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Preferential sites for the integration and disruption of human papillomavirus 16 in cervical lesions

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ABSTRACT

Background: Persistent infection with high-risk human papillomavirus (HPV) is necessary to cause cervical cancer, and integrating viral DNA into the host genome may contribute to the process of carcinogenesis. The underlying mechanisms are still unclear.
Objective: In this study, we aimed to investigate the distribution of HPV 16 integration in the host genome and disrupted sites in the viral genome.
Study design: The physical status of HPV 16 genomes in 46 cervical precancerous and cancerous lesions was determined via ligation-mediated chain reaction (DIPS) using 15 previously published primer sets and 12 newly designed primer sets.
Results: A total of 60 viral-cellular junctions were identified in 31 of 46 specimens, and over 80% of the integration sites in the human genome were located in regions of repetitive elements. The proportion of LSIL-, HSIL-, and SCC-containing integration sites near cancer-relevant genes was 10%, 18.8%, and 33.3%, respectively. The frequency of viral gene disruption was significantly higher ($P < 0.05$) in the *L2* gene than in other regions of the viral genome.
Conclusion: There are sites of preferential HPV 16 integration. The integration sites tend to be located in repetitive regions of the host genome, and some sites are found near cancer-relevant genes. In addition, the HPV 16 genome is more likely to be disrupted in the *L2* gene locus.

1. Background

Persistent infection with high-risk human papillomavirus (HPV) is necessary to cause cervical cancer,[1] the third-most common cancer in females worldwide, with approximately 275,000 deaths each year.[2] The HPV genome is a double-stranded circular DNA of approximately 8 kilobases (kb) that can exist in an episomal form or be integrated into the host genome.[3] The frequency of integration increases with the progression of cervical lesions.[4–8] After HPV integration, the expression of viral oncogenes *E6* and *E7* increases in cervical cancer due to the increased stability of their mRNAs.[9] These continuously expressed oncogenes interact with and inactivate the tumor suppressors *p53* and *RB*.[10,11] In addition, these oncogenes can cause centrosomal abnormalities, aberrant mitotic spindle pole formation, and chromosomal instability.[12–15] DNA replication originating from the integrated HPV genome is triggered when the viral replication proteins *E1* and *E2* are present in the viral integrated sequence, which results in cross-chromosomal translocations in the host genome and may facilitate the formation of HPV-dependent cancer cell.[16,17]

The existence of sites of preferential HPV integration has been controversial. During HPV integration, the viral genome may be disrupted between the *E1* and *L1* genes, though the majority of disruptions have been documented to be located in the *E1* and *E2* genes.[4,6–8,18] Researchers believe that integration sites are

Abbreviations: HPV, human papilloma virus; BCCs, benign cellular changes; LSIL, low-grade squamous intraepithelial lesions; HSIL, high-grade squamous intraepithelial lesions; SCC, squamous cell carcinoma; DIPS, detection of integrated papillomavirus sequences; *URR*, upstream regulatory region; *LINE*, long interspersed nuclear elements; *SINE*, short interspersed elements; *LTR*, long terminal repeat elements; nt, nucleotide.

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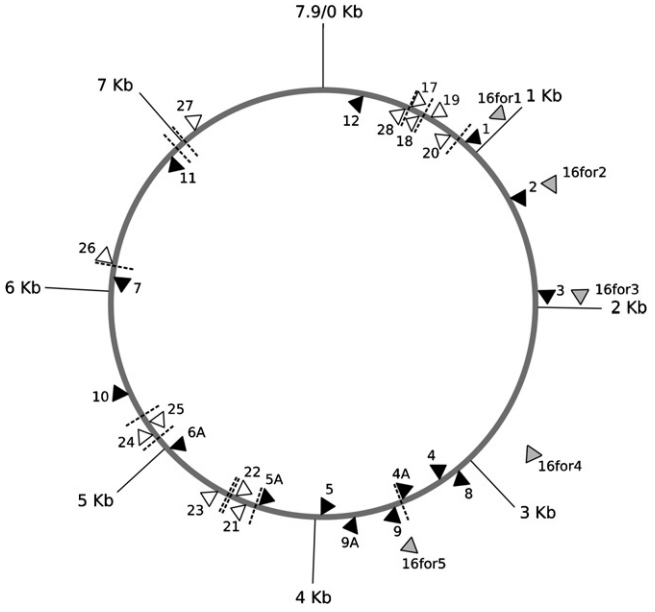

Fig. 1. Primers used for the DIPS analysis of HPV 16. Dotted lines denote the restriction sites via endonuclease *Sau3AI*. Solid black arrowheads and gray arrowheads indicate the primers published by Matovina et al.[7] and Luft et al.,[6] respectively. Hollow arrowheads represent the new primers designed in this study. Numbers near the arrowheads represent the names of primers.

randomly distributed across the host genome, but there are reports of accumulated HPV integration events in the *MYC* locus and common fragile sites.[19–21] A recent study demonstrated identical viral-cellular fusion transcripts in different samples.[22] Therefore, the phenomenon of non-random integration of HPV DNA is intriguing. It also remains unclear whether only singular integration events are necessary for the process of cervical transformation. To better understand the underlying mechanisms of HPV integration in cervical carcinogenesis, we performed an extended analysis of genomic integration loci and viral gene disruption sites using the Detection of Integrated Papillomavirus Sequences (DIPS-PCR) method.[6] In addition to the 15 published nested primer sets,[7] 12 newly designed primer sets were utilized in this study (Table 1 and Fig. 1). The new primer sets allowed us to detect additional integration sites in cervical lesions and define new disruption sites across the entire HPV 16 genome.

2. Objective

To better understand the underlying mechanisms of HPV integration in cervical carcinogenesis, we evaluated liquid cytology cervical samples for evidence of preferential sites for HPV16 integration and viral genome disruption.

3. Study design

3.1. Cell lines and clinical specimens

Cervical cancer cell lines SiHa and CaSki were purchased from the Cell Resource Center, IBMS, CAMS/PUMC (Bejing, China) and cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum. A total of 338 specimens were obtained from outpatients aged 21–66 years (mean age: 40.4 ± 9.2 years; median age: 41.0 years) attending the Department of Obstetrics and Gynecology between June 2009 and March 2010. Cervical samples were collected using an endocervical Cytobrush and were then placed into 20 ml of PreservCyt Solution (Cytyc Corp., Boxborough, Massachusetts, USA) and stored at 4 °C. Cervical

Table 1
Detail information of the new DIPS-PCR primer sets for HPV 16.

Linear PCR			Exponenitial PCR			Expected amplicons				
Primer	5′ nucleotide	5′→3′ sequence	Primer	5′ nucleotide	5′→3′ sequence	Primer sets used	Annealing temperature (°C)	5′ nucleotide	Target *Sau3AI* restriction site	Size (bp)
17	530	TCAAGAACACCTAGAGAAACCCAGC	17N	534	GAACACGTAGAGAAACCCAGCTGT	17/17N	60	534	621	136
18	587	TGCAATGTAGGTGTATCTCCATGCA	18N	572	TCTCCATGCATGATTACAGCTGGGT	18/18N[a]	60	572	621	92
19	643	TTAAGTGACAGCTCAGAGGAGGAGG	19N	665	AGGATGAAATAGATGGTCCAGCTGG	19/19N	60	665	870	254
20	834	GCACACAATTCCTAGTGTGCCCA	20N	820	GTGTGCCCATTAACAGGTCTTCCA	20/20N[a]	60	820	870	243
21	4397	TTTTGGTGGGTTAGGAATTGGAACA	21N	4418	AACAGGGTCGGGTACAGGCCG	21/21N	60	4418	4520	151
22	4511	AGGGGGTCTTACAGGAGCAAGTGT	22N	4490	TGTATCTGTAGCTGTGGCAGGCC	22/22N[a]	60	4490	4520	173
23	4588	GTGCACCAACATCTGTACCTTCCAT	23N	4600	CTGTACCTTCCATTCCCCCAGATGT	23/23N	60	4600	5072	521
24	5103	CATAGGCCAGCATTAACCTCTAGGC	24N	5107	GGCCAGCATTAACCTCTAGGCG	24/24N	60	5107	5234	176
25	5141	CCTAATGCCAGTACCCCTAGAGGT	25N	5135	GCCAGTACCCCTAGAGGTTAATGC	25/25N[a]	60	5135	5234	107
26	6152	GATCCCCATGTACCAATGTTGCAGT	26N	6164	CCAATGTTGCAGTAACTCCAGGTGA	26/26N	60	6164	6951	836
27	7047	ACAAGCAGGATTAAAGGCCAAACCA	27N	7101	ACCCACCACCTCATCTACCTCTACA	27/27N	62	7101	524	1377
28	512	TACATCGACCGGTCCACCGA	28N	504	CCGGTCCACCGACCCCTTATATTA	28/28N[a]	62	504	524	1339

[a] Antisense primer sets were designed to detect the 5′ viral-cellular junctions. Nucleotide positions of primers, and their downstream *Sau3AI* sites were indicated according to HPV 16 East Asian type, of which the Genbank accession numbers was AF534061.1.