

Research Article

No Evidence of Human Papillomavirus in Patients with Breast Cancer in Hong Kong, Southern China

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Several studies have suggested that viral oncogenesis is one of the etiologic factors of breast cancer, while others are provocative, however, their association remains controversial. The purpose of this study was to investigate whether the human papillomavirus (HPV) DNA is present in the blood and tissue samples of breast cancer patients in Hong Kong. A total of 102 patients with breast tumour tissues and adjacent normal tissues were available and recruited unselectively. Both DNA and RNA were extracted from those samples, and real-time quantitative PCR was performed to detect HPV-16, with 18 sequences targeting the E6 and L1 regions. Results showed that HPV DNA sequences were absent in all the blood and breast tissues. These data argue against the role of oncogenic HPV in the pathogenesis of breast cancer. Additional lines of evidence need to be obtained in order to assess the possibility of breast cancer prevention using HPV vaccines.

1. Introduction

The incidence of breast cancer in Hong Kong is 1 in 20. Although this is lower than that of Western population, breast cancer is still the most common cancer in Chinese women, and the incidence is rising. Despite decades of research, the etiologic factor(s) for human breast cancer remains unclear. Many studies have identified risk factors such as age, diet, hormonal balance, and genetic predisposition, but a clear underlying cause for the disease has not been established. Emerging evidence suggests that breast cancer most likely is a multifactorial disease consisting of many different causes and factors [1]. Thus, the possibility that a virus is etiologically involved in breast cancer has not been eliminated.

Human papilloma virus (HPV) is widely known to be a major instigator for both cervical cancer [2] and a subset of cancers of the head and neck [3]. However, the involvement of the virus in breast cancer remains inconclusive. Previous reports have shown that HPV DNA was detected in breast cancer specimens from diverse populations around the world.

The prevalence of HPV positive breast cancer in these studies was reported to vary from 4% to 86% [4]. Although further clarification is required, there have also been promising results in the correlation between breast cancer and HPV [5]. In 2009, Heng et al. [6] substantiated the presence of DNA pertaining to high-risk HPV subtypes 16 and 18 in the specimens of breast cancer cells. Furthermore, Heng et al. found that the oncogenic properties of these HPV-associated breast cancers were comparable to HPV-associated cervical cancer. This evidence suggested a relationship between HPV-16 and HPV-18 and breast cancer and also highlighted that the pathogenesis of cervical cancer caused by HPV-16 and HPV-18 may share a similar mechanism to cause breast cancer. The latter theory has been further exemplified with the discovery of koilocytosis (a condition where cellular alteration leads to an appearance of haloed nuclei) in breast cancer cells. These cellular changes have been well documented in HPV-associated cervical cancer cells [2], and the presence of koilocytes in breast cancer provides additional evidence that HPV may

play a causal role instead of being merely a passenger in the pathogenesis of the disease [7].

As the aetiology of most breast cancers remains unclear, and that ethnic differences are also increasingly coming to play a role to complicate the multifactorial causes of breast cancer, it is important to verify these observations in Hong Kong breast cancer populations which mainly comprise of Asian ethnic background, and the majority of them are Chinese. The HPV detection rate in breast cancer is 20%, which is consistent with previous publications reporting the presence of HPV in breast cancer worldwide with a prevalence ranging from 4 to 86% [4]. In the present study, we used our developed assays to evaluate the current prevalence of HPV sequences in breast tumor and normal breast tissue specimens from Hong Kong patients. This study is enormously beneficial to our local community. The increased use of HPV vaccination to prevent cervical cancer is already in place. If it is proven that HPV infection does play an important role in the development of breast cancer, HPV vaccinations can also be implemented in the prevention strategies for breast cancer.

2. Materials and Methods

2.1. Study Population. Over 150 Chinese women, the age range is from 38 to 72, with breast cancer have been recruited with informed consent through the Hong Kong Hereditary Breast Cancer Family Registry (Hong Kong Registry), Queen Mary Hospital, and other hospitals in Hong Kong. Preoperative blood samples, corresponding breast tumor tissues, and their surrounding noncancerous breast tissues from this patient cohort were collected if available. Samples were collected under protocols approved by the Institutional Review Boards (IRB) of the University of Hong Kong. Although 150 breast cancer patients were recruited, only 102 patients had both peripheral blood and paired adjacent normal/tumor tissues for qPCR assays.

2.2. Detection of HPV Sequences by Real-Time Quantitative PCR and RT-PCR. Detection of HPV sequences were performed using LNA-based probe qPCR and SYBR green qPCR. Both RNA and DNA were extracted from tumors, their corresponding normal breast tissues, and blood samples using RNeasy and QIAamp DNA mini extraction kit (Qiagen), respectively. DNA and RNA qualities were assessed by NanoDrop spectrophotometer. Amplifications were conducted together with internal reference sequences of the β -globin gene (for DNA detection) and β -actin (for RNA detection). qPCR and qRT-PCR assays were used for HPV DNA and RNA detection, respectively. For HPV RNA detection, 100 ng (from blood) and 50 ng (from tissues) of RNA were reverse transcribed to cDNA using reverse transcription kit (Qiagen). For HPV DNA detection, 20 ng (from blood) and 10 ng (from tissues) of DNA were used. Single target real-time qPCR and qRT-PCR are performed using LC480Q Probe Master kit (Roche) in Roche LC480 (Roche). PCR primers targeting both L1 and E regions of HPV-16 and HPV-18 have been used for this project. Each sample was run in duplicates for analysis.

2.3. HPV Subtyping by DNA Sequencing. To confirm the presence of HPV viral sequences and rule out those amplification of nonspecific sequences, the amplified products of the positive breast cancer samples would be sequenced. PCR products were purified from agarose gels using PCR Clean-Up Purification kit (Qiagen) and sequenced on an ABI PRISM 3100 (Applied Biosystems).

3. Results and Discussion

The mean age of breast cancer patients was 52 years with the range from 38 to 72 years. No patient presented a history of HPV infection of cervical cancer. In this study, 102 breast cancer patients recruited: 94 had invasive ductal carcinoma (IDC), and 8 had ductal carcinoma in situ (DCIS). The following is the distribution of IDC patients with TNM stage: stage 1, $n = 28$; stage 2, $n = 55$; stage 3, $n = 8$; and stage 4, $n = 3$. Our results showed that all tested samples were negative for the presence of both E6 and L1 region sequences of both HPV-16 and HPV-18. In order to examine whether other HPV subtypes were present, we performed qPCR with universal primer set targeting the L1 common region. Interestingly, we could not detect any amplification in our samples, indicating the absence of HPV sequences in breast cancer samples.

Our results seemed to be contradicting with other studies, reporting HPV positivity in breast cancer. However, the results observed from these papers are not coherent. The inconsistency may be due to the following: (1) the study populations were not from the same origin; (2) high risk or low risk HPV was sometimes detected and is not the same in all cases; (3) HPV positivity frequency varies among individuals. Positivity of certain HPV subtype was also detected in normal samples without breast cancer [8–14].

On the other hand, several studies failed to detect HPV positivity in breast carcinoma. Wrede et al. [15] studied HPV subtypes 6b, 11, 13, 16, 18, 30, 31, 32, 33, 45, and 51 in 95 women with breast cancer without detecting any of the subtypes. Bratthauer et al. [16] researched the HPV subtypes 6, 11, 16, and 18 in 13 IDC, 15 papillomas, and 15 papillary carcinomas cases, and there was no evidence of HPV infection. Czerwenka et al. [17] also showed no association of HPV in 20 cases of Paget's disease. Furthermore, Lindel et al. [18] used six different primers, including a total of 40 subtypes 16, 18, 31, 33, and 45 in 81 Swiss women cases with breast cancer, and no positivity of HPV was detected. De Cremoux et al. [19] studied the prevalence of subtypes of high (16, 18, 33, and 45) and low risks (6, 11) in 50 breast cancers in women in France by PCR with the general primer GP5+/GP6+; none of these subtypes were detected in both cases.

This may be due to the lack of standardized technique to detect the presence of HPV, since there are different types of primers for different HPV subtypes. False negatives and false positives may occur when PCR overestimates the association between HPV and breast cancer because it cannot indicate which types of cells the virus has infected. Contamination while handling the sample may be partially responsible for the high frequencies of HPV positivity that were reported in

several papers. Other risk factors might affect the outcome of the results. For example, studies have shown that women under the age of 25 have a higher prevalence of HPV positivity detection with a linear decrease rate as age increases [20]. Storage of specimens may affect the result since some researchers found that positive specimens became negative after being frozen at -70°C for 3 months [21]. Demographic features and genetic backgrounds may contribute to the geographic difference and HPV infection in breast cancer. To conclude, our results are consistent with the null or limited proportion of HPV sequences explored from breast cancer patient samples.

A definitive relationship between human breast cancer and HPV infection has not been determined in Hong Kong breast cancer patients. It seems unlikely that high-risk genital HPV plays a role in breast oncogenesis. However, we cannot exclude the possibility that HPV infection may cause breast cancer in other areas or in other races and HPV itself is still a pathogenic virus which causes other different kinds of cancers, in particular, cervical cancer. Therefore, more studies are essential to proof or exclude the possibility of HPV being an etiological risk factor for breast cancer.

4. Conclusions

A total of 102 patients with breast normal and tumor tissues were used unselectively. Both DNA and RNA were extracted from those samples and real-time quantitative PCR was performed to detect HPV-16 and HPV-18 sequences targeting the E6 and L1 regions. Results showed that HPV DNA sequences were absent in all the blood and breast tissues samples. These data did not show the presence of oncogenic HPV in the breast cancer tissues. Additional lines of evidence need to be obtained in order to assess the possibility of breast cancer prevention using HPV vaccines.

Abbreviations

DCIS:	Ductal carcinoma in situ
qRT-PCR:	Quantitative reverse transcription-polymerase chain reaction
HPV:	Human papillomavirus.

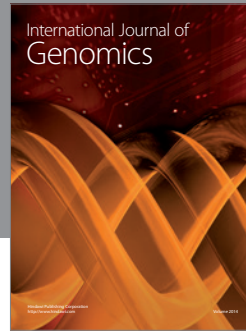
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