Variants of the Long Control Region of Human Papillomavirus Type 16 in Chinese Cervical Cancer*

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Abstract **Objective** Human papillomavirus (HPV), especially HPV16, plays a very critical role in the development of the malignant tumor and pre-cancer lesion. Many researches have indicated that the mutation of long control region (LCR) of HPV16 is closely related to the cervical cancer and the mutation shows the variance in different regions, ethnic populations. Based on this, DNA sequence of HPV16 LCR in cervical cancer tissue specimen is analyzed to provide gist for molecular carcinogenic mechanism of cervical cancer. **Methods** In the present study, 52 Chinese Han people's cervical cancer specimens are detected by means of PCR and DNA sequencing. **Results** There are 25 (25/52) nucleotide sequence variations have been detected. They are 7197G→T, 7199T→C, 7518G→A, 7665T→A, 7674G→T, 7493→T, 24C→G, 94G→A, 7711T→G, 7268C→T, 7285A→C, 7486→G, 7657A→G, 7727A→C, 7839C→T, 24C→T, 180T→G, 7155G→T, 7170T→C, 7173A→C, 7175T→C; 7191G→T, 7199T→G. Among which nine (9/25) nucleotide sequence variations occur at the binding sites of known cells and viral transcription factors and three(3/9) specimen variations occur at the same nucleotide (7518 G→A). These binding sites are YY1(7518G→A; 7518G→A; 7518G→A; 7486→G), TEF-1(7191G→T; 7197G→T), NF1(7674G→T; 7711→G), Oct-1 (7839C→T). **Conclusion** Nucleotide sequence variation of YY1 may be one of the features for LCR nucleotide sequence variations of HPV16 for those cervical cancer patients in Chinese han population.

Key words Cervical Cancer; HPV16; Long Control Region; Genetic Expression

Many researches have indicated that HPV is closely related to cervical cancer and cancerization. HPV may infect epithelia of skin and mucosa and thus induce cells' proliferation and the pathological tumor-like changes of mammilla. Among the patients of cervical cancer, the detection rate of HPV is more than 90 percent [1, 2]. Among more than 80 sub-types of HPV, HPV16 infection is mostly connected with the malignant pathological changes of cervix and its detection rate is 60% or so. The research about HPV16 becomes more and more popular.

long control region (LCR)of HPV16 is located between the end of L1 region and the beginning of E6 region, and it is the vital regulation region for viral gene transcription. Although LCR does not contain major open reading frames, yet there is a distinct amplification sub-region at the centre of LCR, which can activate and regulate virus replication at the epidermis cells by promote the activity of P97 [3]. Enhancer is made up of distinct binding sites for many transcription activation factors, including AP1, NF1, OCT-1, NF-IL6, SP1, TEF-1, TEF-2 and TT1 etc, by controlling HPV's genetic expression and duplication, which plays important roles during the occurrence and development of cervical cancer [4]. The following view is generally supported by many researches that the relationship between HPV16 LCR gene variation and the occurrence of cervical cancer is much closer than that with other region's variation[5, 6].

China is the area where cervical cancer is highly popular. HPV has a high detection rate in Chinese cervical cancer tissues, which is the major pathogenic mi-

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croorganism. Many binding sites for transcription factors of numerous cells and virus rejoining are located at HPV16 LCR.

Nucleotide sequence variations of LCR binding sites affect the function of activating and regulating the activity of HPV16 enhancer, which plays an important role in the occurrence of cervical cancer. Whether HPV16 LCR gene variation exists and whether it contains varied binding sites for viral or cellular transcription factors needs to be studied in order to explore its effect upon the occurrence of cervical cancer and provide hinters for further exploration into disease-causing mechanism.

Based on this, DNA sequence of HPV16 LCR in cervical cancer tissue specimen is analyzed to provide gist for molecular carcinogenic mechanism of cervical cancer.

MATERIAL AND METHODS

Tissues

52 cervical cancer tissues were collected from de-

Table 1 HPV16 LCR sequence variation

Case	Variation Sites	Reference Sequence	Variation Sequence	Virus or Cell Factors Sites bound by
2	7197	G	T	TEF-1
2	7199	T	C	
3	7518	G	A	YY1
3	7665	T	A	
3	7674	G	T	NF1
5	7493	-	T	
5	24	C	G	
5	94	G	A	
8	7711	T	G	NF1
8	7518	G	A	YY1
11	7268	C	T	
11	7285	A	C	
13	7486	-	G	YY1
13	7518	G	A	YY1
13	7657	A	G	
16	7727	A	C	
17	7839	C	T	Oct -1
21	24	C	T	
21	180	G	T	
24	7155	G	T	
25	7170	T	C	
27	7173	A	C	
27	7175	T	C	
27	7191	G	T	TEF-1
29	7199	T	G	

Add: Nucleotide sequence sites 7431-7433 shift from GC to CGG and A deletion at 7861, are found by comparing with the original one (published but not revised nucleotide sequence) and thus not counted variation.

Table 2 LCR´s common binding sites for viral or cellular factors

Factors	TEF−1	NF1	YY1	AP−1	Oct −1	E2
Binding sites	7189, 7466, 7617, 7688, 7705, 7738, 7821	7476, 7557, 7590, 7678, 7714, 7745, 7770	7438, 7483, 7517, 7600, 7619, 7647, 7706, 7822, 7983	7306, 7634, 7651, 7814	7469, 7735, 7839, 7975	7454, 7861 7942, 7957
Sites sequence	YRCATDBYDB	TTGGC	MCATNKT	TKWNTMA	AANWGYAB	ACC(N)6GGT

Add: K=G or T; W=A or T; M=A or C; R=G or A; Y=T or C; B=G, T, C; D=G, A, T; N=G,A,T,C.

partment of Gynecologic Oncology, Cancer Hospital of Shanxi Province of China. All the specimens had never received radiological or chemical treatment and were confirmed(one section of resected tissues) to be cervical cancer tissues by department of pathology in Cancer Hospital of Shanxi Province. After resection they were immediately storied at −70℃ refrigerator until use. Before colletion, the basic information such as age, career, the stage of oncology, pathological types *et al.* was considered. All specimens were consented by the patients.

DNA extraction

Reagent kit for microextraction (obtained from Weitejie Gene and Biol−Tech. Ltd) contained reagents, 2ml Microfuge tube, Filter and DNA extraction tube, etc.

Primer

HPV16 LCR primers (designed and synthesized by Beijing Aoke Company)were 5´−CAC CCA CCA CCT CAT CTA CCT CT−3´ (nt7099−7121) and 5´−CTC ACG TCG CAG TAA CTG TTG CT −3´ (nt182−204), producing a 1026bp fragment.

PCR

50μl PCR mixture contained: Taq DNA polymerase5μl, 25pmol/l upper −stream primer 2μl, 25pmol/l down −stream primer 2μl, 10nmol/l 4 × dNTP2μl, 10 ×Buffer25mmol/l 5μl, 25mmol/l$MgCl_2$4μl, template DNA 2μl. PCR conditions: DNA was first denatured at 94℃ for 10 min, and then 35 cycles amplification were carried out in a thermal cycler as follows: 1 min for denaturation at 94℃, annealing at 62℃, extension at 72℃, with 10 min for an initial denaturation at 94℃ and a final elongation at 72℃.

Product assay

The PCR products were analyzed on a 2% agarose gel stained with ethidium bromide.

DNA sequence analysis

PCR product was purified by Mini−DNA purification kit(Beijing Saibaisheng Gene Tech Ltd. Company). After purification, PCR product was mapped by ABI gene sequence analyzer, measured by Shanghai Jikang Biology Ltd. Company. All the specimen receives forward and reverse sequencing and the result is analyzed by contig software. The DNA segment length is 1026bp. The nucleotide −sites variants were found by comparison between the spliced complete HPV16 LCR sequence and the original one, NCBI gene bank: K02718.

RESULTS

In this study, 25 nucleotide sequence variations derived from 13 cervical cancer specimens (each corresponds with one patient) were found by the comparison with reference sequence. They were 7197G →T, 7199T →C, 7518G →A (3 Specimens), 7665T →A, 7674G→T, 7493→T, 24C→G, 94G→A, 7711T→G, 7268C→T, 7285A→C, 7486→G, 7657A→G, 7727A→C, 7839C →T, 24C →T, 180T →G, 7155G →T, 7170T →C, 7173A →C, 7175T →C, 7191G →T, 7199T→G. See table 1 and figure(1−6).

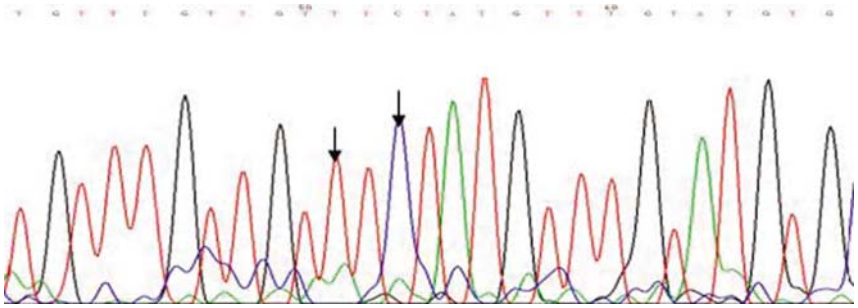

Fig. 1 LCR Gene 7197, 7199 Coden variation(7197G→T,7199 T→C. Case2, 7197 is TEF−1factor binding site)

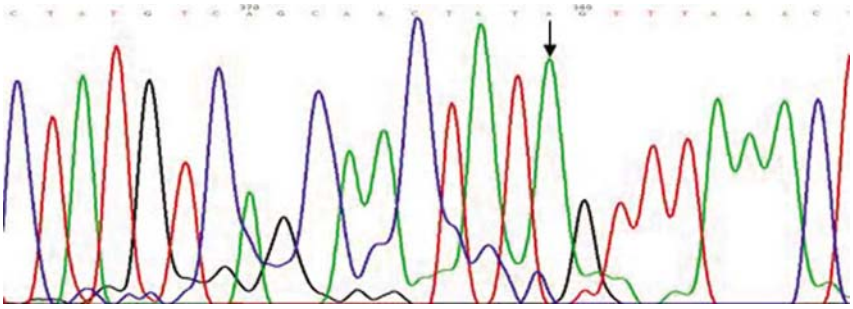

Fig. 2 LCR Gene 7518 Coden variation(7518G→A.Case 3, YY1factor´s binding site)

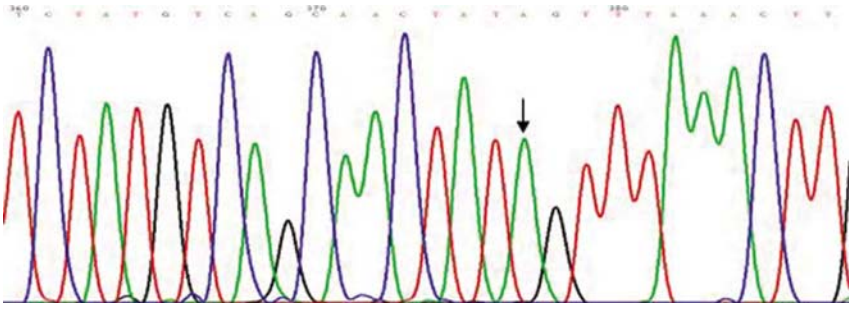

Fig. 2 LCR Gene 7518 Coden variation(7518G→A.Case 8,YY1 factor´s binding site)

transcription factors. Of which, 3 happened at the same binding site (7518 G→A) for the binding factor YY1; the rest happened at the binding sites for TEF −1 (7191G→T; 7197G→T), NF1(7674G→T; 7711T→G), Oct −1 (7839C→T) and another binding site for YY1 (7486→G).

DISCUSSION

HPV−16 LCR are located between the end code of L1 region and the beginning of E6 region with 833bp length from bp7155 to 82, which accounts for 10.54% of the total length of HPV16. LCR does not contain ORF, but contains considerable binding sites for viral or cellular transcription factors，which regulate the expression and duplication of HPV16, see table 2.

There are 4 E2 protein binding sites within LCR. They divide LCR into three fragments according to the location of NO.1 and No.2 binding sites: 5´fragment, central fragment and 3´fragment. Numerous binding sites for E2 protein and viral or cellular factors are located at the central and 3´fragments, see picture 1−6.

In this research it has been found that the two variations of binding sites for NF1 cellular transcription fac-

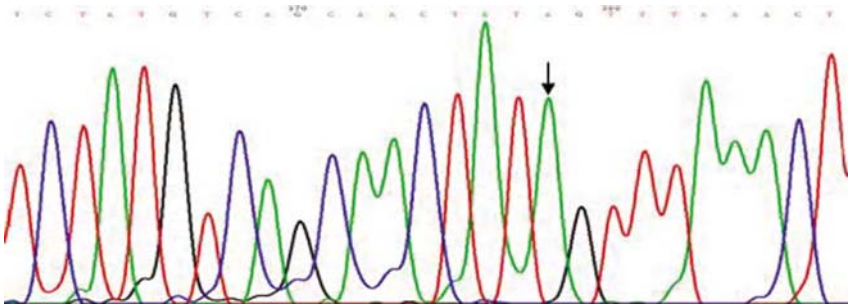

Fig. 4 LCR Gene 7518 Coden variation(7518G→A.Case 13, YY1 factor´s binding site)

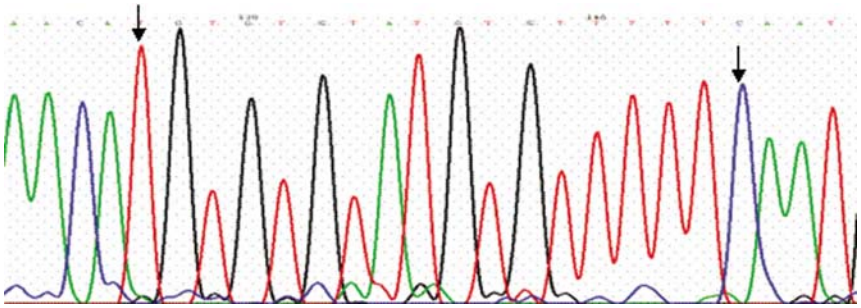

Fig. 5 LCR Gene 7268, 7285 Coden variation(7268C→T,7285A→C. Case 11)

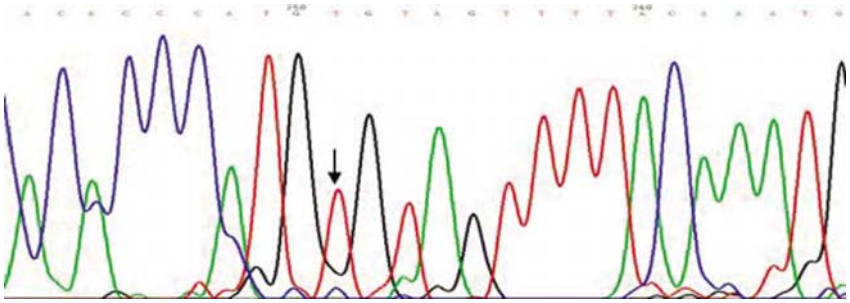

Fig. 6 b LCR Gene 7839C→T Coden variation (7839C→T.Case 17, Located at Oct−binding site)

tors occurred at the central fragment of LCR (7674G→T; 7711T→G). Nucleotide sequence variation at the binding sites will inevitably result in structural change. NF1 as a cellular transcription factor has a highly specific structure for the binding sites. The change of structure may bring about failed NF1 binding and other binding factors may take the place of it. The promoter may be increased or inhibited and the expression of HPV gene may be influenced by it.

In this research it has also been found that the varied site of Oct−1 is (7839C→T), and its variation increases activity of enhancer, and is apted to the occurrence of cancer.

The structures of binding sites for TEF−1, TEF−2 and YY1 are quite similar and overlapped[7]. The majority of the binding sites for YY1 factor located in HPV enhancer overlap with the binding sites of TEF−1. It has been found in this research that nucleotide sequence variations of two TEF−1 binding sites (7191G→T; 7197G→T) occur outside the binding sites of YY1 factor, and did not interleave, thus with unchanged nucleotide sequence of YY1 factor´s binding sites, won´t exert any effect upon the activity of YY1 factor.

It has been found that the variations of binding sites

for YY1 factors occur in three specimens in this research. They are 7518 G→A, 7486→G. In these 3 specimens, the variation of 7486→G for YY1 factors occurs once and the variation of 7486→G for YY1 factors occurs in all 3 specimens. The variations of YY1 factors could relieve the normal inhibition and promote the expression and duplication of HPV16 virus gene. The frequently occurred variation of 7518 G→A for YY1 factors has not been found in other areas (may be it occurs in some areas but has not been found or we have not read the article about this), therefore it may be one of markers of HPV16 LCR for the cervical cancer in Chinese han population. We will do further reacher to determine the relationship between the variation of 7518 G→A for YY1 factors and the cervical cancer in Chinese han population.

Seven site variations of viral or cellular transcription factors´ binding sites in LCR have been found. These seven variations are attribute to four factors (TEF-1, YY1, NF1, Oct-1) and three of these seven site variations occurred between 7660 to 7890. it has been illustrated that variations occurred between 7660 to 7890 could enhance the activity of promoter [8]. Mutations at 24(C→G)and 7199(T→C) happened twice at the non-binding sites and the other mutations at the binding sites happened only once. However, the mutation of binding sites for AP-1 and E2 protein variation were not found in this research.

K. Kurvinen [2] found that the two mostly appeared mutations nucleotide for HPV16 LCR are 7139 (A→C) and 7521 (G→A), the variation rate amounts to 37% of HPV16 infected cervical cancer patients. It can be inferred that these two site mulations exist worldwide. Smits HL [9] found in the early research that these mutations are very popular among Hollanders and Barbadians. However these two point variations are not found in this research. Maybe these are due to the difference between Chinese and Europeans.

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