Human Papillomavirus 16E6 Oncogene Mutation in Cervical Cancer

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ABSTRACT

Objective: Cervical cancer (CC) is the second most common type of cancer in women worldwide, after breast cancer. High-risk human papillomaviruses (HR-HPVs) are considered to be the major causes of cervical cancer. HPV16 is the most common type of HR-HPVs and HPV16 E6 gene is one of the major oncogenes. Specific mutations are considered as dangerous factors causing CC. This study was designed to find mutations of HPV16 E6 and the relationship between the mutations and the happening of CC.

Methods: The tissue DNA was extracted from 15 biopsies of CC. Part of HPV16 E6 gene (nucleotide 201-523) was amplified by polymerase chain reaction (PCR) from the CC tissue DNA. The PCR fragments were sequenced and analyzed.

Results: The result of PCR showed that the positive rate of HPV16 E6 was 93.33% (14/15). After sequencing and analyzing, in the 13 out of 14 PCR fragments, 4 maintained prototype (30.77%), 8 had a same 350G mutation (61.54%), and 1 had a 249G mutation (7.69%).

Conclusion: This study suggest that there is a high infection rate of HPV in cervical cancer and most of the HPV16 E6 gene has mutations. Those mutations may have an association with the development of cervical cancer.

Key words: Cervical cancer; Human papillomavirus; E6 gene; Mutation

INTRODUCTION

Cervical cancer (CC) is the second leading cause of death from cancer in women worldwide, after breast cancer[1]. Human papillomaviruses (HPVs) are considered to be the major causes of CC worldwide and are detected in more than 99% of the cases[2,3]. In recent studies the mutations of HPV16 E6 gene and the protein coded by it are considered to have a direct relationship with the development of CC. The mutations of HPV16 E6 are different with respect to the different geographical regions over the globe. This study was designed to find mutations of HPV16 E6 and the relationship between the mutations and the happening of CC.

MATERIALS AND METHODS

Main Reagents

H.Q.&Q tissue DNA extract kit (U-gene, NO. JYZ-2-1-2); DL2000 DNA Marker (NO. D501A), dPNT Mixture(NO. D4030A) and TaKaRa Taq(NO. DR100A) were purchased from DaLian TaKaRa Biotechnology Co. Ltd.

DNA Extraction

Fifteen specimens of CC patients (age range,
24-65 years; mean age, 41 years) were collected from the Maternal and Child Health Hospital of Gansu Province and Lanzhou General Hospital of PLA, from 2007 to 2008. All patients had not received radiotherapy or chemotherapy. All cases were reviewed and diagnosed as CC by 3 associate chief physicians of pathology department. And the specimens were stored in -60°C freezer.

From each specimen 30mg tissue were cut to extract genomic DNA using DNA extraction box (H.Q.&.Q Tissue) according to the manufacturer's instructions.

Primer Synthesis and PCR Amplifications

According to Clere[4], HPV16 E6(nt201-523) primer were designed and synthesized by TaKaRa (DaLian) Biotechnology CO., Ltd. The primers (NO. LZD174) were 5'-GCAAGCAACAGTTACTGCGA GT-3' (sense, nt201-223) and 5'-GCAACAAGAC ATACATCGACCG-3' (antisense, nt501-523).

DNA amplifications for HPV16 E6 detection were performed with specific primers previously described. Amplification was carried out in 25μl of reaction mixture containing 10×PCR buffer, 0.25mmol/L of each dNTP, 10pmol of sense and antisense primers, 0.5μg genomic DNA and 2.5U Taq DNA polymerase. The PCR mixtures were initially denatured for 4 min at 94°C and subjected to 30 cycles of amplification. Each cycle included a denaturing step at 94°C for 1 min, an annealing step at 50°C. A final elongation step was performed for another 5 min at 72°C. The PCR products were analyzed by 1% agarose gel electrophoresis with ethidium bromide (EB) staining for visualization of DNA under ultraviolet light by comparison with a DL2000 DNA Marker.

HPV16 E6 Sequencing

PCR fragments of the 14 samples were sent to Sangon (Shanghai) Biotechnology Co. for sequencing. The sequences of the fragments were checked according to the E6 gene sequence in GENEBANK.

RESULTS

HPV16 E6 Gene in Cervical Cancer

Of the 15 samples, 14 maintained HPV16 E6 gene. In Figure 1 it shows the PCR products of 5 samples analyzed on 1% agarose gel.

![Figure 1. Electrophoresis of PCR products of cervical cancer.](image)

M: DL2,000 DNA Marker; PCR fragments of sample 2, 3, 6, 7, 8

Sequence Analysis of HPV16 Oncogene E6

Of the 15 PCR fragments, 14 contained E6 gene. The positive rate of HPV16 E6 was 93.33% (14/15). 13 out of the 14 PCR fragments were successfully sequenced, and 4 of them maintained prototype 30.77% (4/13), 8 (61.54%) had one same mutation (nt350) which caused amino acid variations, resulting in the amino acid substitution of valine for Leucine. One of these 13 cases (7.69%) had one mutation (nt249) caused the amino acid substitution of glycine for Aspartic acid. The results of 15 cases are showed in Table 1 and the sequence of No. 5 and No. 15 samples are shown in Figure 2 and Figure 3.

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Table 1. The mutations of 15 cervical cancer samples