A research for the relationship between human papillomavirus and human uterine cervical carcinoma

II. Molecular genetic and ultrastructural study on the transforming activity of recombinant retrovirus containing human papillomavirus type 16 subgenomic sequences

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Summary. In order to elucidate the role of HPV-16 in the development of genital cancer, NIH3T3 cells were transfected by HPV-16 whole genome and its two early genes, E6-E7. Besides ordinary calcium phosphate/DNA coprecipitation technique, a newly designed recombinant retrovirus containing the HPV-16 genome or subgenomes was used to infect cells for transfer of the target genes. The transforming activities have been demonstrated to be most efficient when a bioengineering technique of this kind is used. HPV-16 DNA was proved to have transforming potential for NIH3T3 cells, and the DNA of HPV-16 was proved to undergo multi-site integration into transformed cells and nude mice tumour cells. The E6-E7 open reading frames are sufficient for transforming NIH3T3 cells independently in vitro, which implies that E6-E7 open reading frames are transforming genes or even viral oncogenes of HPV-16. The RNA transcribed by the E6-E7 of HPV-16 was expressed in transformed cells and in tumour cells of nude mice. The use of a recombinant retrovirus for gene transfer in this study is much more efficient than that of calcium phosphate/DNA coprecipitation. The lack of a tissue-culture system suitable for HPV replication in vitro makes HPV gene recombination into a specially engineered retrovirus for viral-mediated gene transfer of particular significance for the possible application of viral carcinogenesis, both in vitro and in vivo, for basic and clinical research.

Key words: HPV - Transforming activity - Recombinant retrovirus - Viral oncogene

Introduction

In recent years, there has been increasing evidence that human papillomaviruses (HPV) are emerging as the major agents for the etiology of human cervical carcinoma through clinical, epidemiological, molecular biological and tumour virological studies (Stanbridge et al. 1981; Fukushima et al. 1985; Howley 1989; zur Hausen 1985; Gao et al. 1988). More than 60 HPV genotypes have been isolated and characterized, and the heterogeneity of HPV types is considered to reflect the adaptation of these viruses in specific differentiated tissues (zur Hausen 1989). HPV-6, 11, 16, and 18 have been found in neoplastic lesions of the genital tract (Pecoraro et al. 1989).

In our laboratory, a molecular hybridization study showed that 60.4% of biopsies obtained from Chinese patients with cervical cancer contained a sequence homologous to HPV-16 DNA; it is therefore the most prevalent viral type in Chinese women (Si et al. 1987). Our earlier work also showed that the early-region E6 and E7 genes of HPV-16 might play a key role in the carcinogenesis of cervical cancer (Si et al. 1990).

Clinical observations and recent experimental results have suggested that the development of cervical cancer, like other cancers, involves multistage processes and multiple etiological agents (Weinstein 1988). In order to elucidate the role of HPV-16 in the development of genital cancer, the present study was designed to transfected cultured NIH3T3 cells with HPV-16 whole genome and its two subgenomic genes, E6 and E7, separately. In addition to the ordinary calcium phosphate/DNA coprecipitation technique, the method of a newly designed recombinant retrovirus containing the HPV-16 genome or subgenomes was used to infect cells for transferring the target genes. The transforming activities have been demonstrated to be more efficient when this kind of bioengineering technique is used compared with the results of ordinary methods.
The transforming activity of HPV-16 was first observed in mouse NIH3T3 cells (Yasumoto et al. 1986; Noda et al. 1988; Bedell et al. 1987). Several laboratories have shown that much more recalcitrant cells, both of fibroblast and epithelial origin, are efficiently transformed by HPV DNA singly or cooperatively with other agents such as oncogene EJ-ras, etc. (Tsunokawa et al. 1986; Matlashewski et al. 1988). Up to now, no report has been found on the use of a recombinant retrovirus as a vector for HPV gene transfection.

Materials and methods

Plasmid preparation

A plasmid pSV2-neo/HPV16, containing the whole HPV-16 genome, cloned into the BamHI site of pSV2-neo, and plasmids HZIP-16 and HZIP-16K, containing the total early genes (6.6 kb) as well as the E6–E7 (1 kb) subgenomic sequences in plasmid pZIP-NeoSV(X), were kindly provided by McCance (McCance et al. 1988) and Matlashewski (Matlashewski et al. 1987) (Figs. 1 and 2).

Recombinant plasmids pSV2-neo/HPV16, HZIP-16, HZIP-16K and pZIP-NeoSV(X) were transfected into E. coli by a modified method of Mandel (Mandel and Higa 1970).

Preparation of pSV2-neo, pZIP-NeoSV(X) vector plasmids

The pZIP-NeoSV(X) vector consists of the Moloney murine leukemia virus (M-MuLV) transcriptional unit, derived from an integrated M-MuLV provirus, and PBR322 sequences necessary for the propagation of the vector DNA in E. coli. HZIP-16K was hydrolysed into two parts [pZIP-NeoSV(X)] and E6–E7] by BamHI after electrophoresis. The pZIP-NeoSV(X) part was collected and ligated into a circular plasmid by T4 ligase, pSV2-neo was collected after pSV2-neo/HPV16 had been digested by BamHI.

The amplification and purification of plasmids were performed as described by Mandel and Cai (Cai 1987).

NIH3T3 and ψ-2 cells culture. NIH3T3 cells were maintained in Dulbecco modified eagle medium (DMEM) supplemented with 10% calf serum, 0.2 mmol/l glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin. Cells were grown in a humidified 5% CO₂ incubator at 37°C and fed every 3 days.

ψ-2 cells were cultured in the same conditions as NIH3T3 cells.

Transfections

pSV2-neo/HPV16 plasmid transfection into NIH3T3 cells was performed by the calcium phosphate/DNA coprecipitation technique as described by Wigher (Wigher et al. 1978) and Davis (Davis et al. 1986). After transfection for 72 h, NIH3T3 cells were digested by trypsin and cultured in DMEM with G418 (400 μg/ml) until anti-G418 clone formation.

HZIP-16, HZIP-16K and pZIP-NeoSV(X) were transfected into ψ-2 cell by calcium phosphate/DNA coprecipitation. The ψ-2 cell line contains integrated copies of the M-MuLV provirus genome, which provides all the trans functions necessary for the encapsidation of a recombinant genome, whereas it is unable to encapsidate its own RNA. ψ-2 cells were digested by trypsin 72 h after infection and cultured in DMEM medium containing G418 (400 μg/ml), until the formation of an anti-G418 cell clone that could produce defective retrovirus (pZIP-ψ2, HZIP16-ψ2 and HZIP16K-ψ2 cells). After culture for 18 h, the culture fluid of cloned ψ-2cells (pZIP-ψ2, HZIP16-ψ2, HZIP16K-ψ2) cells containing a large number of virions (viral fluid) was harvested, filtered through a 0.45-μm microporefilter, then added into NIH3T3 cell culture medium at 37°C in a 5% CO₂ incubator. After 2 h, some fresh medium was added to the NIH3T3 cell culture flask. The transformed NIH3T3 cells (HZIP16-3T3 and HZIP16K-3T3 cells) could be formed by infection with recombinant retrovirus after culture in G418-containing (400 μg/ml) DMEM medium for selection of transformed cells.