



# Detection of human papillomavirus DNA in fine needle aspirates of women with breast cancer

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## SUMMARY

**Background:** HPV infection is the most commonly distributed sexually transmitted disease. Human papillomavirus has also been linked to malignant tumors of many human organs. The presence of viral DNA in breast cancer cells is controversial. The aim of the present study was to investigate the presence of HPV-DNA in a group of Greek women with breast carcinoma.

**Methods:** Liquid cytology specimens from 35 malignant breast cases and 35 cases with benign breast lesions were investigated by PCR (clinical arrays technique). In addition, *in situ* hybridization was performed on all HPV positive cases.

**Results:** HPV-DNA was detected in 17.14% of the carcinoma cases and HPV16 DNA was present in 83.3% of them. All benign breast lesions were negative for HPV-DNA.

**Conclusion:** Our report confirmed the presence of HPV in breast cancer cells while the most prevalent type was HPV16. More studies are necessary in order to elucidate the pathogenesis of HPV and a possible way of prevention of some breast cancers.

**Key words:** Breast Neoplasms; Polymerase Chain Reaction; DNA Probes, HPV; Human papillomavirus 16

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## INTRODUCTION

Human papillomaviruses (HPVs) are double stranded DNA viruses that exhibit a high degree of cellular tropism for squamous cells and were accepted as being carcinogenic and have a direct causal relationship with 95% of all cervical cancers (1-5). More than 100 HPV types have been identified associated with various lesions.

HPV infection is the most commonly distributed sexually transmitted disease and spread easily through genital contact. Two types of genital tract HPV16 and HPV18 are known to cause the majority of cervical malignancies (their E6 and E7 proteins are capable of strong binding).

The HPV virus has eight genes. The early genes E1 and E2 are involved in viral genome replication and transcription, whereas the E5 gene enhances the activity of epidermal growth factor. E6 and E7 control the transcription and late genes. L1 and L2 encode viral capsid proteins.

A significant factor in carcinogenesis of anogenital and other epithelial carcinomas is persistent infections with HR-HPVs (5).

Human papillomavirus has also been linked to malignant tumors of the larynx, skin, penis, as well as mouth (6-9). Additionally, HPV may have an important role in tonsillar and bladder cancers (10, 11).

Lawson and colleagues at the University of New South Wales and the University of Western Australia published the results of a DNA analysis, which showed that HPV18 gene sequences were present in DNA extracted from breast tumors in Australian women (12). They believed that the evidence is far from conclusive and more research is needed in order to investigate the precise role that this virus plays in breast oncogenesis. However, other scientific reports suggest no viral association with breast cancer pathogenesis (13-16).

The aim of the present study was to investigate the presence of HPV-DNA in a group of Greek women with breast carcinoma.

## MATERIALS AND METHODS

Thirty-five cases of breast cancer and 35 cases of benign breast lesions were selected from the files of the Department of Clinical Cytology.

Liquid cytology specimens were used in all cases collected in vials containing PreservCyt Solution (Cytoc Corp. Boxborough, MA) and stored at 4°C before the DNA extraction.

A total number of 70 women with a median age of 43 years participated in this study.

HPV detection and genotyping was performed with the clinical arrays kit (Genomica) according to the manufacturer's protocol from 1 ml of liquid cytology specimens. For the DNA extraction and purification after centrifugation of the samples for 10 minutes and 12000 rpm, 25 µl of proteinase K solution were added and incubated for 1-3 hours at 56°C in a water bath until the samples were completely lysed. Then, 200 µl of the lysis buffer were mixed with the samples that were incubated at 70°C for 10 min. DNA purification was performed using a DNA purifying column for each sample. The pelleted DNA was resuspended in 100 µl of Elution buffer (preheated at 70°C).

Once the extraction was finished, 5 µl of extracted DNA from each sample and 45 µl of a PCR mix PGMY09/PGMY11 generic consensus primers and internal controls were added to the reaction tubes that were placed in the DNA thermocycler. In order to denature the PCR amplification products, the tubes were incubated at 95°C for 10 min. The denaturation was followed by the hybridization and the blocking and addition of conjugate solution. It was important to use a different tip for each sample and for every reagent.

Amplicons were detected by hybridization in a low density microarray containing triplicate DNA probes specific to 35 genotypes: 20 HR-HPV (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73,

82, 85) and 15 LR-HPV (6, 11, 40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84, 89).

The Clinical Arrays Reader analyzed the arrays automatically and the report with the results was provided.

The results were processed by a specific software.

PCR is the most sensitive technique for identification of low viral loads and replaced in situ hybridization (low specificity and sensitivity).

In addition to PCR, in situ hybridization was performed using the Bond Ready-to-use DNA ISH HPV Probe (subtypes 16, 18, 31, 33, 51) Leica Biosystems, UK, and in accordance to the manufacturer's protocol, on all HPV positive cases.

## RESULTS

The 35 selected invasive carcinoma samples displayed a duct cell carcinoma pattern and did not have family history of breast cancer.

The present study detected HPV-DNA in 6 of the carcinoma cases (17.14%), whereas 26 cases were negative (74.28%).

HPV16 DNA was present in 5 cases (83.3%). The only other high-risk HPV type was HPV18 and it was present in one breast sample (16.6%). All benign breast lesions were negative for HPV-DNA.

All tumor cell samples that had been positive for HPV16 (Figure 1) or HPV18 (Figure 2) by polymerase chain reaction were also positive by in situ hybridization analysis.

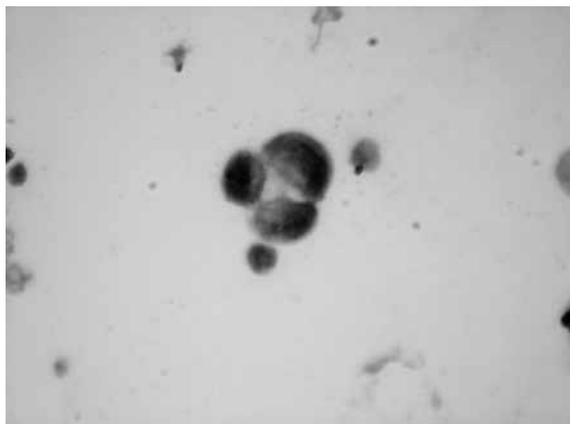


Figure 1. Human papillomavirus detected by in situ hybridization in tumor cells (magnification x40)

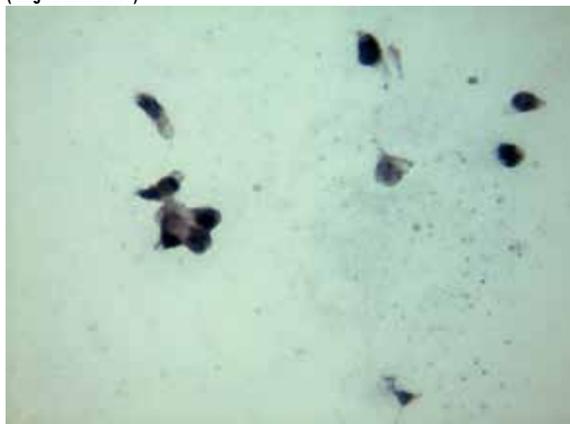


Figure 2. HPV in cancer cells of ductal carcinoma demonstrated by in situ hybridization (40x objective)

## CONCLUSION

It is well accepted that cancers of different human organ sites, other than cervical cancer harbor HPV-DNA (17-20).

There are known risk factors associated with breast cancer such as hormones, alcohol, cigarette smoking, family history, and others that have not been identified such as viral infections. Epstein-Barr virus (EBV) (21) and mouse mammary tumor virus (MMTV) (22) have been suggested to be related to this cancer, as well as HPV. There are studies evaluating the controversial presence of Human papillomavirus in breast lesions. Those studies have demonstrated 24.7%-85% breast cancer cases positive for HPV-DNA (12, 16, 17, 23-25), whereas others found no association between Human papillomavirus presence and breast cancer (13, 14).

Our report confirmed the presence of HPV in breast cancer cells and the most prevalent type was HPV16. We have not found viral DNA in benign lesions.

There are differences in published reports concerning the types of HPV and this fact may be due to demographical reasons. The presence of viral DNA in breast cancer cells leads to the possibility of tumorigenic role for high-risk HPV.

Lawson et al. reported that cell surface-to-surface contact mainly during sexual activities is required for HPV transmission and modulation of cellular pathways (12, 26).

Since the mechanism is not clear, it is quite probable that additional cofactors are needed to immortalize and transform the infected breast cells (24).

More studies are necessary in order to elucidate the etiological role and pathogenesis of HPV in breast cancer, as well as the possibility of preventing some breast cancers by vaccination against HPV.

## Conflict of interest

We declare no conflicts of interest.

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