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

Smaroula N. Divani\(^1\), Angeliki M. Giovani\(^2\)

SUMMARY

Background: HPV infection is the most commonly distributed sexually transmitted disease. Human papillomaviruses have 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 cancer.

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 HPV16/18 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

INTRODUCTION

Human papillomaviruses (HPVs) are double stranded DNA viruses that exhibit a high degree of cellular tropism for squamous cells and are 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 (26). The 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 E6 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 papillomaviruses has also been linked to malignant tumors of the larynx, skin, penis, as well as mouth (6-9). Additionally, HPV may have cofactors are needed to immortalize and transform the infected breast cells (24). Since the mechanism is not clear, it is quite probable that additional cofactors are needed to immortalize and transform the infected breast cells (24).

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 (Cytyc Corp, Boxborough, MA) and stored at 4\(\text{°C}\) 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 lysis buffer were mixed with the samples that were incubated at 70\(\text{°C}\) for 10 min. DNA purification was performed using a DNA purifying column kit (Genomica) according to the manufacturer’s protocol from 1 ml of liquid cytology specimens. In order to denature the PCR amplification products 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\(\text{°C}\) before the DNA extraction. The results were processed by a specific software.

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, cigarettes 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. These 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).

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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 v-raf and v-src expression (their E6 and E7 proteins are capable of strong binding). A significant factor in carcinogenesis of anogenital and other epithelial cancers is persistence (their E6 and E7 proteins are capable of strong binding).

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 (16.7%). 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.

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 29 cases were negative (74.28%).

CONCLUSION

It is well accepted that cancers of different human organ sites, other than cervical cancer favour HPV-DNA (17-20). There are known risk factors associated with breast cancer such as hormones, alcohol, cigarettes 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. There 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.

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.

REFERENCES

Increased mean corpuscular volume as a predictor of response during bevacizumab treatment

Aneta Lida Żygulska, Krzysztof Krzemieniecki

SUMMARY
Background: Remission during sunitinib (a multikinase inhibitor and antiangiogenic drug) treatment correlates with appearance of macrocytosis. There are some suggestions that bevacizumab, an antiangiogenic drug, may result in macrocytosis as well. There are no published data available on the influence of bevacizumab on macrocytosis. This paper attempted to answer the question: does bevacizumab induce macrocytosis being a predictor of the response?

Methods: Between August 2008 and August 2011, 53 patients (29 male and 24 female) were treated with bevacizumab in the combination with chemotherapy at the Oncological Department, University Hospital in Krakow, Poland. Efficacy of bevacizumab was assessed on the basis of the computer tomography scans performed every 3 months within the period of 12 months. Concurrently, mean corpuscular volume (MCV) was evaluated and correlated to the response of the treatment.

Results: The percentage increase of MCV compared to baseline at 3, 6, 9 and 12 months was 3.7%, 9.2%, 8.7% and 11.8% respectively. The mean value of baseline MCV was 85.3 fl. The mean value of MCV at 3, 6, 9 and 12 months was 90.5 fl, 93 fl, 91.8 fl and 93.1 fl respectively. Macrocytosis did not occur in our study but an increase of MCV was observed within bevacizumab therapy. It was closely related to the response of the treatment. It seems that an increase of MCV can be a predictive agent of bevacizumab response.

Conclusion: Bevacizumab does not induce macrocytosis. Increased MCV after treatment with bevacizumab is related to the treatment response. MCV can be a predictor of the treatment response during bevacizumab treatment. A small number of the observed patients requires further investigations.

Key words: Antibodies, Monoclonal; Humanized; Erythrocyte Indices; Antineoplastic Agents; Treatment Outcome

INTRODUCTION
Bevacizumab is a humanized monoclonal antibody binding and neutralizing all isoforms of vascular endothelial growth factor (VEGF). VEGF is the most powerful pro-angiogenic factor. Multikinase inhibitor – sunitinib is the next antiangiogenic drug. Remission in the course of treatment with sunitinib correlates with appearance of macrocytosis (1). Mean corpuscular volume (MCV) ranges from 82 to 92 fl. Macrocytosis is the next antiangiogenic drug. Remission in the course of treatment with sunitinib correlates with appearance of macrocytosis (1). Mean corpuscular volume (MCV) ranges from 82 to 92 fl. Macrocytosis is the next antiangiogenic drug. Remission in the course of treatment with sunitinib correlates with appearance of macrocytosis (1). Mean corpuscular volume (MCV) ranges from 82 to 92 fl. Macrocytosis is the next antiangiogenic drug. Remission in the course of treatment with sunitinib correlates with appearance of macrocytosis (1). 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