



In Vivo and *In Vitro* Studies Suggest a Possible Involvement of HPV Infection in the Early Stage of Breast Carcinogenesis via APOBEC3B Induction

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Abstract

High prevalence of infection with high-risk human papilloma virus (HPV) ranging from 25 to 100% (average 31%) was observed in breast cancer (BC) patients in Singapore using novel DNA chip technology. Early stage of BC demonstrated higher HPV positivity, and BC positive for estrogen receptor (ER) showed significantly higher HPV infection rate. This unique association of HPV with BC *in vivo* prompted us to investigate a possible involvement of HPV in early stages of breast carcinogenesis. Using normal breast epithelial cells stably transfected with HPV-18, we showed apparent upregulation of mRNA for the cytidine deaminase, APOBEC3B (A3B) which is reported to be a source of mutations in BC. HPV-induced A3B overexpression caused significant γ H2AX focus formation, and DNA breaks which were cancelled by shRNA to HPV18 E6, E7 and A3B. These results strongly suggest an active involvement of HPV in the early stage of BC carcinogenesis via A3B induction.

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Introduction

Breast cancer (BC) is one of the major health issues faced by women globally, ranking first in mortality (GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012). Epidemiology gave the first hints for an involvement of viral agents in specific human cancers [1]. Since the mid-20th century when mouse mammary tumor virus (MMTV) was discovered to cause mammary cancers in mice, some viruses, especially lately HPV, have been suspected to play an etiological role in BC. A number of molecular epidemiological studies conducted have accumulated data relating HPV to BC and indicated that HPV DNA is present at a high frequency in BC samples but is rare in normal breast tissues [2–8]. However, results from these studies have been rather varied and sometimes contradictory. Although some authors from these previous studies suggested an etiological role for HPV in BC, no clear explanation had been proffered for the causative mechanism other than the high rate of HPV positivity as the sole reason with a precedent premise that high-risk HPV has been established as a cause of CC. On the other hand, other researchers held an opposing view of these epidemiological results. For instance, Khan et al. concluded that it is unlikely for integrated HPV to be etiologically involved in

the development of BC since the viral load was very low (a geometric mean of 5.4 copies per 10⁴ cells) [2]. Many recent reports also showed that HPV genome could be detected in non-genital cancers such as head and neck (oesophageal, laryngeal and tonsil), lung, urothelial, breast and colorectal cancers [9,10].

HPV is a DNA virus with a circular double stranded configuration, with more than 100 subtypes found to date [11,12]. Most of the known HPV types cause no symptoms in general, although some types can cause warts, while others are believed to lead to cervical cancer [13] and other forms of genital cancers (vulvar, vaginal, anal, and penile) [14]. The HPV genome is composed of 8 genes (E1, E2, E4, E5, E6, E7, L1 and L2) and a long control region (LCR) which has promoter and replication origin for replication [15]. In the pre-cancerous lesions of cervical cancer, most HPV genomes persist in an episomal state whereas in many high-grade lesions, these viral genomes are found integrated into the host chromosome. Amongst various types of HPVs, 15 types including HPV-16 and HPV-18 are categorized as high-risk oncogenic HPVs based on the fact that they account for over 90% of cervical cancer [16]. E7 protein of high-risk HPVs has a high affinity to human retinoblastoma (Rb) proteins, and this leads to enhanced pRb degradation, resulting in the activation of E2F