

INTRODUCTION

Carcinoma of the uterine cervix is the most common cancer of women in developing countries. For almost 100 years it has been recognized that most cervical cancer and its precursor lesions have many characteristics of sexually transmitted diseases. A number of sexual factors, such as initial intercourse at an early age, number of sexual partners and history of sexually transmitted diseases have shown to be risks for developing cancer of the uterine cervix (Boyd and Doll, 1964; Barron and Richart, 1968; Roitkin, 1973; Kessler, 1974; Davesa, 1984). However, these observations suggested that sexually transmitted agents may play a part in the genesis of the cancer. Because of this venereal association, studies on the etiology of cervical cancer were focused mainly on a variety of infectious agents. In the past, most common infectious agents, including *Trichomonas vaginalis*, *Chlamydia trachomatis*, cytomegalovirus and herpes simplex virus, were studied extensively by many investigators. Although initial studies suggested that some of those agents were associated causally with cervical cancer, more detailed investigations into this relationship found that it was unlikely to be a causative. It now appears that the association is due to the fact that both are related to the sexual habits of the population at risk. In contrast to those agents, lesions induced by HPV infection have morphological features in common with certain cervical cancer precursor lesions. Currently, for different reasons, human papillomaviruses (HPVs) were the only viral agents receiving great interest from many investigators, and their causal role in carcinogenesis has also been supported by several studies.

Human papillomaviruses (HPVs) are common sexually transmitted pathogens. They are a group of small DNA viruses that infect skin (cutaneotropic type) and mucosal epithelium (mucotropic type). Until now, there have been more than 70 HPV genotypes identified. Cutaneotropic viruses are almost always associated with benign warts that are usually found on infected hands or feet. Mucotropic viruses infect the anogenital tract preferentially at varying degrees in association with malignancy,

particularly in cancer of the uterine cervix. However, some types of virus are associated with benign lesions, especially cervical condylomata.

The oncogenic potential of papillomaviruses was first observed in cottontail rabbits infected with a cottontail rabbit papillomavirus (CRPV) that induces hornlike papillomas lesions (Shope *et al*, 1933). Research on the papillomavirus was hampered for a long time by the fact that they could not be propagated in laboratory conditions. Not until the development of the recombinant DNA technology in the late 1970s, were the first papillomavirus genomes successfully cloned in bacteria (Law *et al*, 1978; Gissmann *et al*, 1985). Much of the current understanding of HPV-related carcinogenesis derived from manipulations of cloned viral DNA *in vitro*. It was clear that the high-risk types of those HPVs have an ability to transform and this function resided in an early gene region, particularly in the E6 and E7 open reading frames (ORFs). Expression of both E6 and E7 ORFs is required for full transformation and maintenance of the malignant phenotype.

Studies concerning the state of viral DNA in cervical cancer cells and cell lines, which derived from cervical cancer, showed that most of the viral DNA were integrated into the genome of those cancer cells, particularly in malignant lesions, whereas, episomal forms were detected mostly in premalignant states. However, the integrated DNA was truncated at various degrees. While the regulatory E2 ORF was invariably deleted, the E6 and E7 ORFs were consistently retained and expressed. Thus, this observation indicated the requirement of E6 and E7 ORFs in malignant transformation *in vivo*. So far, at least four species of E6/E7 mRNA have been identified in HPV-16 infected cells; three spliced transcripts, E6*I, E6*II and E6*III; and an unspliced full-length E6 transcript. Functional analysis of those transcripts suggested that E6*I encoded the E7 protein while E6*II and unspliced E6 putatively encoded E6 proteins. The encoding potential of the E6*III is still unknown. The generation of E6/E7 mRNA by splicing appears to be one of the major factors differentiating HPVs of a high and low carcinogenicity. However, it is not known whether these transcription patterns correlate with the phenotype of infected cells. Also, it is unclear whether there are any

clinical implications. Although some investigators failed to correlate the patterns of viral transcription in either the histological type of tumors or FIGO stages (Rose *et al*, 1995), the quantitative analysis of specific transcripts, E6*I/E6*II, in cancer-derived cell lines showed convincing results. The E6*I was found in 97.1% of total spliced transcripts, while the E6*II comprised only 2.9% of the spliced product (Hsu *et al*, 1992, 1993).

Infections by most genital HPVs range from subclinical or asymptomatic, at which the virus stays latent in the basal cells, to those of severe diseases such as carcinoma. Before developing to carcinoma the virus needs a long latency period of approximately 3 to 10 years in infected persons, during which, the viral genomes persist and are consistently detected. Thus, the detection of viral DNA from cervical specimens alone may not provide a sufficient prognostic value for cancer development. Active expression of specific viral genes, which are responsible for malignant transformation, may provide more direct information about the transition of the diseases from a latency state to cancer. This study has been interested in identifying the prognostic indicator that could be used in conjunction with HPV DNA detection to provide a guidance for the management of HPV infected persons.

In this study, HPV DNA extracted from cervical cell scrapes and tissue biopsy samples obtained from individuals with cancer or a normal cervix were amplified and genotyped by using the polymerase chain reaction (PCR) based restriction fragment length polymorphism (RFLP) technique. For genotyping, 4 restriction endonuclease, *Rsa* I, *Dde* I, *Hae* III and *Hinf* I were used. The RFLP patterns were analyzed by comparison with the patterns obtained from the referent HPV prototypes. The expression of HPV E6/E7 genes from HPV-16 and -18 containing cells were analyzed further by using the reverse transcriptase polymerase chain reaction (RT-PCR) technique. The fluorescent-dye labeled primer, which was incorporated into RT-PCR product fragments, was used as an aid in the quantitative analysis of the transcripts. The fluorescent-dye labeled DNA fragments were analyzed by using an automatic genetic analyzer (ABI Prism 310 Genetic Analyzer, Perkin Elmer USA) with a computer program, GeneScan™ Analysis 2.1 Software (Perkin Elmer USA). The data obtained from each

sample were computed, and it displayed the relative amount of HPV-16 or -18 E6/E7 transcriptions in graphic pictures. The ratio of E6*I to E6*II was calculated and analyzed together with histological types of cancer.

Aims of the study

1. To evaluate the prevalence and genotype distribution of HPV infection in cervical samples.
2. To evaluate the relationship between HPV E6 and E7 gene expression and tumor histology.
3. To develop the sensitive, quantitative technique for E6/E7 mRNA detection.