HPV16 E6 gene polymorphisms and the functions of the mutation site in cervical cancer among Uygur and Han women in Xinjiang

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Abstract
Objective: This study aimed to: 1) investigate the status and genotype distribution of human papillomavirus (HPV) in infected Uygur and Han women in Xinjiang; 2) elucidate the variation of the HPV16 E6 gene sequence in the cervix of Uygur and Han women in Xinjiang; and 3) analyze the HPV16 E6 gene polymorphism site and relationship with the development of cervical cancer.

Methods: A total of 2879 samples of cervical mucus from the exfoliated cells of Uygur and Han women were collected for an epidemiological analysis of HPV. Genomic DNA was extracted from the cervical HPV16-positive tissues of 110 Uygur and Han women, and E6 was amplified by PCR and sequenced. The HPV16 E6 sequence was analyzed using the European standard as the prototype, and an evolutionary tree analysis was performed. HPV16 E6-295T/350T-GV230, HPV16 E6-295G/350G-GV230, and HPV16 E6-295T/350G-GV230 were stably transfected into human cervical cancer C33A cells. HPV16 E6 protein expression was confirmed using a direct immunofluorescence assay. CCK8 and clonogenic assays were used to analyze C33A cell proliferation. Both a transwell and cell scratch assay were used to study C33A cell migration and invasion. C33A cell apoptosis was analyzed using FACS experiments. SPSS17.0 statistical software was used for statistical data processing. P < 0.05 was considered statistically significant.

Results: The total HPV infection rate was 26.390% (760/2879), whereas the Uygur infection rate was 22.87% (196/857) and the Han infection rate was 27.89% (564/2022) (P < 0.05). HPV16, HPV 52, and HPV 53 were associated with higher detection rates in Uygur, whereas HPV16, HPV52, and HPV58 exhibited a higher detection rate in Han. HPV-infected women from Uygur and Han commonly exhibited a single infection. A total of 14 mutation sites were identified in the HPV16 E6 gene by sequencing 110 HPV16-positive samples, including eight missense and six synonymous mutations. Among these, 65 cases of E6 genes were mutated at nucleotide 350 (T350G) with the corresponding amino acids changing from leucine to valine (L83V) and a mutation rate of 59.09%. Moreover, there were seven cases of an E6 gene mutation at nucleotide 295 (T295G) with corresponding amino acid changes from aspartic to glutamic (D64E) and a mutation rate of 6.36%. It is important to note that these seven cases of HPV16 E6 T295G mutations were accompanied by the E6 T350G mutation.
Phylogenetic analysis showed that there were HPV16 European (Ep), European variant (E), and three Asian (As) types in Uygur and Han women. No African (Af) and Asian American (AA) types were observed. When HPV16 E6 295T/350T, 295G/350G, and 295T/350G GV230 eukaryotic expression vector(s) were stably transfected into cervical cancer C33A cells, they were found to promote cellular proliferation, migration, invasion, and inhibit apoptosis. The 295T/350G-GV230 had the strongest effect on C33A cells and 295G/350G-GV230 was significantly stronger than 295T/350T-GV230 (P < 0.05). Conclusions: The positive HPV infection rates differed between the Uygur and Han groups in Xinjiang, and the genotype distribution of HPV infection was different. Between the Uygur and Han women in Xinjiang, the main types of HPV16 infection were European (E) and Asian (As). After stably transfecting C33A cells with a eukaryotic expression vector for different polymorphism sites (295T/350T, 295G/350G, and 295T/350G), the 295T/350G mutation site promoted the proliferation, migration, and invasion of C33A cells to a greater extent than 295G/350G; however, 295G/350G had a stronger effect than 295T/350T.

Full-text
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Figures
Figure 1. Phylogenetic tree analysis of the HPV16 E6 gene

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The expression of HPV16 E6 in C33A cells by indirect immunofluorescence (200×). Following the transfection of C33A cells with a GV230 empty vector, HPV16 E6 prototype vector, HPV16 E6-G295/G350 mutation vector, HPV16 E6-T295/G350 mutation vector, the red fluorescence of the vector was measured. The GV230 empty vector group was used as a control group and displayed no red fluorescence, whereas red fluorescence was observed in the other three experimental groups. The HPV16 E6-T295/G350 mutation group contained the highest fluorescence.
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Figure 3

The effect of an HPV16 E6 mutation on the proliferation of C33A cells. A. The proliferation of the HPV16 E6 prototype group at different times compared to the empty vector NC group. B. The proliferation of the HPV16 E6-G295/G350 mutant group at different times compared with the empty vector NC group. C. The proliferation of the HPV16 E6-G350 mutant group at different times compared with the empty vector NC group. * P < 0.05; ** P < 0.01 were considered to be statistically significant.
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Figure 4. Effect of an HPV16 E6 mutation on the formation of C33A cell clones. a. GV230-transfected NC group; b. HPV16 E6 prototype group; c. HPV16 E6-G295/G350 mutation group; d. HPV16 E6-G350 mutation group. * P < 0.05; ** P < 0.01 were considered statistically significant.

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Figure 5. The effect of an HPV16 E6 mutation on the migration and invasion of C33A cells. After C33A cells were transfected with a GV230 empty vector, HPV16 E6 prototype vector, HPV16 E6-G295/G350 mutation vector, and HPV16 E6-G350 mutation vector, a transwell invasion assay and migration assay of the C33A cells were performed. * P < 0.05; ** P < 0.01 were considered statistically significant.

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Figure 6. The effect of an HPV16 E6 mutation on the apoptosis of C33A cells. a. GV230 transfected NC group; b. HPV16 E6 prototype group; c. HPV16 E6-G295/G350 mutation group; d. HPV16 E6-G350 mutation group. * P < 0.05; ** P < 0.01 were considered statistically significant.

Figure 6

The effect of an HPV16 E6 mutation on the apoptosis of C33A cells. a. GV230 transfected NC group; b. HPV16 E6 prototype group; c. HPV16 E6-G295/G350 mutation group; d. HPV16 E6-G350 mutation group. * P < 0.05; ** P < 0.01 were considered statistically significant.
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Figure 7

Nude mouse xenograft tumor experiment. a. Comparison of the final tumor volume of the nude mice after death. b. Tumor growth curve. c. The tumor weight of the nude mice after death.
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