論 文 要 旨

THE PREVALENCE OF THE HUMAN PAPILLOMAVIRUS AND ITS COFACTORS IN BREAST CARCINOMAS TO EVALUATE ITS ROLE IN BREAST CARCINOGENESIS

（乳がんにおけるヒトパピローマウイルスの分布および発がん過程におけるその役割と関連要因に関する検討）

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ABSTRACT

Purpose of this study

Recent studies have revealed a possible association with human papillomavirus (HPV) in the pathogenesis of breast cancer. To investigate the etiological role of HPV in breast cancer, we examined the presence, genotype, viral load, and physical status of HPV in breast carcinomas (BCs) collected from different countries.

Subjects and methods

The present study examined 307 BCs collected from Japan (n=124), Pakistan (n=61), India (n=11), Mexico (n=63), and Chile (n=48). Cervical carcinoma (CC) samples from Japan and Pakistan were also used for comparison. HPV presence was examined by PCR using SPF10 primers, and primer sets targeting the *E6* region of HPV-16, -18, and -33. The INNO-LiPA HPV genotyping kit was used to determine genotype. Real-time PCR analysis was used to examine viral load and physical status of HPV DNA. The $p16^{INK4a}$ and p53 expressions were analyzed by immunohistochemistry assays.

Results

1. HPV detection rate

HPV DNA was detected in 26 (21%), 17 (28%), 4 (36%), 8 (13%), and 5 (10%) BCs from Japan, Pakistan, India, Mexico and Chile, respectively. The difference of HPV detection rate among these countries was statistically significant (P=0.048). In CCs, HPV was detected in 72 (85%) Japanese and 77 (94%) Pakistani cases. The presence of HPV was significantly different between the breast and CC ($P < 0.001$). The most frequently detected HPV genotype was HPV-16 in both sites, and its frequency was 54 (90%) and 125 (84%) carcinomas of breast and cervix, respectively. The detection rate of HPV-16 in all HPV-positive cases was not different between breast and CC.

2. Distribution of HPV genotypes

In all HPV-positive cases with BCs, the frequency of low-risk HPV genotype (P=0.021) and HPV multiple infection (P=0.032) was significantly different among the five countries. Most cases with low-risk HPV infection were revealed to harbor multiple HPV types (93%), and the highest frequencies of low-risk types and multiple infections of HPV were observed in Japanese series (46% for both). Multiple infections were also found in 2 (12%) and 1 (13%) BCs of Pakistan and Mexico, respectively. In CC, multiple infection was more frequently found when compared to BCs (P < 0.001): 91% in Pakistan and 15% in Japan. The observed difference between the two countries was statistically significant (P < 0.001).

3. HPV detection in normal tissue and breast milk

In 19 normal epithelium specimens adjacent to 19 HPV-16-positive BCs, 10 (53%) were HPV-16-positive. However, three (5%) of the normal breast tissue specimens adjacent to HPV-negative BCs were also HPV-positive. Nipple specimens adjacent to eight HPV-16-positive Mexican BCs were also examined, and one nipple specimen was HPV-16-positive. In addition, ten clostrum and 25 breast milk samples from Japan were also examined for HPV presence. One (10%) clostrum specimen was positive for HPV-16 and two (8%) breast milk samples were positive for HPV. However, only low-risk types of HPV were detected in milk samples.

4. Viral load and physical status

To clarify the etiological involvement of HPV in carcinogenesis of the breast, further analyses were conducted. Real-time PCR analysis suggested the presence of integrated-form of viral DNA in all HPV-16-positive BCs from all countries, and estimated viral load was low with geometric mean of 5.4, 1595, 158, 5.3 and 6402 copies per 10^4 cells in carcinomas of Japan, Pakistan, India, Mexico and Chile, respectively. The geometric mean of HPV-16 viral load was significantly different among these countries (P < 0.001). All HPV-16 positive CCs were found integrated into the host genome except two cases, which were suspected to have only episomal HPV-16. The geometric mean of HPV-16 viral load in cervical cancers of Japan and Pakistan was 410,000 and 2,370,000 copies per 10^4 cells, respectively. The geometric mean of viral load in the CC was higher than BC and the difference was statistically significant.

5. Immunostainning

The $p16^{INK4a}$ expression was significantly high in HPV-positive CCs (P = 0.031). Although a similar association was found in BC, their relationship was not statistically significant. On the other hand, p53 expression tended to decrease in HPV-positive CCs (P = 0.110), while there was no trend in BC. These differences of $p16^{INK4a}$ and p53 expressions between cervical and BCs were statistically significant (P = 0.013 and P < 0.001, respectively).

Conclusion

In conclusion, the relatively low HPV copy number and infection rate in breast cancer suggest that HPV is unlikely to play as an essential role in the carcinogenesis of breast cancer as in genital neoplasia. However, since oncogenic HPVs were consistently detected in BC and most of the detected HPV-16 DNA was considered integrated into the host genome, HPV infection may play some roles in the carcinogenesis of a subset of BCs.