction, or abnormal hematology; ii) those including participants with severe infections or related diseases, or incomplete pathological information regarding diagnoses; iii) articles with missing data or duplicate reports. This study was approved by the Ethics Committee of Fudan University, Shanghai, China.

Search strategy

Publications relating to *TP73* status in cervical cancer patients and healthy controls were identified by searching through PubMed, Embase, Cochrane Library, China National Knowledge Infrastructure, and Chinese Biomedical Literature databases between January 2010 and June 2014. The following keywords were used: cervical cancer; gene polymorphism; p73; correlation.

Data extraction

Two investigators independently carried out the database searches. In cases of disagreement or uncertainty as to whether an article conformed to the above criteria, researchers reached a decision by discussion or the adjudication of a third investigator. The information extracted principally included: the main dataset of the study, general statistical information regarding research object, baseline data comparability, specific and practical interventions, final outcomes, and main research findings. Articles consisting of abstracts only and reviews were excluded.

Inclusion assessment

The Cochrane Handbook for Systematic Reviews of Interventions version 5.0 was applied for literature evaluation, particularly in regard to: i) the experimental methods used and whether the data were randomly distributed; ii) whether the blind method was adopted; and iii) the presence of dropout or absence of follow-up, and whether intention-to-treat was applied.

Genotyping

Peripheral venous blood samples (3 mL) were taken and treated with 0.8 mL citric acid anticoagulant. DNA was extracted using a genomic DNA purification kit (Sigma-Aldrich, St. Louis, MO, USA), following the manufacturer protocol. Polymerase chain reaction (PCR) with confronting two-pair primers was employed for *TP73* G4C14-A4T14 polymorphic classification. The two sets of primers used were as follows: F1: 5'-CCA CGG ATG GGT CTG ATC C-3'; R1: 5'-GGC CTC CAA GGG CAG CTT-3'; F2: 5'-CCT TCC TTC CTG CAG AGC G-3'; R2: 5'-TTA GCC CAG CGA AGG TGG-3'.

Statistical analysis

RevMan 5.0 software (RevMan, USA) was used for data analysis. Numerical data are reported as odds ratios with 95% confidence intervals, and P-values below 0.05 were considered statistically significant. The chi-square test was used to assess the heterogeneity of included studies, with $\alpha < 0.1$, while I^2 was applied to quantitatively analyze heterogeneity. Where $P \geq 0.1$ and $I^2 \leq 50\%$, no statistically significant heterogeneity was considered to be presented, and a fixed-effect model was applied.

RESULTS

Genotype and allele frequency distribution in case and control groups

Samples from 180 patients and 180 healthy control subjects were collected. *TP73* G4C14-A4T14 genotype and allele frequencies showed no significant differences between these groups ($P > 0.05$; Table 1).

Table 1. *TP73* G4C14-A4T14 genotype and allele frequency.

Group	Genotype count (%)			Allele count (%)	
	GC/GC	GC/AT	AT/AT	GC	AT
Controls	114 (63.3)	55 (30.6)	11 (6.1)	283 (78.6)	77 (21.4)
Patients	103 (57.2)	67 (37.2)	10 (5.6)	273 (75.8)	87 (24.2)

TP73 PCR genotyping

PCR analysis revealed that GC/GC genotype samples produced a 193-bp fragment, while AT/AT samples produced a 270-bp fragment, and GC/AT samples produced two fragments of 193 and 270 bp (Figure 1).

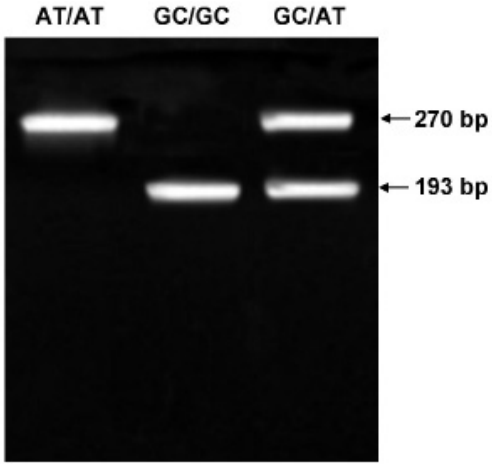

Figure 1. A sequencing image relating to tumor protein p73 gene polymerase chain reaction products.

Basic characteristics and quality evaluation of the included studies

Twelve articles were retrieved and 3 articles were selected after the evaluation of their titles, abstracts, and full texts (Craveiro et al., 2012; Jha et al., 2012; Wang et al., 2012a). *TP73* status in 8452 cervical cancer patients and 8326 healthy controls was included in our study (Table 2).

Table 2. Data regarding the *TP73* G4C14-A4T14 polymorphism meta-analysis.

Author	Country	Ethnicity	Genotype			HWE	Expected power (%, $\alpha = 0.05$)
			GC/GC	GC/AT	AT/AT		
Wang et al. (2012a)	China	Asian	107/128	100/80	11/12	0.913	48.5
Craveiro et al. (2012)	Portugal	European	95/119	38/48	8/9	0.164	37.1
Jha et al. (2012)	India	Asian	71/77	28/19	2/4	0.062	25.5

HWE = Hardy-Weinberg equilibrium.

Meta-analysis

TP73 expression in 8452 cervical cancer patients and 8326 participants with healthy cervixes were used for meta-analysis. The heterogeneity test returned a P value of 0.86, therefore a fixed-effect model was applied. A forest plot of the corresponding research results

was generated. The *TP73* AT/GC genotype distribution showed a statistically significant difference between the case and control groups (Figure 2; $Z = 1.85$, $P < 0.05$), indicating that *TP73* expression is clearly higher in cervical cancer tissues than in corresponding healthy cervical squamous epithelium.

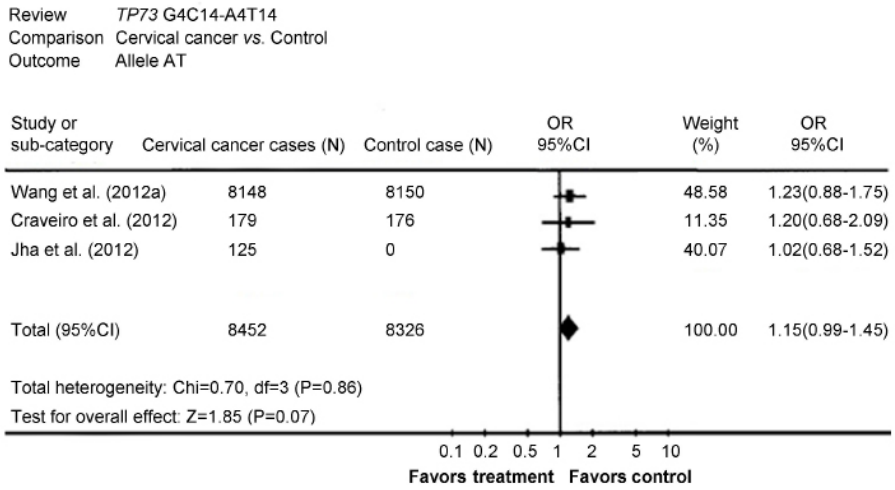

Figure 2. Meta-analysis of the relationship between the tumor protein p73 (*TP73*) G4C14-A4T14 polymorphism and cervical cancer. d.f. = degrees of freedom; OR = odds ratio; CI = confidence interval.

DISCUSSION

Oncogene activation is the genetic basis of tumorigenesis, while tumor suppressor genes are capable of inhibiting cell growth and cancerization. *TP73*, which was the first member of the *TP53* gene family associated with human tumors, receives much attention from numerous researchers as a candidate tumor suppressor gene. It is located in the 1p36.2 to 1p36.3 region, the deletion of which is often associated with human oncogenesis (Decrion-Barthod et al., 2010; Adaramoye et al., 2011; Singh and Singh, 2011; Zhang et al., 2013). Some scholars have found that *TP73* is overexpressed in many malignancies, including skin, breast, colorectal, and ovarian cancers, while its expression is relatively low in corresponding healthy tissue (Finzer et al., 2004; Lee et al., 2006; Das Purkayastha and Roy, 2011; Chen et al., 2013). Using immunohistochemical techniques, several reports have revealed that the TP73α isoform is expressed in non-proliferating normal cervical epithelial cells, and not in proliferating cells (Matsha et al., 2007; Dong et al., 2008; Singh and Singh, 2008; Wakatsuki et al., 2008; Cheung et al., 2010). In addition, TP73α is minimally expressed, or not at all, in cervical cancer tissue.

Among colon cancer patients, it has been demonstrated that the AT/AT genotype is present at a higher frequency (Liu et al., 2006), while GC/AT and AT/AT genotypes have been correlated with the occurrence of lung cancer and head and neck squamous cell carcinomas (Oh et al., 2008). Craveiro et al. (2004) investigated the relationship between the *TP73* 2nd exon polymorphism and cervical cancer, and found that the GC/AT genotype in cervical cancer patients is associated with both early menarche and increased parity. Furthermore, polymorphism in the 72nd codon of this gene has been shown to take part in human papilloma

virus (HPV)-related cervical cancers. Das and Somasundaram (2006) suggested that the HPV E6 protein does not bind to TP73, allowing the latter to play its tumor-suppressive role, and speculated that the relationship between *TP73* polymorphism and HPV infection is low. Other scholars have pointed out that the binding of the HPV E6 protein to *TP73* may occur upon *TP53* mutation, in which case, HPV E6 is able to inhibit TP73 activity (Roperch et al., 2008; Wang et al., 2012b). At present, a firm conclusion regarding the relationship between *TP73* 2nd exon polymorphism and cervical cancer susceptibility remains to be reached, and further research incorporating large sample sizes is needed.

Meta-analysis is a method enabling the systematic evaluation of results of multiple independent studies with the same research objectives. To a certain degree, it is able to provide a comprehensive analysis using multiple investigations of small sizes to effectively increase sample size and inspection efficiency. It not only strengthens statistical inferences by negating the impact of single small studies, but also synthesizes various research conclusions through its improved estimation of effects, making it more accurate than individual research efforts. With this in mind, in the present study we found that the *TP73* AT/GC allelic distribution was significantly different between the case and control groups (Figure 2; $Z = 1.85$, $P < 0.05$) under a fixed-effect model, indicating that the AT allele may elevate cervical cancer risk.

In conclusion, TP73 status may play an important role in the occurrence and development of cervical cancer. Therefore, *TP73* testing might be useful in the screening and diagnosis of cervical cancer and precancerous lesions. At present, research into the correlation between *TP73* polymorphisms and cervical cancer incidence is lacking, particularly large, multicenter, case-control studies. Using a meta-analysis of the relevant literature, our investigation found that the *TP73* AT allele may be associated with cervical cancer tumorigenesis in the Asian population. As the combined sample size was not large, and to achieve a more representative sample of the Asian population, further larger studies are needed to confirm this correlation.

Conflicts of interest

The authors declare no conflict of interest.

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