Introduction

Cervical cancer ranks as the fourth most prevalent cancer in the world; in 2018, there were approximately 570,000 new cases of cervical cancer with 311,000 deaths [1, 2]. Among cervical cancer-caused deaths, approximately 85% occur in underdeveloped or developing countries, and the death rate in low- and middle-income countries is 18 times higher than that in developed countries [3].

Human papillomavirus (HPV) infection has been shown to be the main aetiologic factor for cervical cancer, and studies have shown that the number of people newly infected with HPV is 6.2 million in the US each year [4, 5]. HPV is a highly heterogeneous small DNA virus and can be categorized into low-risk and high-risk types. Persistent infection with high-risk HPV can lead to precancerous lesions and cervical cancer. According to the literature, among all HPV infection-caused cervical cancers, HPV 16 is the most common type [6-10].

HPV mainly infects basal keratinocytes through the injured skin or mucosal surface. With viral DNA replication, up to 50-100 viral copies can be made per cell [11, 12]. Initially, genome replication is in the free stage. When infected basal cells differentiate into cells above the basal cell, replication and expression of early and late viral genes and encoded gene products are activated in terminally differentiated cells; having been assembled in the squamous epithelial cell, viruses are released and infect other cells nearby [13, 14]. This mechanism of HPV virus infection and replication in host epithelial cells can lead HPV to escape the host’s immune surveillance [14].

The HPV genome is closed double-stranded DNA that is divided into three regions: noncoding upstream regulatory region, open reading frames, and late coding region. The early genes include E1-E7, which encode viral replication proteins. Among these genes, the HPV E7 area is directly related to the carcinogenic properties of HPV [15]. The primary function of E7 is to inactivate two important tumour suppressor genes, p53 and Rb. The inactivation function of HPV E7 can lead to cell proliferation, immortalization and malignant transformation [14]. Mutations of HPV16 E7 are widespread and show regional characteristics, and the five major germline mutation groups are European type (E), Asian type (As), Asian-American type (AA), African-type 1 (Af1), and African-type 2 (Af2) [13-15].

Xinjiang is located in northwestern China. This region has a high incidence of cervical cancer. Uyghur women are at a particularly high risk of cervical cancer [16-18]. In Uyghur women in Xinjiang, the incidence of cervical cancer has been reported to be 459/100,000-527/100,000, which is significantly higher than the incidence in any other ethnic group of China [19]. Although numerous studies on HPV16 E7 have been reported to date, the characteristics of its mutations are not consistent. In addition, to the best of our knowledge, mutations in HPV16 E7 among Uyghur women in Xinjiang have not been reported.

Based on the aforementioned findings, this study investigated the mutation status of HPV16 E7 among Uyghur women in Xinjiang. The results of this study may provide a theoretical basis for preventive vaccine development in related regions and enrich the data regarding the pathogenesis of cervical cancer.
Polymorphisms of human papillomavirus type 16 E7 in Uyghur women with cervical cancer

Materials and Methods

Samples

A total of 80 cervical cancer patients who visited the Xinjiang Uygur Autonomous Region People’s Hospital from December 2016 to August 2018 were consecutively enrolled in this study. Their median age was 48 years (20-60 years). All patients were Uyghur women from southern Xinjiang who had long-term residence in this region. Before recruitment, they had never received any treatment related to cervical cancer, such as radiotherapy, chemotherapy, surgery, or immunotherapy. The pathological diagnosis of cervical cancer was in accordance with that indicated in the literature [20].

This study was approved by the Ethics Committee of the People’s Hospital of Xinjiang Autonomous Region, with an approval number of 20050126. Written informed consent was obtained from all patients.

Sampling

Approximately 20-40 μg of fresh cervical tissue was collected and then stored at -80 °C. DN10-whole blood, a cell genomic DNA extraction kit (centrifugal columnar), DNA Marker I, and GoldView nucleic acid dye were purchased from the Bo Maide Beijing Science and Technology Development Co., Ltd., China. The DNA polymerase chain reaction (PCR) kit and SK2492 were purchased from Shanghai Biological Engineering Technology Services Ltd., China.

DNA extraction

The tissue sample was ground evenly. Then, DNA extraction was performed in accordance with the manufacturer’s instructions. The quality of the extracted genome was verified using polymerase chain reaction (PCR) amplification.

PCR

Primers were synthesized by Shanghai Biological Engineering Technology Engineering Service Co. Ltd., China. β-β-Globin was used as the internal control. The sequences of the primers are summarized in Table 1.

The 25 μl reaction system contained 2.5 μl 10 × PCR buffer, 2.5 μl of dNTP (2 mM), 1.5 μl of MgCl2 (25 mM), upstream and downstream primers of the target gene (20 μM; 0.5 μl for each), 0.2 μl of Taq DNA polymerase (5 U/μl), 2 μl of DNA template, and 15.8 μl of deionized distilled water. The amplification conditions for HPV16 L1 consisted of predenaturation at 95 °C for 5 min, 35 cycles of 94 °C for 45 s, 57 °C for 45 s at 45 °C for 45 s, and a final extension at 72 °C for 10 min. If the electrophoresis results showed a band at 150 bp, HPV16 infection was observed in the sample. Samples with positive bands at 150 bp were selected. The amplification conditions for HPV16 E7 included 95 °C for 5 min followed by 35 cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 45 s, and a final step of 72 °C for 10 min. GoldView nucleic acid dye was used for staining, and the samples were observed under ultraviolet light.

DNA sequencing

The amplified PCR products were sent to Shanghai Sangon Biological Engineering Service Co. Ltd for sequencing. DNAMAN software was used to analyse the sequencing results. The German standard strain (AF534061) in GenBank was used as a prototype of HPV16 E7 for comparison.

Statistical analysis

SPSS 22.0 was used for data processing. The mean value was expressed as X ± S. Counts and percentages were used for mutation frequency calculation. The chi-square test or Fisher’s exact probability was used for the hypothesis test. p < 0.05 was considered significantly different.

Results

HPV16 infection

According to the PCR and gel electrophoresis results, 60 out of the 80 total samples were HPV16 L1 positive, with an HPV16 L1 infection rate of 75% (Figure 1A).

The samples with HPV16 L1 positivity were further subjected to PCR amplification and gel electrophoresis. All HPV16 L1-positive samples were also HPV16 E7-positive (Figure 1B).

HPV16 E7 mutations

The 60 HPV16 E7-positive samples were submitted for sequencing, and 54 were successfully sequenced (Figure 2). E7 sequences were then compared with the HPV16 sequences from the German reference strains in the gene sequence database (AF534061), and then germline mutations were classified in accordance with the known distribution (21, 22) of HPV16 E7 mutants: Ep (prototype sequence), As (A3979C, A4042G, A4042T, A647G, T846C), E (A3979C, A4042G), Af (G3868A, C3991T, T789C, T795G), and AA (T732C, T789C, T795G). Among the 54 HPV16 E7 sequences, 6 had E7 gene mutations, with a total of 9 mutation positions. One case had 2 mutation positions, and 4 had one mutation position. The specific mutations are summarized in Table 2. Among the 9 mutation positions, 3 were missense mutations, and 6 were silent mutations.

Discussion

Numerous studies have shown that E7 is one major transforming gene of HPV16 [23-28]. Therefore, it is the key factor that causes cervical cancer. This study focused on the characteristics of the HPV16 E7 gene and its status in Uyghur women with cervical cancer in Xinjiang.
### Table 1. — Primer sequences and PCR length of HPV16 E1, E7 and β-globin.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Sequence</th>
<th>Reverse Sequence</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-globin</td>
<td>TGA CGT GGA CAT</td>
<td>CTG GAA GGT GGA</td>
<td>203 bp</td>
</tr>
<tr>
<td></td>
<td>CCG CAA AG</td>
<td>CAG CGA GG</td>
<td></td>
</tr>
<tr>
<td>HPV16L1</td>
<td>TGCTAGTGCTTATGC</td>
<td>ATTTACTGCAACATTTG</td>
<td>152 bp</td>
</tr>
<tr>
<td></td>
<td>GCAA</td>
<td>GTAC</td>
<td></td>
</tr>
<tr>
<td>HPV16 E7</td>
<td>CGATGTATGTCTTGTC</td>
<td>TTACATCCCGTACCCCTT</td>
<td>407 bp</td>
</tr>
<tr>
<td></td>
<td>GAG</td>
<td>TCTT</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. — Agarose (1.5%) gel electrophoresis of the PCR products. A, HPV16 L1. B, HPV16 E7.

The HPV E7 oncoprotein is a small acidic protein that consists of 98 amino acids, with a molecular weight of 21 kDa. It is mainly located within the nuclear matrix. According to its homology to adenovirus E1A, it can be divided into three conserved regions: conserved region (CR) I (1-16 amino acids), CR II (17-38 amino acids), and CR III [29]. Among them, the 22-26 amino acids in CR II are LX-CXE, which constitute the recognition site for PRb1 as well as its family protein members. The binding ability of E7 of high-risk HPV types with PRb is far greater than that of low-risk types. The 31-38 amino acids constitute the recognition site for protein kinase II. CR III contains two Zn2+ binding sequences, which is the binding region for the formation of E7 protein dimers. The Zn2+ binding sites are located at amino acids 58-61 and 91-94, with a structure of Cys-XX-Cys. Mutants of cysteine in C58G and C91G can sup-
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Table 2. — The variable sites of HPV16 E7.

<table>
<thead>
<tr>
<th>Number</th>
<th>Base (amino acid) sites</th>
<th>Branch Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>647 (29)</td>
<td>663 (34)</td>
</tr>
<tr>
<td>German standard strain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC10</td>
<td>A Asparagine</td>
<td>G Glutamate</td>
</tr>
<tr>
<td>CC17</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CC21</td>
<td>G Serine</td>
<td>A</td>
</tr>
<tr>
<td>CC32</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CC59</td>
<td>G Serine</td>
<td>—</td>
</tr>
<tr>
<td>CC80</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Variation number</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Note: “—”, no change.

Figure 2. — The sequencing results of HPV16 E7.

press the formation of E7 protein dimers, thus completely destroying the transforming activity of the E7 protein [30-32]. In this study, the sequencing of the 54 cases of HPV16 E7 showed 2 cases had changes in the 29th amino acid and 1 case had changes in the 77th amino acid. However, all these cases had no mutations in the pRb1 binding site. Studies have reported that HPV16 E7 was relatively conservative compared with E6 [33, 34]. However, inconsistency arises here. According to some studies conducted in Japan, Korea and Indonesia, the mutation rate of HPV16 E7 was high in patients with cervical cancer (65%-75%) [35, 36]. Wu et al. [37] analysed the mutation of HPV16 E7 oncogenes in 233 hospitalized patients with HPV16-infected cervical cancer from Nanchang, Jiangxi, and Guangdong, Zhuhai, China, and found that the HPV16 E7 mutation rate in these patients was 100%. According to another study carried out among cervical cancer patients from northeastern China, the variation rate of HPV16 E6 was 63.46%, whereas that of E7 was 84.62% [38]. In this study, HPV16 E7 was sequenced in 54 cases of cervical cancer. The results showed that 88.9% of them had no mutation, and HPV16 E7 was relatively conservative. According to Duensing and Munger [39], changes in E7 may result in changes in encoded proteins in biological and carcinogenic characteristics. Given that Uyghur women in southern Xinjiang have a high incidence of cervical cancer, our finding may be reasonable to explain why Xinjiang Uygur women exhibit the features of high incidence and high mortality of cervical cancer but with a low HPV infection rate. Hua et al. [40] analysed HPV16 E7 variation in Hubei and found that the HPV16 E7 mutation rate was high, with the mutation hot spots of T846C and A647G (2/54). Liu et al. [41] conducted a study on HPV16 E7 variations in patients with cervical cancer from Guangdong and found that the mutations included T846C (68%), A647G (68%), C666T (50%), G823A (4%), and A844C (4%). Yang et al. [42] found that the hot spot of HPV16 E7 mutations in cervical cancer patients from Beijing was A647G (31.9%). In this study, the main mutation of E7 in Uygur women with cervical cancer from southern Xinjiang was 647. This finding was not consistent with mutations in other areas. However, our finding was basically consistent with those reported from Hong Kong, Korea, and India, according to which the A647G mutation rates in these regions were 72% [43], 58.0% [44], and 37.8% [45], respectively. The results of our study suggest that the HPV16 E7 oncogene was highly conserved, which is consistent with the literature [46]. By comparing the genetic variations of 296 cases of the HPV16 E7 genome with those of high-risk HPV31 E7 and HPV73 E7, Mahboobeh found that HPV16 E7 had four mutation sites, and all the
mutations were synonymous, which did not cause changes in amino acids. However, the E7 mutations of HPV31 and HPV73 were located at 7 sites, 4 of all of which were missense, which can cause changes in the amino acid [46]. The results of this study and those in the literature may serve as evidence for the feature of high evolutionary selection of HPV16 and may also explain why HPV16 E7 is highly carcinogenic.

This study had some limitations. First, the sample size of this study was small, and only 80 patients were enrolled. Therefore, the results of this study need to be verified by studies with a larger sample size. Second, all samples in this study were from a local hospital in southern Xinjiang. To further verify the results of this study, multi-centre research needs to be carried out in the future.

Conclusions
In conclusion, HPV16 E7 is relatively conserved in Uygur women with cervical cancer, and its mutation rate is low. Since HPV16 E7 possesses high antigenicity, it is possible to develop HPV vaccines based on the features of HPV16 E7.

Acknowledgments
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Conflict of Interest
The authors have no conflicts of interest to declare.

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Corresponding Author:
MAYINUER NIYAZI, Ph.D.
No 91 Tianqi Road, Urumqi 830001, China
e-mail: mayinniya@163.com