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ORIGINAL ARTICLE

Prevalence of human papillomavirus infection in esophageal and cervical cancers in the high incidence area for the two diseases from 2007 to 2009 in Linzhou of Henan Province, Northern China

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Abstract The etiological role of human papillomavirus (HPV) in cervical cancer has been well established. However, it is inconclusive whether HPV plays the same role in esophageal carcinogenesis. In this study, we detected HPV infection in 145 frozen esophageal tissues, including 30 normal epithelium (ENOR), 37 dysplasia (DYS) and 78 invasive squamous cell carcinoma (ESCC), and in 143 frozen cervical tissues composed of 30 normal epithelium (CNOR), 38 intraepithelial neoplasia (CIN) and 75 invasive squamous cell carcinoma (CSCC). The patients and symptom-free subjects enrolled in this study were from a high-incidence area for both ESCC and CSCC, Linzhou City, Northern China, from 2007 to 2009. The HPV infection analysis was conducted by using an HPV GenoArray Test Kit. We found that the high-risk HPV types accounted for more than 90 % of the HPV-positive lesions of esophagus and cervix tissues. The prevalence of high-risk HPV types increased significantly during the progression of both esophageal and cervical carcinogenesis (positive rate in esophageal tissues: 33 % ENOR, 70 % in DYS and 69 % in ESCC; positive rate in cervical tissues: 27 % in CNOR, 82 % in CIN and 88 % in CSCC; $P < 0.001$, respectively). Infection with the high-risk HPV types increased the risk for both DYS and ESCC by 4-fold (DYS vs. ENOR: OR = 4.73, 95 %CI = 1.68-13.32; ESCC vs. ENOR: OR = 4.50, 95 %CI = 1.83-11.05) and increased the risk for both CIN and CSCC by 12-fold and 20-fold (CIN vs. CNOR: OR = 12.18, 95 %CI = 3.85-38.55; CSCC vs. CNOR: OR = 20.17, 95 %CI = 6.93-58.65), respectively. The prevalence of high-risk types in ESCC patients was lower than that in CSCC patients ($P = 0.005$) and was significantly associated with the degree of ESCC tumor infiltration ($P = 0.001$). HPV 16 was the most prevalent subtype in both esophageal and cervical tissues. Single HPV infection increased significantly along with the progression of ESCC and maintained

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a high level in cervical tissues, regardless of whether they were CNOR or CSCC tissues. Our results showed that infection with HPV, especially the high-risk types, was positively associated with both esophageal and cervical cancers, suggesting that HPV also plays a role in the etiology of ESCC in the high-incidence area.

Abbreviations

BCH	Basal cell hyperplasia
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
CNOR	Cervical normal
CSCC	Cervical squamous cell carcinoma
DYS	Dysplasia
ENOR	Esophageal normal
ESCC	Esophageal squamous cell carcinoma
HPV	Human papillomavirus
PCR	Polymerase chain reaction
SCC	Squamous cell carcinoma

Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most common cancers in China with the incidence ranked fifth and the mortality ranked sixth in all cancer sites [1, 2]. Linzhou City in Henan Province, Northern China, is well known in the world for its striking high incidence of ESCC, where it exceeds 100 per 100,000 individuals [3, 4], which is much higher than the average incidence in China (22/100,000) [2]. In contrast, the incidence of ESCC is less than 20/100,000 in Puyang City in Henan Province, which is less than 200 kilometers from Linzhou City [4]. Such remarkable geographic variation in the incidence of ESCC suggests that environmental factors have significant influence on esophageal carcinogenesis. Tobacco smoking, drinking of alcohol, low vegetable intake, and low fruit intake have been estimated believed to be responsible for 46 % of esophageal cancer mortality and incidence in China [5].

An association between HPV infection and gastrointestinal (GI) cancers including ESCC [6–17], gastric cancer [18, 19] and colorectal cancer [20, 21] has been reported in the past decades. However, its role in the development of GI cancers except anal cancer [6] has been debated for years. Esophageal carcinogenesis has been recognized as complex, multistage processes in which various factors participate. In the past decades, although numerous studies have indicated that HPV infection is associated with esophageal carcinogenesis [8–18], the rates of HPV infection varied from 0 % to 67 % for ESCC [6–17] and from 15.7 % to 66.7 % for esophageal precancerous lesions [11–14], even in the same areas with high incidence of ESCC. One possible reason for the discrepancies is that different methods were used to detect HPV infection in the previous studies, including light microscopy, electron microscopy, immunochemistry, polymerase chain reaction (PCR), *in situ* hybridization, Southern blot, and hybrid capture II [15]. Another reason is possibly that samples used in these studies were collected or stored in different ways, with biopsy [11–13] or surgical [14, 16] specimens stored frozen or formalin-fixed and embedded in paraffin.

Interestingly, the role of HPV in the pathogenesis of cervical cancer has been well established [6]. The incidence rate of cervical cancer is still extremely high in parts of rural China [22, 23], such as Linzhou City in Henan Province, Yangcheng City in Shanxi Province, and Shihezi City in Xinjiang Province. It is of interest to note that these areas also have high incidence of ESCC. Furthermore, the main histological type of cervical cancer is squamous cell carcinoma (SCC), which is also the main histological type of esophageal cancer in China. Moreover, the predilection site of both ESCC and cervical SCC (CSCC) is the junction of the squamous and columnar epithelium. In addition, both ESCC and CSCC have similar multi-stage histopathological progression patterns: for ESCC, esophageal normal squamous cell epithelia (ENOR) progresses to basal cell hyperplasia (BCH), to dysplasia (DYS), to carcinoma in situ (CIS), and eventually to invasive carcinoma; and for CSCC, cervical normal squamous cell epithelia (CNOR) progresses to BCH, to cervical intraepithelial neoplasia (CIN), to CIS, and finally to invasive carcinoma. The similarities in geographic distribution of the cancer incidence and histopathology suggest that environmental risk factors might play an etiologic role in both esophageal and cervical carcinogenesis. It is now well understood that persistent cervical infection with high-risk HPV types is a critical cause of the development of cervical cancer and its immediate precursor lesions [24].

A systemic analysis of HPV infection in a large number of samples using sensitive DNA detection techniques should be helpful in elucidating the role of HPV in esophageal carcinogenesis. In the current study, we examined the prevalence of 21 HPV types, including fifteen high-risk types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68) and six low-risk types (HPV6, 11, 42, 43, 44 and CP8304) by using an HPV GenoArray Test Kit. The infection rate was compared among esophageal and cervical tissues with different degrees of precancerous and cancerous lesions.

Materials and methods

Study subjects

Normal subjects and patients with esophageal precancerous lesions

Normal subjects and patients with esophageal precancerous lesions were recruited from mass surveys for screening of high-risk individuals and early detection for ESCC in the high-incidence area for ESCC in Linzhou City, which was carried out from 2009 to 2011 by Henan Key Laboratory for Esophageal Cancer Research, The First Affiliated Hospital of Zhengzhou University. Based on cellular morphological changes and tissue architecture, esophageal biopsy tissues were classified as normal, BCH, DYS and CIS [25]. A total of 67 symptom-free subjects were finally enrolled in this study, including 30 individuals with normal esophageal epithelia (16 males and 14 females with a mean age of 56 ± 11 and 60 ± 10 years, respectively) and 37 patients with DYS (20 males and 17 females with a mean age of 49 ± 12 and 51 ± 10 years, respectively).

Patients with esophageal squamous cell carcinoma

Seventy-eight patients with ESCC (40 males and 38 females with a mean age of 59 ± 9 and 56 ± 9 years, respectively) were recruited from Linzhou Esophageal Cancer Hospital from 2009 to 2011. All of the patients had received surgical treatment, and no chemotherapy or radiotherapy was performed before the surgery. All of the patients were confirmed to have ESCC by histopathology.

Normal subjects and patients with cervical precancerous lesions

Normal subjects and patients with cervical precancerous lesions were recruited by physical examination of patients who agreed to undergo colposcopy examination at The First Affiliated Hospital of Zhengzhou University from 2009 to 2011. The patients enrolled in this study included 68 symptom-free women from Linzhou City who underwent cervical biopsy or loop electrosurgical excision procedure cone biopsy. The cervical biopsy specimens were analyzed by the pathology department of the hospital. There were 30 individuals with normal cervical epithelia (mean age: 45 ± 10 years) and 38 patients with cervical intraepithelial neoplasia (CIN; mean age: 46 ± 10 years).

Patients with cervical squamous cell carcinoma

Seventy-five patients with CSCC (mean age: 47 ± 11 years) who were also from Linzhou City received surgical treatment at The First Affiliated Hospital of Zhengzhou University. No chemotherapy or radiotherapy was performed before the surgery. All of the patients were confirmed at the pathology department of the hospital to have CSCC.

Collection of tissue samples

Both biopsy materials and surgical specimens were collected and processed using a standardized protocol designed to minimize the possibility of tissue contamination by environmental HPV [8].

Biopsy procedures

Tissues from esophagus and cervix of symptom-free subjects were collected by biopsy under endoscopy. A questionnaire was completed for each participator in one-on-one interviews. Esophageal biopsies were taken from all visible lesions and the standard sites in the mid-esophagus and cardia. Cervical biopsies were taken from all visible lesions and the regular biopsy at the 3, 6, 9, 12 points of the junction of squamous and columnar epithelium. Generally, at least two biopsy samples of each point were collected. One piece was fixed in 80 % ethanol and embedded in paraffin for pathological diagnosis, and the other one was stored at −80 °C for HPV detection.

Surgical sample collection procedures

Tumor tissues including ESCC and CSCC were collected after surgery; half of the surgically resected sample was fixed with formalin and embedded in paraffin, and the other half was stored at −80 °C. Demographic data, personal information, and family histories of ESCC and CSCC patients were obtained from patient medical records.

This study was reviewed and approved by the Institute Research Ethics Committee of the Zhengzhou University and Linzhou Esophageal Cancer Hospital. Informed consent was obtained from all participants before their tissue samples were used.

Histopathological examination

Histological assessment was made by at least two pathologists who were unaware of the HPV DNA status. The esophageal biopsy tissues from esophagus were diagnosed using criteria described previously [25]. The criteria used for histopathological examinations and TNM staging of esophageal cancer specimens were based on the UICC criteria of 2002 [26]. The cervical intraepithelial neoplasia terminology was adopted for classification of cervix biopsy tissues. The criteria used for histopathological

examinations and TNM staging of cervical cancer specimens were based on the FIGO criteria of 2009 [27].

HPV detection and genotyping

DNA extraction

Approximately 50-100 mg of frozen tissue from each sample was used for DNA extraction using a Puregene Tissue Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. The extracted DNA was stored at −80 °C until use.

HPV DNA detection and genotyping

β-actin (forward primer, 5′-GAA GAG CCA AGG ACA GGT AC-3′; reverse primer 5′-CAA CTT CAT CCA CGT TCA CC-3′) was amplified as an internal control to confirm the quality of the DNA by polymerase chain reaction (PCR). The amplification produced a 292-bp product. Only β-actin-positive samples were used for HPV testing in the subsequent study.

The β-actin-positive samples were used for HPV testing using a HPV GenoArray Test Kit (HybriBio Ltd., Beijing, China). This assay could accurately detect 21 HPV types simultaneously, including 15 high-risk types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68) and six low-risk types (HPV6, 11, 42, 43, 44 and CP8304). A positive control for HPV 18 was used as quality control in the kit. A total 20 μl of PCR reaction mix was used for flowthrough hybridization analysis according to the manufacturer's protocol. The final results were determined based on colorimetric change. Positivity was designated as visualization of one or more blue spots at the position of each HPV type probe on the membrane.

Polymerase chain reaction (PCR) amplification targeting the HPV E6/E7 gene

For all of the samples of esophageal and cervical cancers that were positive for HPV16 subtypes when tested using the HPV GenoArray kit, the presence of HPV DNA was confirmed by PCR using type-specific primers for the HPV *E6/ E7* gene region of HPV16.

HPV16 *E6/E7* primers were designed using OLIGO 5.0 (National Bioscienses, USA) according to the full-length sequence of HPV16 genomes in the GenBank database. The sequences of the primers were as follows: forward, 5′-GTA ACC GAA ATC GGT TGA ACC C-3′; reverse, 5′-CAT AAA ACC ATC CAT TAC ATC CCG-3′. All PCR reactions were performed in a total volume of 20 μl as described previously [16]. The amplification produced a 120-bp product. PCR products were separated on a 1.5 % agarose gel. The gel was then stained with ethidium bromide and visualized under UV illumination.

Statistical analysis

All statistical analyses were carried out using the SPSS 17.0 software package, using the χ^2 test or Fisher's exact probability test to compare categorical variables. The Spearman correlation test was used to examine the correlation between HPV-positive rates and the progression of carcinogenesis. The association of HPV infection and disease progression was assessed using unconditional logistic regression and expressed as odds ratios (OR) with 95 % confidence intervals (CI). All tests were two-tailed. $P < 0.05$ was considered statistically significant.

Results

A total of 288 samples were successfully genotyped using an HPV GenoArray Test Kit. All of the samples of esophageal (n = 56) and cervical (n = 62) cancers that were positive for HPV16 subtypes by using the HPV GenoArray kit were confirmed by subsequent type-specific PCR analysis (data not shown).

Genotypes detected in esophageal tissues

The prevalence of high-risk types was significantly higher in both ESCC (69 %, 54/78) and DYS (70 %, 26/37) tissues when compared with ENOR tissues (33 %, 10/30) ($P < 0.001$) (Table 1). Infection with high-risk types increased the risk of DYS and ESCC by 4.5-fold and 4.7-fold, respectively (ESCC vs. ENOR: OR = 4.50, 95 %CI = 1.83-11.05; DYS vs. ENOR: OR = 4.73, 95 %CI = 1.68-13.32). A correlation was found between infection with high-risk types and the progression of ESCC ($r = 0.29$, $P < 0.001$).

Although multiple-HPV-type infection was frequently identified in HPV-positive esophageal normal tissues (8/11, 73 %), single-HPV-type infection was more frequent in DYS (22/28; 79 %) and ESCC tissues (41/60, 68 %) than in ENOR tissues (1/10; 10 %) ($P = 0.008$).

Distribution of HPV genotypes in esophageal tissues

The distribution of the HPV genotypes in the 99 cases of HPV-positive samples (ENOR: 11; DYS: 28; ESCC: 60) is summarized in Table 2. The high-risk HPV types accounted for almost 90 % of the HPV-positive esophageal lesions. HPV types 16, 18, 58, 31, and 52 were the most common genotypes infecting esophageal tissues. It is noteworthy that HPV16 was the most prevalent type and

Table 1 Prevalence of HPV infection in cervical and esophageal tissues▲

Diagnosis	N	High-risk					Low-risk			Single		Multiple	
		n	(%)	*P*	OR	(95 %CI)	n	(%)	*P*	n	(%)	n	(%)
Esophageal													
ENOR	30	10	(33)				1	(3)		3	(27)	8	(73)
DYS	37	26	(70)	**0.003**	4.73	(1.68-13.32)	2	(5)	0.69	22	(79)	6	(21)
ESCC	78	54	(69)	**0.001**	4.50	(1.83-11.05)	6	(8)	0.42	41	(68)	19	(32)
Cervical													
CNOR	30	8	(27)				2	(7)		9	(90)	1	(10)
CIN	38	31	(82)	**<0.001**	12.18	(3.85-38.55)	3	(8)	0.95	22	(65)	12	(35)
CSCC	75	66	(88)	**<0.001**	20.17	(6.93-58.65)	4	(5)	0.89	65	(93)	5	(7)

P values were presented in bold if less than 0.05

ENOR, esophageal normal; DYS, esophageal dysplasia; ESCC, esophageal squamous cell carcinoma

CNOR, cervical normal; CIN, cervical intraepithelial neoplasia; CSCC: cervical squamous cell carcinoma

▲ *P*-values relative to NOR

Table 2 HPV type distribution in HPV-positive patients

Diagnosis	N	High-risk												Low-risk			
		16		18		58		31		52		33		11		6	
		n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Esophageal																	
ENOR	11	11	(100)	8	(73)	8	(73)	2	(18)	0	(0)	0	(0)	2	(18)	0	(0)
DYS	28	8	(29)	6	(21)	5	(18)	3	(11)	2	(7)	0	(0)	0	(0)	2	(7)
ESCC	60	54	(90)	12	(20)	6	(10)	4	(7)	0	(0)	0	(0)	4	(7)	5	(8)
Cervical																	
CNOR	10	8	(80)	6	(60)	6	(60)	2	(20)	4	(40)	4	(40)	2	(20)	4	(40)
CIN	34	22	(65)	5	(15)	8	(24)	4	(12)	3	(9)	0	(0)	0	(0)	3	(9)
CSCC	70	62	(89)	7	(10)	11	(16)	7	(10)	5	(7)	0	(0)	0	(0)	5	(7)

ENOR, esophageal normal; DYS, esophageal dysplasia; ESCC, esophageal squamous cell carcinoma

CNOR, cervical normal; CIN, cervical intraepithelial neoplasia; CSCC: cervical squamous cell carcinoma

was detected in all of the positive esophageal normal tissues and 90 % (54/60) of the ESCC tissues. HPV 18 and 58 were the other two most common high-risk types in esophageal tissues. HPV11 and 6 were the most common low-risk types.

Genotypes detected in cervical tissues

The detection rate of high-risk types was 28 % (8/30) in normal cervical tissues, and it increased strikingly to 82 % (31/38) in CIN and 88 % (66/75) in CSCC tissues ($P < 0.001$) (Table 1). Infection with high-risk HPV types significantly increased the risk of CIN by 12-fold (OR = 12.18, 95 %CI = 3.85-38.55) and 20-fold for CSCC (OR = 20.17, 95 %CI = 6.93-58.65). There was a good correlation between infection with high-risk HPV types and the progression of CSCC ($r = 0.51$, $P < 0.001$).

Single-HPV-type infection was much more prevalent in cervical lesions when compared with multiple-HPV-type infection ($P = 0.001$). More than 90 % of HPV-infection-positive cervical normal and CSCC tissues showed single-HPV-type infection (90 % [9/10] in CNOR; 93 % [65/70] in CSCC), which was much higher than that in CIN tissues (65 %; 22/34).

The distribution of HPV genotypes in cervical tissues

The distribution of HPV genotypes in the 114 HPV-positive samples (CNOR: 10; CIN: 34; CSCC: 70) is summarized in Table 2. The high-risk HPV types accounted for almost 92 % of the HPV-positive cervical lesions. HPV

Table 3 Association between infection with high-risk HPV types and clinical pathological characteristics of ESCC and CSCC patients[#]

Characteristic	N	Positive		Negative		χ^2	*P*
		n	(%)	n	(%)		
ESCC patients							
Gender							
Male	49	34	(69)	15	(31)	0.002	0.969
Female	29	20	(69)	9	(31)		
Age (years)							
<60	39	27	(69)	12	(31)	0.000	1.000
≥60	39	27	(69)	12	(31)		
Infiltration degree							
T1 + T2	16	6	(38)	10	(62)	14.206	**0.001**
T3	27	17	(63)	10	(37)		
T4	35	31	(89)	4	(11)		
Lymph node metastasis							
Positive	30	23	(77)	7	(23)	1.265	0.261
Negative	48	31	(65)	17	(35)		
Differentiation	0						
High	10	6	(60)	4	(40)	0.564	0.754
Middle	56	40	(71)	16	(29)		
Low	12	8	(67)	4	(33)		
Tumor stage							
I	48	31	(65)	17	(35)	1.265	0.261
II	30	23	(77)	7	(23)		
CSCC patients[※]							
Age (years)							
<45	27	23	(85)	4	(15)	0.491	0.484
≥45	27	21	(78)	6	(22)		
Differentiation							
High	5	4	(25)	1	(75)	0.245	0.885
Middle	25	22	(84)	3	(16)		
Low	24	21	(59)	3	(41)		
Lymph node metastasis							
Positive	10	9	(64)	1	(36)	0.095	0.757
Negative	44	38	(62)	6	(38)		
Clinical stage							
I	34	30	(67)	4	(33)	0.117	0.733
II	20	17	(56)	3	(44)		

P values were presented in bold if less than 0.05

[#] The high-risk HPV types include 16, 18, 58, 31 and 52. HPV 16 was the most prevalent subtype

[※] The clinical pathological information for 21 patients was missing and was not included in statistical analysis

types 16, 18, 58, 31, 52, and 33 were the most common genotypes found in cervical tissues. Similar to esophageal tissues, HPV16 was the most prevalent type, which was detected in more than 80 % of the HPV-positive cervical normal and CSCC tissues (80 %; [8/10] in CNOR; 89 % [62/70] in CSCC). HPV 18 and 58 were the other two most common high-risk types in cervical tissues, likewise in esophageal tissues. HPV11 and 6 were the most common low-risk types.

Relationship between HPV infection and clinical-pathological characteristics of ESCC and CSCC patients

The prevalence of high-risk HPV types in CSCC patients (88 %) was higher than that in ESCC patients (69 %) ($P = 0.005$) (Table 1). However, there was no significant difference in normal epithelium or precancerous lesion tissues between esophagus and cervix (ENOR vs. CNOR = 33 % vs. 27 %, $P = 0.787$; DYS vs. CIN = 70 % vs. 82 %, $P = 0.115$).

We then analyzed if there was a relationship between HPV infection and clinical-pathological characteristics of both ESCC and CSCC patients. For ESCC patients, the detection rate of high-risk HPV types increased significantly with the degree of tumor infiltration (T1&T2: 42 %; T3: 67 %; T4: 93 %) ($P = 0.001$) (Table 3). However, no significant association was found between infection with high-risk HPV types and gender, age, lymph node metastasis, differentiation, or clinical stage in ESCC patients.

There was no significant difference between infection with high-risk HPV types and the clinical-pathological characteristics we analyzed, including gender, age, infiltration degree, lymph node metastasis, differentiation, or clinical stage in CSCC patients.

Discussion

In the present study, we determined the prevalence of HPV infection in frozen epithelium tissues from esophagus and cervix with lesions of different degrees. A strength of the current study is that the normal epithelium and precancerous lesion tissues were collected from symptom-free subjects who underwent gastroscopy or colposcopy for examination. All of these subjects were biopsied using an endoscope and histologically diagnosed as esophageal normal epithelia (n = 30), esophageal dysplasia (n = 37), cervical normal epithelia (n = 30), or cervical intraepithelial neoplasia (n = 38). All of the subjects enrolled in this study, including symptom-free subjects and patients with ESCC or CSCC, came from Linzhou City, a high-incidence area for both ESCC and CSCC.

It is of particular interest that our results indicated that infection with HPV was positively associated with both esophageal and cervical carcinogenesis. The rate of infection with high-risk HPV types was relatively low in normal tissues from esophagus (33 %) and cervix (27 %) but was strikingly higher in precancerous lesions (70 % in

esophageal dysplasia, 82 % in CIN) and continued to be observed at a high rate during the progression from precancerous lesions to carcinoma in both esophagus and cervix (69 % in ESCC; 88 % in CSCC). With the infection of high-risk HPV types, the risk for both DYS and ESCC increased by 4-fold, while the risk for both CIN and CSCC increased by 12-fold and 20-fold, respectively. The high-risk HPV types accounted for more than 90 % of the HPV-positive lesions from esophagus and cervix.

The detection rate of HPV infection in esophageal tissues with lesions of different degrees in this study was apparently much higher than that in previous reports [6, 7, 14]. The association between HPV infection and the progression of ESCC was evaluated in cytologic and endoscopic tissues from symptom-free subjects from Linzhou City who underwent endoscopy, using Hybrid Capture II (HC2) [7]. HPV positivity was identified in 13 % of subjects without squamous dysplasia, 8 % (8/102) with mild dysplasia, 7 % (6/83) with moderate dysplasia, 16 % (6/38) with severe dysplasia, and none of the subjects (0/4) with invasive cancer [6, 7]. A total of 1876 formalin-fixed, paraffin-embedded specimens from 700 patients with ESCC from Anyang City were analyzed for HPV sequences by screening ISH [6, 14]. Approximately 17 % of the ESCC samples were positive for HPV, but it was much rarer in the surrounding dysplastic epithelia (5.6 %) and only infrequently present in the resection margins (0.2 %) [14]. A recent meta-analysis study [28] examined paraffin-embedded ESCC specimens in which HPV infection was detected by the PCR approach and found that the average HPV prevalence was 46.9 % (95 % CI: 43.8 %-50.0 %), vary from 8.3 % to 69.8 % in different locations in China. Although Gao et al. [7] used a large number of samples from asymptomatic subjects in their study, only four of them were diagnosed as invasive ESCC samples, which limited their ability to explore the role of HPV infection in ESCC progression. In addition, both cytologic and endoscopic samples with different degree of esophageal lesions were used to determine the presence of HPV infection. The limited number of cytologic samples might limit the sensitivity of HPV detection. Chang et al. [14] analyzed HPV infection in primary tumor, adjacent esophageal mucosa, and surgically resected margin tissues from ESCC patients who underwent esophagectomy for invasive ESCC. However, it is questionable whether the tissues from ESCC patients that were diagnosed as "normal" or "dysplasia" could be considered truly "normal" or "dysplasia", because the worst result is always used for pathological diagnosis in the clinic. Furthermore, Gao et al. [7], Chang et al. [14] and the meta-analysis [28] used 80 % ethanol/formalin-fixed, paraffin-embedded tissues for HPV detection, whereas our study used frozen tissues. The different methods for storing tissues might lead to inconsistencies in HPV detection. Moreover, we applied an HPV GenoArray Test Kit in this study, which can detect 21 HPV types simultaneously. To date, there have been no reports in which this method was used in cancer research except for cervical cancer [29], where high-risk HPV types were found in 83 % (40/48) of Jewish Israeli patients with invasive cervical cancer. This is similar to our results, which showed that 88 % (66/75) of the CSCC patients were infected with high-risk HPV types.

The prevalence of multiple HPV infections was 73 % in HPV-positive esophageal normal tissues. However, the rate of single HPV infection increased along with the progression of ESCC (79 % in DYS; 68 % in ESCC), which was similar to the results of ESCC patients from Anyang City (66 %) [17]. This suggests that HPV subtypes might commonly be present in esophageal mucosa, even before morphological changes can be detected. However, the persistence of single-HPV-type infection, especially the high-risk types, might play an etiological role in esophageal carcinogenesis. When compared to esophageal tissues, single-HPV-type infection was maintained in a high rate in cervical tissues from normal epithelium from CIN to CSCC. The prevalence of multiple HPV infections in CSCC tissues was 7 %, which was within the reported range of 1.5 % to 39.6 % worldwide [30].

HPV 16 was the most frequent single-type infection in both esophageal and cervical tissues, accounting for 52 % of the 145 esophageal specimens and 64 % of the 143 cervical tissues we analyzed. HPV 16 was present in 69 % (54/78) of ESCC tissues, which was consistent with the previous studies in Linzhou City [12]. HPV16 infection accounted for 83 % of cervical cancer, which is similar to what has been observed in Israel (81 %), North America (79 %) and Europe (78 %) [29, 31]. The consistency between our results and previous studies indicates that the HPV GenoArray Kit we used in this study has high sensitivity and good agreement in HPV detection.

Although there was no difference in HPV infection rates in normal epithelium or precancerous lesions between esophagus and cervix, they were higher in CSCC than in ESCC. This is consistent with what has been observed for esophageal and cervical cancer patients [32] from Chaoshan District in Guangdong Province, China, which is also a high-incidence area for ESCC. Our results suggest that HPV infection might have the same effect in the transition from normal epithelia to precancerous lesions in both esophagus and cervix. When the precancerous lesions transit to invasive carcinoma, HPV infection, especially with the high-risks types, could play a much more important role in the cervix than in the esophagus.

In addition to HPV, infection with other oncogenic viruses such as herpes simplex virus 1 (HSV-1), Epstein-Barr virus (EBV), and cytomegalovirus (CMV) has also been suggested to be associated with cancer worldwide

[33]. Furthermore, HSV-1, EBV and CMV infection [34, 35] have been suggested to play a role in esophageal carcinogenesis. However, in contrast to HPV, the presence of CMV, HSV and EBV analyzed in patients with ESCC from Linzhou City in Henan Province has indicated that CMV, HSV and EBV are highly unlikely to be involved in the pathogenesis of ESCC in this area [34]. Interestingly, in Shantou City [35], another high-incidence area for ESCC in southern China, the infection rates of HSV-1 and EBV were 30.0 %, in ESCC mucosa, but these viruses were not detected in normal mucosa, suggesting that infection with HSV-1 or EBV might be an etiological factor in EC. The role of these viruses in the development of ESCC needs to be studied further.

Conclusion

The present study showed that HPV infection, especially with the high-risk types, was positively associated with both esophageal and cervical carcinogenesis. It is possible that HPV plays a role in the etiology of ESCC in high-incidence areas. Additional work is needed to explore the molecular mechanisms associated with HPV infection in esophageal carcinogenesis.

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Conflict of interest The authors declare that they have no conflict of interest.

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