GENETIC INSTABILITY IN CERVICAL CARCINOMA

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ABSTRACT

Objective: The role of human papillomavirus (HPV) in the development of cervical carcinoma has been clearly established but other factors could be involved in cervical tumorigenesis such as loss of heterozygosity (LOH) and microsatellite instability (MI). The aim of the present study was to investigate the genetic instability in cervical carcinoma tissues and provide evidence for discovering new tumor suppressor genes and screening diagnostic molecular marker of cervical carcinoma. Methods: Fifty primary cervical carcinoma samples from high-incidence area were analyzed by PCR for HPV16 infection, LOH and microsatellite instability. Results: HPV16 was detected in 88% of the cases. Sixty-six percent of total cases showed LOH with no more than 3 different loci per case. The highest frequency of the allelic loss was found in D18S474 (18q21, 40.5%). MI was detected in 4 cases (8%) only. Conclusion: Different percentages of LOH on specific chromosomal regions were found and MI was very infrequent in cervical carcinoma. The putative suppressor gene(s) could be located on specific chromosome regions such as 18q, and genetic instability could be involved in cervical tumorigenesis.

Key words: Cervical carcinoma, Human papillomavirus, Genetic instability, Loss of heterozygosity, PCR[1]

Cervical carcinoma is one of the most common malignancies in women in developing countries. Many studies have linked the presence of specific types of human papillomavirus (HPV) such as HPV16 and 18 with the development of cervical carcinoma[1]. However,HPV infection alone is probably insufficient for complete neoplastic transformation of the cervical epithelium. Additional genetic alterations, especially involving tumor suppressor gene (TSGs) seem to be required for development and progression of cervical carcinoma[2,3]. Inactivation of p53 and Rb proteins by E6 and E7 proteins from integrated oncogenic HPV strains is an important component of TSG inactivation in the vast majority of HPV-positive cervical carcinoma[4-7]. Other mutations or deletions due to genetic instability including loss of heterozygosity (LOH) and microsatellite instability (MI) are frequently accompanied by loss of the remaining allele, leading to homozygous inactivation of the gene. Several studies have shown that at specific chromosomal sites such as 1p, 1q, 4p, 5p, and 17p genetic changes are frequently associated with development of cervical carcinoma[8-10]. However, data about the role of genetic changes (besides HPV infection) in the progression of cervical carcinoma are meager. Knowledge of these genetic changes in conjunction with the viral status of cervical cancer might lead to improvement methods of prognosis and development of more effective therapeutic strategies.

Genetic instability has been studied in different tumors, such as colon, gastric, breast, et al. On the other hand, the oncogenic potential of LOH or MI in cervical tumorigenesis is not yet completely understood. Moreover, a combined molecular, viral, and cytogenetic status of cervical carcinoma samples from high-incidence area in China was undone. This study was set to investigate the correlation between HPV infection, genetic instability and cervical carcinoma.

MATERIALS AND METHODS

Tumor Samples

Fifty untreated primary cervical carcinoma and the corresponding peripheral blood samples from patients were collected. All samples were obtained from Wufeng

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County, a high incidence area of cervical carcinoma in Middle West of China. Clinically, the 50 tumors were classified as follows: stage 0 (in situ cancer), 16 cases; stage I 10 cases, stage II, 16 cases; stage III, 8 cases. Histologically, the 50 cases consisted of 48 squamous cell carcinomas and 2 adenocarcinomas. The ages of the patients ranged from 34—76 years.

DNA Amplification and Analysis

High molecular weight DNA from tumor and peripheral blood samples was isolated using standard procedures of proteinase K-SDS digestion and phenol-chloroform extraction[11].

HPV16 is the major infection factor in-patients with cervical carcinoma in China. Each tumor samples were analyzed by PCR for HPV16 E7 gene sequence, using primers: sense, 5'CGAGATCACATGGAGAGAAACC-CAGCT 3'; antisense, 5' ACGCCTCCGAG AGGAT-CAGCCATGGTAGAT 3'. The size of the amplified sequences was 312 bp. The amplification reaction mixture consisted of 2 μl (200ng) of DNA, 5 μl of 10×PCR buffer, 50 μM of each dNTP, 10 pmol each of the primer, 1 U Taq DNA polymerase (Biostar), and water to make a final volume of 50 μl. An overlay of 40 μl mineral oil was then applied. The amplification reaction was electrophorezed through 1.5% agarose gel and analyzed by UV after staining with ethidium bromide.

Both normal and tumor samples were analyzed using a panel of 8 microsatellite markers whose names,chromosomal location, and heterozygosity are detailed in Table 1. The amplification was performed in a 20 μl reaction mixture containing 1 μl (100ng) of sample DNA, 2 μl of 10×PCR buffer, 2 mM $MgCl_2$, 100 μM of each dNTP, 20pmol each of the primer, 1 U Taq DNA polymerase (Bio-star), and water to make a final volume of 20 μl. An overlay of 20 μl mineral oil was then applied. The amplification products (5μl) were electrophorezed through 2% agarose gels and analyzed by UV after staining with ethidium bromide. The PCR products (5μl) were then diluted with an equal volume of formamide to which 0.25% xylencyanol and 0.25% bromphenol blue were added, denatured at 95℃ for 10 min and run on 6% polyacrylamide gels containing 7 M urea. Electrophoresis was conducted in 1×Tris-boric acid-EDTA buffer.

LOH was considered to have occurred either when an allelic band was present in the control lane but absents in the tumor lane, or when it was one half or less the density of the corresponding band in the tumor lane[12,13]. MI was detected by a shift in the mobility of one or both alletes and extra CA repeat bands in tumor DNA loci in invasion cancer and in situ cancer was examines by χ^2 test.

RESULTS

In this study, HPV16 was detected in 44 out of 50 cases (88%). No significant differences were found between the rate of HPV infection and clinical stages. LOH was observed at one or more loci in 33 of LOH on specific chromosomal regions were found. The most common chromosomal aberrations was detected on 18q21 and 3p21. The highest frequency of the allelic loss was found in D18S474 (18q21, 40.5%) and the lowest frequency of LOH was found in D18S35 (8.7%) (Table 1 and Figure 1—4). In our study, MI was only detected in 4 cases (8%). In addition, we compared the frequency of LOH in patients at histopathological stage 0 (in situ cancer) with histopathological stage I—III (invasion cancer) and found that the LOH frequency of D18S474 was very high in invasion cancer (51.85%), but low in situ cancer (20%) (Table 2).

Table 1. Loss of heterozygosity on chromosomes 3, 6, 11 and 18 in cervical carcinoma

Sites	Chromosome position	Het	Number of effective information*	LOH(%)
D3s1478	3p21.2—3p21.3	0.88	41	13(31.7%)
D3s1766	3p14.3—21.1	0.76	40	6(15.0%)
D6s260	6p23	0.84	43	10(23.3%)
D11s925	11q23—24	0.84	39	7(17.95%)
D18s35	18q21.1—21.31	0.78	46	4(8.7%)
D18s474	18p21.1	0.82	42	17(40.5%)
D18s64	18q21.32	0.73	36	6(16.7%)
D18s68	18q22.1	0.78	44	12(27.3%)

*: The genomes of tumor and control tissue from the same sample were both successfully amplified.
LOH: Loss of heterozygosity; Het: heterozygosity;
χ^2= 18.54, P<0.01