Immunohistochemical and Electron Microscopic Demonstration of Human Papillomavirus in Dysplasia of the Uterine Cervix

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TOKI, T., OIKAWA, N., TASE, T., SATO, S., WADA, Y., YAJIMA, A. and HIGASHIIWAI, H. Immunohistochemical and Electron Microscopic Demonstration of Human Papillomavirus in Dysplasia of the Uterine Cervix. Tohoku J. exp. Med., 1986, 149 (2), 163-167 —— Twenty-five cases of dysplasia of the uterine cervix were studied for the presence of human papillomavirus (HPV) by means of immunohistochemical and electron microscopic techniques. Serial sections of the same histological specimen were examined in each case. HPV was detected in 14 cases by both immunohistochemistry and electron microscopy, while 10 cases were negative with both methods. In only one case, there was a discrepancy in the results derived from these two methods. It was concluded that the relation between HPV infection and cervical dysplasia was confirmed and that immunohistochemical and electron microscopic methods led almost to the same result in detecting HPV in cervical dysplasia. —— cervical dysplasia; human papillomavirus; immunohistochemistry; electron microscopy; koilocytosis

Recently, it has become evident that human papillomavirus (HPV) plays an important role in the development of malignant lesions of the uterine cervix (Zur Hausen 1977). In some cases of condylomatous and dysplastic lesions of the cervix, HPV antigens have been detected using the immunoperoxidase technique (Woodruff et al. 1980; Ferenczy et al. 1981; Kurman et al. 1981; Morin et al. 1981; Syrjänen 1983) and HPV particles have been demonstrated by electron microscopy (Hills and Laverty 1979; Morin and Meisels 1980; Reid et al. 1980; Ferenczy et al. 1981). There have been few reports, however, in which these two methods were simultaneously applied to cervical lesions (Ferenczy et al. 1981). The aim of the present study was to demonstrate HPV using both immunohistochemical and electron microscopic techniques in the same histological specimen of cervical dysplasia and to compare the results derived from these two different

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MATERIALS AND METHODS

Twenty-five cases of cervical dysplasia were selected from a series of 460 dysplasias discovered by mass screening for cervical cancer in Miyagi Prefecture, Japan in 1983. Among the 25 cases, 15 cases had been found to be HPV antigen-positive, as described in our previous report (Toki et al. 1985). Histological specimens obtained by target biopsy had been fixed with 10% formalin and embedded in paraffin. They were serially recut into 3-µm sections for further immunoperoxidase study and into 10-µm sections for ultrastructural study.

Immunohistochemical studies

Deparaffinized 3-µm sections were incubated sequentially with normal goat serum, rabbit anti-papillomavirus (Dakopatts, USA), goat anti-rabbit IgG, and rabbit PAP complex. Bound peroxidase was visualized by immersing the slices in a solution of 0.02% diaminobenzidine with 0.005% hydrogen peroxide.

Ultrastructural studies

The 10-µm sections were deparaffinized and rehydrated. They were then post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.2, for 1 hr, dehydrated and embedded in epoxy resin using gelatine capsules. The embedded blocks were cut using a diamond knife at about 100 nm thickness on MT-5000 ultramicrotome (Dupont Instruments-Sorvall, USA). The sections were then mounted onto Formvar-coated monopore grids. At least 30 cells were observed in each case using a Hitachi H-700 electron microscope at 175 kV.

RESULTS

Immunohistochemically, positive staining for HPV antigen was observed in the nuclei of superficial cells of dysplastic epithelium, mainly in the region of koilocytosis (Figs. 1a and b). Among the 25 cases, 15 were positive for HPV, exhibiting the same pattern as that noted in our previous report.

Fig. 1. Immunohistochemical staining for papillomavirus antigen in a case of cervical dysplasia.

a, Nuclear localization of the viral antigens. (PAP stain and hematoxylin, ×80)
b, Higher magnification of positive cells. (×320)
Fig. 2. Electron micrographs of HPV particles in a case of cervical dysplasia.
   a, Crystalline sheets of virus particles. Note the large perinuclear space devoid of cytoplasmic granules. (original magnification $\times 15,000$)
   b, Higher magnification of the particles. (original magnification $\times 30,000$, Bar = 80 nm)

Fig. 3. HPV particles scattered in the nucleoplasm. (original magnification $\times 15,000$)
Fig. 4. Perinuclear HPV particles. (arrows, original magnification $\times 20,000$)
By electron microscopy, virus particles appeared round and about 50 nm in diameter. They were observed mainly in the nuclei of the superficial cells. The pattern of their distribution showed some variation, the particles appearing either in crystalline arrays (Figs. 2a and b) or scattered (Fig. 3) in the nucleoplasm. Some particles were located outside the nuclear membrane (Fig. 4), but these perinuclear particles were considered to have escaped from a nucleus during specimen processing. The particles were observed mainly in koilocytotic cells, particularly in those that were positive for HPV antigen by the immunohistochemical technique. These koilocytotic cells had a large perinuclear space devoid of cytoplasmic granules as shown in Fig. 2a. The fine structure of the viruses was not clear, because the specimens had been fixed with formalin.

Consequently, the virus particles were detected in 14 of the 15 cases of dysplasia with HPV antigen, while they were not demonstrated in 10 cases of HPV-negative dysplasia (Table 1).

**DISCUSSION**

Recently, a growing body of evidence has been accumulated to support the view that HPV infection has a close relationship with the development of cervical malignancies (Zur Hausen 1977). There are several methods to detect the HPV infection in the tissue; immunohistochemistry, electron microscopy and in situ DNA hybridization being representative (Pfister 1984). Among these methods, the immunoperoxidase technique is the simplest, followed by electron microscopy.

In the present study, we tried both the immunohistochemical and electron microscopic techniques in serial tissue sections of cervical dysplasia to evaluate the applicability of these techniques for the detection of HPV in surgical specimens. The results clearly indicated that not only viral capsid antigens but also viral particles could be demonstrable in formalin fixed tissues and the result of immunohistochemical study was in good agreement with that of electron microscopic observation. Scrutiny of electron microscopic survey also proved that this technique was no less sensitive than immunohistochemistry contrary to the notion by Ferenczy et al. (1981). They had claimed higher sensitivity of immunoperoxidase method in a study on condylomatous lesions of the cervix but it should be noted that the specimens they examined by electron microscopy were apparently

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**TABLE 1. Correlation between immunohistochemical and electron microscopic detection of HPV in cervical dysplasia**

<table>
<thead>
<tr>
<th>Immunoperoxidase</th>
<th>HPV-positive</th>
<th>Negative</th>
<th>Total</th>
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<tr>
<td>HPV-positive</td>
<td>14</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>HPV-negative</td>
<td>0</td>
<td>10</td>
<td>10</td>
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different from ones used for immunohistochemistry. It may also be mentioned that the presence of scattered particles (Fig. 3) could easily be overlooked.

Koilocytosis has been thought to be a pathognomonic sign of HPV infections (Meisels et al. 1982). Our observation of the occurrence of viral antigens and particles mainly in koilocytic cells would be a further support for this hypothesis. Although a high incidence of HPV infection in the present study confirmed the existence of an intimate relationship between HPV infection and cervical dysplasia, clinical implication of HPV infections in the development of cervical malignancies needs to await further investigations.

Acknowledgments

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References