

Original Article

The Expression of HPV E6/E7 mRNA *In Situ* Hybridization in HPV Typing-negative Cervical Cancer

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Summary: High-risk human papillomavirus (HPV) persistent infection is the major tumorigenesis factor for cervical cancer (CC). However, the incidence of HPV-negative CC is 5% to 30% with different HPV detection methods. High-risk HPV E6/E7 mRNA *in situ* hybridization (RISH) can detect HPV-driven tumors. Our study aimed to explore whether HPV typing-negative CC was caused by HPV infection. The tissues of CC patients with HPV typing results, collected from cervical biopsies, conization, or hysterectomies, were submitted to RISH using RNAscope chromogenicin. Immunohistochemistry was performed to evaluate the expression of p16INK4a and Ki-67. A total of 308 women with HPV typing results were enrolled, and 30 (9.74%) cases of HPV typing were negative. In HPV typing-negative CCs, 28/30 (93.3%) were positive for RISH, which contained 22/22 (100%) squamous cell carcinomas and 6/8 (75%) adenocarcinomas. RISH was positive in 278/278 (100%) HPV typing-positive CCs, which included 232/232 (100%) squamous cell carcinomas and 46/46 (100%) adenocarcinomas. Positive RISH in HPV typing-negative CC was significantly lower than in the HPV typing-positive group ($P=0.002$, 95% confidence interval: 0.848–1.027). However, this significant difference only existed in adenocarcinoma. No significant differences were seen in the expression of p16INK4a and Ki-67 (all $P > 0.05$). HPV typing may cause misdiagnosis in 9.74% of CC patients, and HPV E6/E7 mRNA can detect HPV in CC with HPV typing-negative patients. This approach could provide a novel option to accurately detect high-risk HPVs in cervical tumors and help to eliminate the percentage of misdiagnosed HPV-related cases. **Key Words:** Cervical cancer—HPV typing-negative—HPV E6/E7 mRNA *in situ* hybridization—HPV detection.

A causal link between persistent infection with high-risk human papillomaviruses (HR-HPVs) and the development of cervical cancer (CC) is well established and is the cause of 99.7% of CC and >75% of cervical

adenocarcinoma (1,2). Therefore, testing for HR-HPV has been proven to be effective in screening for CC (3–5). However, in the literature, the incidence of HPV-negative CC is ~5% to 30% and may be related to differences in

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Supported by a grant from the National Natural Science Fund of China (No. 81572559) and the Scientific Research Foundation of Weifang Medical University (2017BSQD43).

The authors declare no conflict of interest.

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HPV testing methodology resulting in a high number of false-negative cases (6–8). Therefore, accurate detection of HR-HPVs in cervical lesions is crucial. Current HPV detection methods, which include hybrid capture-II (HC-II), HPV DNA *in situ* hybridization, polymerase chain reaction (PCR), and HPV mRNA *in situ* hybridization (RISH), have varying levels of sensitivity and specificity. The HC-II test, which was a homogeneous hybridization assay using unmarked single-strand RNA probes compatible with the targeted sequences, was practical, but it could not identify the various genotypes. HPV typing assay (PCR) is the most commonly used HPV DNA detection method for persistent HPV infection, which is the key cause of CC. HPV typing tests have been classified according to the expression of HPV L1 capsid proteins, which are one of the main targets of the cellular immune response and are influenced by HPV DNA integration into the human genome. A reduction or loss of capsid antigen production can result in a reduction in the cellular immune response, and previous research revealed that the reduction in L1 capsid antigen was higher in high-grade squamous intraepithelial lesions than in low-grade squamous intraepithelial lesions. HPV typing negativity in CC may be associated with HPV L1 capsid protein loss. Moreover, it cannot distinguish HPV transcriptionally active infections from those defined as “passenger” HPV.

The accumulated data indicated that the viral E6 and E7 proteins are the key factors that maintain the malignant phenotype of HPV-positive cancer cells (9). It is worth pointing out that E6 and E7 are the only viral genes that are always retained and expressed in HPV-positive cancer cells. HPV-positive cancer cells are oncogene addicted in that their growth is dependent on sustained E6/E7 expression. The overexpression of HR-HPV E6/E7 genes is the key causative factor for cervical intraepithelial neoplasias and CC. Quantitative reverse transcriptase PCR to detect HPV E6/E7 mRNA would seem to be an ideal HPV testing method since it reveals that HPV is not merely present but is transcriptionally active. The Food and Drug Administration (FDA) approved PCR to detect E6/E7 mRNA as the “gold standard” for the detection and typing of HPV. However, this assay can be performed exclusively in fresh-frozen tumor tissue and lacks the capacity to specify the detected genotypes; as a result, its use is limited in clinical practice. RISH for detecting HPV E6/E7 mRNA transcripts represents an advance in HPV testing because of the ability to detect the virus in its active transcriptional status in formalin-fixed paraffin-embedded (FFPE) tumor tissues. Leading

research has shown that RISH is a highly specific and sensitive method for detecting HPV in oropharyngeal squamous cell carcinoma (SCC) (10). In addition, our previous research proved the accuracy of RISH in diagnosing cervical lesions (11).

Herein, we describe a cohort of CC patients with negative HPV typing on FFPE material and explore the expression of HPV E6/E7 RISH. RNAscope technology is a new generation of single-molecule RISH analysis technology. A novel probe design strategy and a hybridization-based signal amplification system to simultaneously amplify signals and suppress the background are applied during this procedure. mRNA expression was observed at the single-cell level under a standard bright-field microscope.

MATERIALS AND METHODS

Study Population and Selection

Patients with CC detected by cervical biopsy, conization, or hysterectomies were retrospectively collected from April 2018 to September 2021 at the Affiliated Hospital of Weifang Medical University in China. The exclusion criteria were as follows: (1) not tested for HPV typing; (2) history of cervical treatment, regardless of physical therapy or excision; (3) pregnancy status; (4) chemotherapy and/or radiotherapy; and (5) resistant to anti-HPV treatment. In the cohort, CCs with negative HPV typing

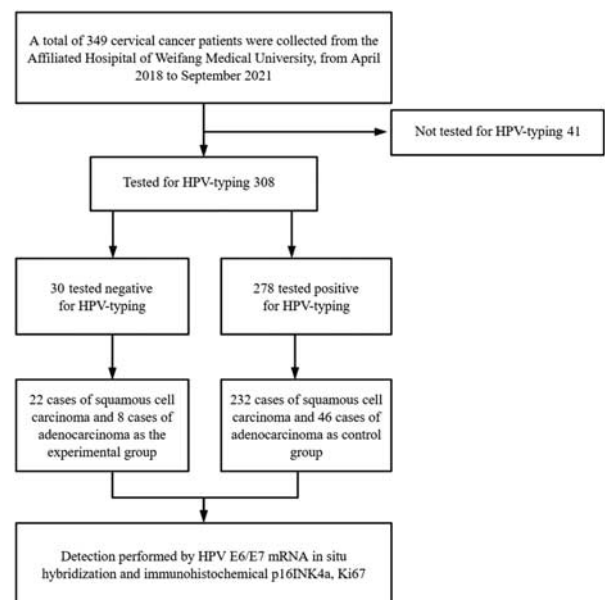


FIG. 1. Flowchart of patient inclusion in the study. HPV indicates human papillomavirus.

were included; CC patients with positive HPV typing in the same period were selected as controls (Fig. 1).

The patients' clinical data, which included age at diagnosis, smoking history, drinking history, fertility status, menopausal status, surgical method, and pathologic diagnosis, were collected and evaluated. Subjects who smoked at least 1 cigarette a day and continuously for >6 mo were considered positive for smoking history. Drinking at least once a month, including social engagements, was considered positive for drinking history.

Depending on the procedure, samples included cervical biopsies, cervical conization, and hysterectomies. The tissue blocks were cut into 5 µm sections on positively charged glass slides for the following assays: (1) hematoxylin and eosin (H&E) staining for morphologic identification; (2) p16INK4a immunohistochemistry (IHC); (3) Ki-67 IHC; (4) RISH; and (5) Hs-PPIB RISH (housekeeping/positive control).

Morphologic Evaluation

Two senior pathologists of gynecology who were blind to the results reviewed the H&E-stained slides independently, and any ambiguity was resolved by coexamination using a multihead microscope. Based on the World Health Organization (WHO) 2014 criteria, the research subjects contained 254 cases of SCC and 54 cases of adenocarcinoma, which contained usual, mucinous, serous, clear cell, and minimal deviation adenocarcinoma.

IHC

IHC was performed on FFPE tissue as per standard protocols, and antibodies against p16INK4a (clone: G175-405; ZSGB-BIO, China) and Ki-67 (clone: MIB1; ZSGB-BIO) were used. Phosphate-buffered saline was used in lieu of the primary antibody as a negative control. A positive control slide was used to ensure the validity of the staining procedure. According to the manufacturer's instructions, the sections were deparaffinized and dehydrated, and antigen retrieval was performed by boiling the slides with EDTA antigen retrieval solution (pH 9.0) in a pressure cooker for 15 min. After blocking endogenous peroxidase with H₂O₂, the sections were incubated with antibodies, followed by incubation with the secondary antibody. The reaction was detected by diaminobenzidine and counterstained with H&E. The IHC results were analyzed independently by 2 pathologists blinded to the samples.

The p16INK4a staining pattern was classified as negative (no staining), patchy (patchy+, focal and uneven staining in the nuclei and cytoplasm), and block-like (block+, diffuse and even staining in the nuclei and cytoplasm in 100% of the tumor cells). p16INK4a block-like was considered positive (12). For Ki-67, the cells with nuclear staining were counted in at least 10 fields per slide, and the average was calculated. In the cancer cell nuclear staining only, continuous staining was considered positive.

HPV E6/E7 RISH

RISH was performed using the RNAscope 2.5 HD Detection Reagent-BROWN and the HR-HPV 18 probe cocktail (Advanced Cell Diagnostics, Hayward, CA) with the HybEZ hybridization system (Advanced Cell Diagnostics) according to the manufacturer's instructions. Target-specific probes were used to detect the E6 and E7 genes of HR-HPV 18 genotypes (HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82). Hs-PPIB was used as the positive control, while normal epithelial cells in the FFPE block were used as the internal negative control. A positive control that showed effective positive staining in the batch of slides effective was used to ensure that the slide processing was successful. The assay was performed according to the supplier's instructions (Advanced Cell Diagnostics). Pretreatment: After being deparaffinized and dehydrated, these sections were serially treated with pretreatment 1 and pretreatment 2 solutions, followed by incubation with pretreatment 3 solution overnight. Hybridization: The sections were hybridized in HR-HPV 18 hybridization solution without a coverslip in a HybEZ Oven (Advanced Cell Diagnostics). Signal amplification: The hybridized probe was performed through the serial application of Amp 1 to 6. Visualization: Diaminobenzidine was used to demonstrate the amplified signal. The sections were counterstained with H&E, dehydrated with graded ethanol and xylene, and mounted with Cytoseal.

Images of the RISH slides were taken at 20× magnification using a Motic light microscope (Motic BA600 Mot, Germany). A positive RISH test result was defined as positive if any of the malignant cells showed brown punctuate dot-like nuclear and/or cytoplasmic positivity (13,14).

Following identification and screening by H&E staining, the effective nucleus was taken as the core while eliminating the identified oversized adherent

TABLE 1. The clinical characteristics of the cervical cancer patients included in the study

Parameters	n (%)
Age [mean \pm SD (range)]	47.0 \pm 9.7 (26–73)
Smoking history	
Yes	0 (0)
No	308 (100)
Drinking	
Yes	0 (0)
No	308 (100)
Fertility status	
Nulliparous	0 (0)
Pluriparous	308 (100)
Menopausal status	
Premenopausal	252 (81.8)
Postmenopausal	56 (18.2)
Presentation	
Contact bleeding	300 (97.5)
Symptomless	8 (2.5)
HR-HPV infection	
Yes	278 (90.3)
No	30 (9.7)
Pathologic diagnosis	
SCC	254 (82.5)
Adenocarcinoma	54 (17.5)

HR-HPV indicates high-risk human papilloma virus; SCC, squamous cell carcinoma.

nuclei and undersized cell fragments to identify the areas with diaminobenzidine staining signals within the cell range and to record the positive patterns. To exclude any possible morphologic influences, the RISH slides were evaluated by 2 pathologists who were blinded to the morphologic diagnoses.

Statistical Analysis

Statistical analyses were performed using SPSS 25.0 (SPSS; IBM). The rate and percentage of expression were used to describe the general situation of the study subject. The χ^2 analysis was used to test for differences in expression rate. A P -value <0.05 was considered statistically significant.

RESULTS

In the total cohort, 308 women with HPV typing results were enrolled, and most CC patients (300/308, 97.5%) had clinical symptoms of contact bleeding, irregular vaginal bleeding, or/and abnormal discharge; conversely, some patients (8/308, 2.5%) had no abnormal symptoms and were diagnosed by the health examination. The clinical characteristics of these patients are presented in Table 1. The mean age at diagnosis was 47.0 \pm 9.7 yr old, with a range of 26 to 73 yr old. The median age was 46 yr old, while the age of most patients at diagnosis was 47 yr old.

TABLE 2. Descriptive statistics of the detection of RISH, p16INK4a, and Ki-67 in different groups

	Research group		Control group		P
	SCC	AC	SCC	AC	
RISH					
+	22	6	232	46	0.002
–	0	2	0	0	
p16INK4a					
+	22	6	227	46	0.291
–	0	2	5	0	
Ki-67					
+	22	7	232	46	0.174
–	0	1	0	0	

– indicates negative; +, positive; AC, adenocarcinoma; RISH, mRNA *in situ* hybridization; SCC, squamous cell carcinoma.

Furthermore, 56 women (56/308, 18.2%) were postmenopausal, and no women were nulliparous. All the patients had a reproductive history of birth at least once, and women of minimum age were included.

HPV typing was negative in 30 cases (9.74%), which included 22/30 (73.3%) cases of SCC and 8/30 (26.7%) cases of adenocarcinoma. The details are presented in Table 2. In the CC patients who were HPV typing-negative, 28/30 (93.3%) were positive for RISH and p16INK4a block+ staining, which included 22/22 (100%) SCCs and 6/8 (75%) adenocarcinomas. RISH was positive in 278/278 (100%) HPV typing-positive CCs, which included 232/232 (100%) SCCs and 46/46 (100%) adenocarcinomas. While 273/278 (98.2%) showed p16INK4a block+ staining, the 5 negative cases were SCC. Positive RISH in the HPV typing-negative CC group was significantly lower than that in the HPV typing-positive group [$P=0.002$, $\chi^2=9.752$, 95% confidence interval (CI): 0.848–1.027]. However, this significant difference only existed in adenocarcinoma, and the incidence of positive RISH in HPV typing-negative SCC was the same as that in the HPV typing-positive group. No significant difference was seen in the expression of p16INK4a and Ki-67 ($P=0.291$, $\chi^2=1.113$, 95% CI: 0.863–1.047 and $P=0.174$, $\chi^2=1.850$, 95% CI: 0.905–1.033).

A total of 232/278 (83.4%) of the patients had a positive HPV typing of SCC, and the positive staining of RISH is presented in Figure 2. The malignant cells showed brown punctuate dot-like nuclear and/or cytoplasmic positivity. p16INK4a positivity showed diffuse and even staining in the nuclei and cytoplasm in 100% of the tumor cells. The representative positive staining of the RISH of adenocarcinoma patients who were positive for HPV typing presented with p16INK4a and Ki-67 strongly positive staining, as

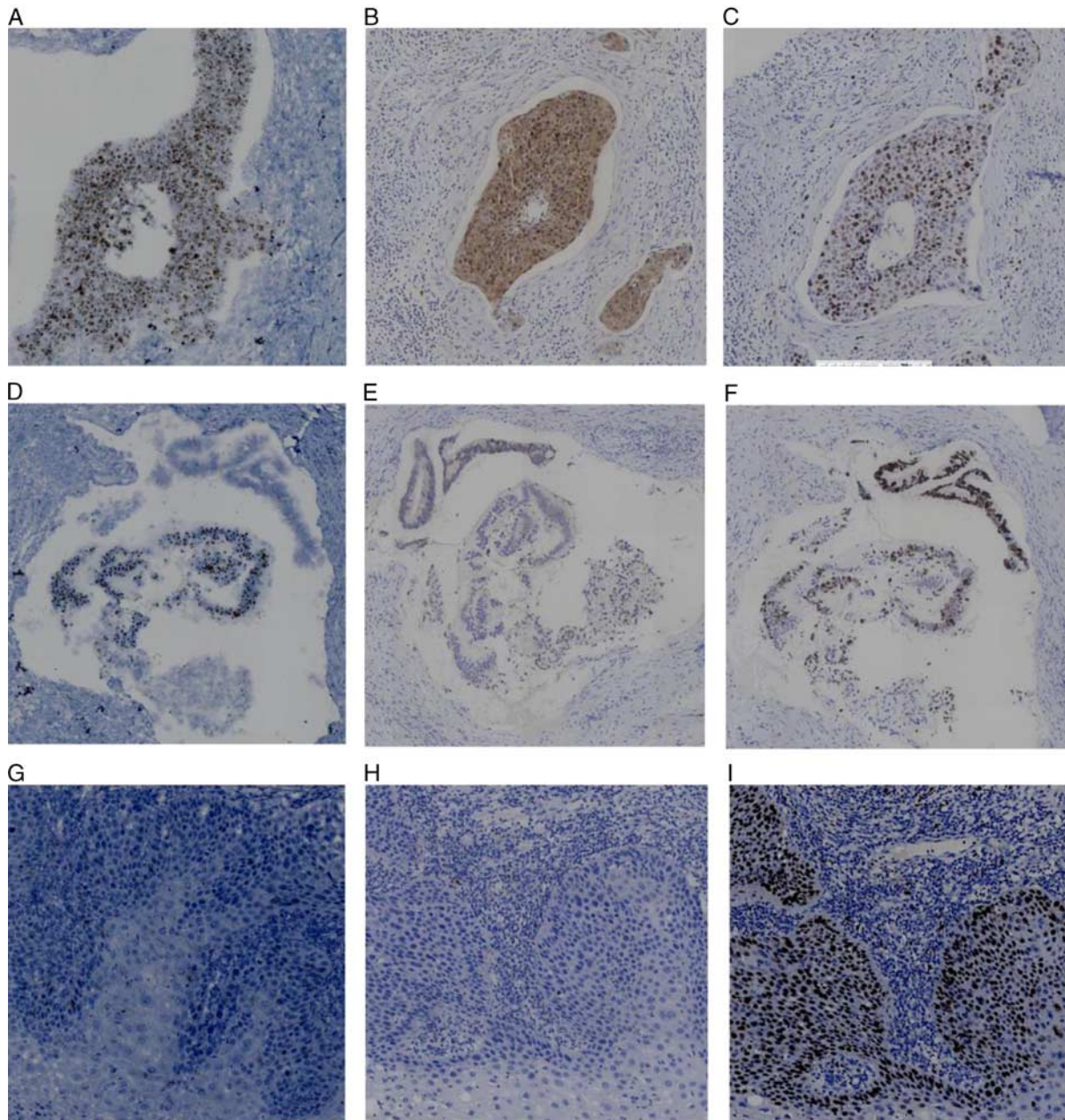


FIG. 2. Human papillomavirus typing-positive cervical cancer staining results. Squamous cell carcinoma specimens (A–C), A: mRNA *in situ* hybridization (+), B: p16INK4a (+), C: Ki-67 (+); adenocarcinoma specimens (D–F), D: mRNA *in situ* hybridization (+), E: p16INK4a (+), F: Ki-67 (+); squamous cell carcinoma specimens (G–I), G: mRNA *in situ* hybridization (+), H: p16INK4a (–), I: Ki-67 (+).

shown in Figure 2. In addition, the expression of RISH and Ki-67 was clearly positive, but p16INK4a was negative, and no block-like staining was observed in SCC with positive HPV typing, as shown in Figure 2.

The characteristics of the RISH-positive staining of cervical SCC and adenocarcinoma patients who had negative HPV typing presented with positive p16INK4a and Ki-67 staining, as shown in Figure 3.

Otherwise, the staining of adenocarcinoma was in conformity, with no positive RISH signals presented. Ki-67 was also negative, while p16INK4a expressed block-like staining, as shown in Figure 3.

In addition, it is worth noting that adenocarcinoma with no staining of RISH and p16INK4a, in which p16INK4a staining was not expressed in every gland, had no patchy or block-like staining in the nucleus or

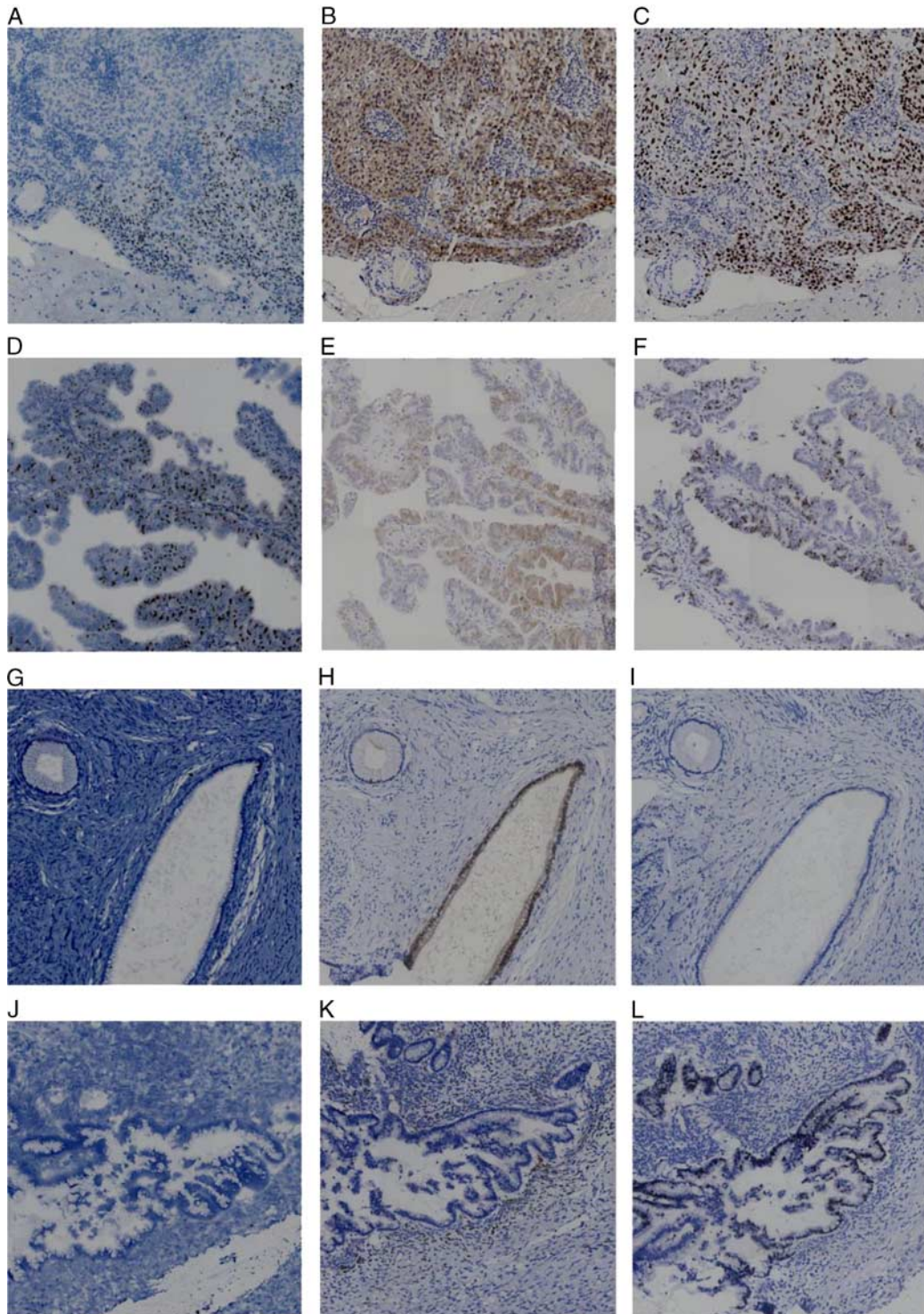


FIG. 3. Human papillomavirus typing-negative cervical cancer staining results. Squamous cell carcinoma specimens (A–C), A: mRNA *in situ* hybridization (+), B: p16INK4a (+), C: Ki-67 (+); adenocarcinoma specimens (D–F), D: mRNA *in situ* hybridization (+), E: p16INK4a (+), F: Ki-67 (+); adenocarcinoma specimens (G–I), G: mRNA *in situ* hybridization (–), H: p16INK4a (+), I: Ki-67 (–); adenocarcinoma specimens (J–L), J: mRNA *in situ* hybridization (–), K: p16INK4a (–), L: Ki-67 (+).

TABLE 3. Statistical descriptions of RISH, p16INK4a, and Ki-67

	Group		Research (%)	Control (%)	P*
	HPV typing-negative	HPV typing-positive			
RISH					
+	28	278	93.3	100.0	1.0
-	2	0			
p16INK4a					
+	28	273	93.3	98.2	1.0
-	2	5			
p16INK4a/Ki-67					
+	27	273	90	98.2	1.0
-	3	5			
RISH/p16INK4a					
+	27	273	90	98.2	1.0
-	3	5			
RISH/p16INK4a/Ki-67					
+	27	273	90	98.2	1.0
-	3	5			

*P-value compared with different detection methods in the research group.

- indicates negative; +, positive; HPV, human papillomavirus; RISH, mRNA in situ hybridization.

cytoplasm of cancer cells, while Ki-67-positive staining is presented in Figure 3.

The positive percentages for RISH were 93.3% and 100% in the HPV typing-negative and HPV typing-positive groups, respectively (Table 3). The different markers in the same group had no statistically significant differences in RISH, p16INK4a, p16INK4a/Ki-67, and different combinations (all $P > 0.05$) (Table 3).

DISCUSSION

In this study, we concluded 2 major findings: (1) HPV E6/E7 mRNA was expressed not only in CC with positive HPV typing but also in the majority of CC with negative HPV typing. HPV E6/E7 mRNA positive expression might reflect the phase of HPV infection, which “contributes to” the development of HPV-associated malignancy. (2) The negative expression of p16INK4a does not exclude HPV infection, and p16INK4a positivity does not necessarily indicate HPV infection.

It is well established that persistent infection with oncogenic HPV subtypes is necessary for the contribution to CC (7) and results in nearly all cases of CC (1). A multicentre, open-label, randomized clinical trial concluded that the primary screening of HR-HPV for CC was effective (15), and HPV typing detection is widely used in CC screening programs at present. However, a previous study in SCC using FDA-approved cervical screening technologies showed that HPV testing

can be negative in patients with SCC diagnosed by liquid-based cytology (16). HPV-negative CC is ~5% to 30% and may be related to differences in HPV testing methodology (6–8). In our study, HR-HPV-negative cases accounted for 9.74% of HPV typing CC, which was nearly consistent with previous studies (6–8). Another study demonstrated that HPV DNA, using different molecular methods in tissue samples, accounted for 45.9% of SCCs, which were previously diagnosed as HPV-negative SCCs on cytology material. Those authors had concluded that the higher percentage of HPV-negative results in some series was due to the high number of false-negative cases considering sampling variability, low virus load, rapidly progressive cancers developing between CC screening intervals, and the rarity of truly HR-HPV-negative SCC (17). Recently, a previous study of the integrated genomic and molecular characterization of CC showed that 95% of CCs are HPV-positive (18). These studies concluded that a subset of patients exists in which HR-HPV is not detectable by current laboratory methods used in the clinic.

In the early stage of HPV infection, the virus is mostly present in transient or latent status, and the HPV genome is in a free state in the nucleus (11,19). HPV E6/E7 genes are regulated by E1/E2 genes, and their expression is inhibited during this stage (11,20,21). HPV E6/E7 mRNA is often undetectable in normal cervical tissues or low-grade squamous intraepithelial lesion (11,22). During the persistent infection stage, HR-HPV DNA integrates into the host genome with the disruption and loss of the E2 gene, which leads to HPV E6/E7 mRNA overexpression and L1 capsid protein loss (9,23). In some studies, an *in situ* hybridization test has been proven to have higher sensitivity and specificity in the detection of HPV infection, and the HPV E6/E7 mRNA test was considered a potential tool (23,24). In our study, the typical HPV E6/E7 mRNA RISH image of CC presented with weak-to-strong nuclear and cytoplasmic dot-like signals within malignant cells. Our study also showed that a subset of women may have a false-negative HPV typing result that may be associated with fluctuations in HR-HPV positivity and HPV L1 capsid protein loss. Moreover, HPV E6/E7 mRNA RISH was negative in 2 (25%) HPV typing-negative cervical adenocarcinomas, which was in accordance with the previous study that CC cases were not all HPV-infected (6,23), and potential contributing factors included infection with an HPV genotype not currently contained in standard testing platforms, variations in sampling, low viral load or decreased transcriptional activity,

excluding E6/E7 genes, host p53, and retinoblastoma protein (Rb protein) mechanisms, and metastatic neoplasms *in vivo* in early or truly HR-HPV-negative carcinoma (6,9,20,23). In contrast to a previous study, which showed that cervical SCC cases were not all HPV-infected (25), all SCCs in our study were positive for RISH, which could reveal that the HPV E6/E7 mRNA test had superior detection in HPV-associated SCC.

Histopathology is the gold standard for the diagnosis of CC. However, pathologists may use some markers to make a differential diagnosis in some cases with equivocal pathologic features, including p16INK4a and Ki-67; however, such markers are not specific. p16INK4a is regarded as a reliable surrogate method to detect HR-HPV with a sensitivity approaching 100% (26), and the use of p16INK4a has been reported in several studies on cytology and histologic samples as a biomarker assisting in the diagnosis of high-grade epithelial cervical lesions (27). In our study, most CCs with p16INK4a-positive staining were 93.3% and 98.2% in the 2 groups, respectively, which was concordant with previous studies reporting p16INK4a-positive staining from 80% to 100% in invasive carcinoma (28). The variation in expression rates may partly depend on the criteria defining positive expression. In this research, nuclear and continuous diffuse cytoplasmic staining of the cells was considered positive, while some studies required nuclear or cytoplasmic staining to be positive (28). However, negative cases were detected in adenocarcinoma. Many previous studies insisted that the overexpression of p16INK4a, as a multitumour suppressor gene, may be closely correlated with HPV oncoprotein E7 inactivating cell cycle regulation Rb protein (29) and was directly involved in the regulation of the cell cycle, inhibiting the activity of cyclin-dependent protein kinase CDK4/CDK6. In our study, we thought that p16INK4a negative staining might be evoked via pathways other than HPV infection through gene mutation or hypermethylation. Currently, the specific mechanism is not fully understood, and the correlation between p16INK4a and HPV infection history remains unclear. It is also controversial whether the p16INK4a expression level can accurately predict the potential risk of precancerous lesions or CC.

Ki-67 positivity is highly dependent on reactive changes and cell proliferation (30). As a nuclear proliferation antigen, Ki-67 is exclusively expressed in the proliferation phase of the cell cycle. Some previous data revealed that the Ki-67-positive staining percentage in normal cervical tissues and cervical intra-epithelial neoplasia and CC tissues gradually increases

with the aggravation of the disease (28). In our study, the levels of Ki-67-positive staining were 96.7% and 100% in the 2 groups, respectively. These results were better concordant with previous studies that found Ki-67 in 90% to 100% of invasive carcinomas (28). Ki-67 and p16INK4a/Ki-67 are the most widely used biomarkers that significantly improve the accuracy of the pathologic diagnosis of cervical lesions (28). The cotest of Ki-67-positive and p16INK4a-positive staining usually indicates that the cell cycle is out of control, which occurs in abnormal cell proliferation. However, our study showed that Ki-67 and p16INK4a/Ki-67 exhibited no significant improvement over p16INK4a alone in the diagnosis of CC. Hence, the routine addition of Ki-67 to p16INK4a was not recommended, which was consistent with the results of previous studies (11). In addition, some cases expressed positive staining of p16INK4a, while HPV E6/E7 mRNA was not expressed, a high correspondence between p16INK4a and the RISH test was shown in the literature; however, even in only a few cases, discordant results were found in our study and some previous studies (13,31–33), which may have resulted from HPV detection methods. In other words, the negative expression of p16INK4a does not exclude HPV infection, and p16INK4a positivity does not necessarily indicate HPV infection (34,35).

The present study has several limitations. As a retrospective study, specimens were taken from samples in the past that were not tested by HC-II, HPV DNA *in situ* hybridization, and HPV E6/E7 mRNA (APTI-MA) to further confirm the presence or absence of HR-HPV. Another limitation was that our study had no detailed history information on HPV infection and had limited available data from Pap screening tests and colposcope examinations, which reflected both the small percentage of patients who were diagnosed with CC who were RISH-negative overall and the fact that the majority of patients who developed CC had inadequate screening. Previous data also revealed that 60% of women diagnosed with CC had never received Pap screenings or had done so at irregular intervals (36). These data would certainly assist further in the interpretation of the correlation of HR-HPV-associated CC (false-negative) and IHC findings. A multicentre, large-scale, and further prospective study with more appropriate and accurate data should be conducted.

CONCLUSIONS

HPV typing tests could cause misdiagnoses in ~9.74% of CC patients, and HPV E6/E7 mRNA

could detect all cervical SCC and most cervical adenocarcinoma with HPV-related status even when HPV typing is negative. This approach could provide a novel approach to accurately detect HR-HPVs in cervical tumors and help to eliminate the percentage of misdiagnosed HPV-related cases.

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