Human Papillomavirus Types and Localization in Adenocarcinoma and Adenosquamous Carcinoma of the Uterine Cervix: A Study by in Situ DNA Hybridization

Toru Tase, Takashi Okagaki, Barbara A. Clark, Dawn A. Manias, Ronald S. Ostrow, Leo B. Twiggs, and Anthony J. Faras

ABSTRACT

Formalin-fixed, paraffin-embedded tissues from 108 cases of invasive carcinoma of the uterine cervix, consisting of 40 cases of adenocarcinoma, 44 cases of adenosquamous carcinoma, and, as a control, 24 cases of squamous cell carcinoma were examined for the presence of human papillomavirus (HPV) DNA by in situ hybridization of high sensitivity using tritium-labeled HPV-2, HPV-6, HPV-16, and HPV-18 DNA probes. This method detects five genome copies of homogeneous HPV DNA per cell. HPV DNA was detected with mixed HPV DNA probes in 17 cases (42.5%) of adenocarcinoma, 16 cases (36.4%) of adenosquamous carcinoma, and in 13 cases (54.2%) of squamous cell carcinoma. The types of HPV DNA in the HPV-positive tissues were also analyzed with each individual probe under high stringency conditions. HPV-18 DNA was detected in all but one case of the HPV DNA-positive adenocarcinoma and one-half of the HPV DNA-positive adenosquamous carcinoma. HPV-16 DNA was detected in one case of the HPV DNA-positive adenosquamous carcinoma, one-half of the HPV DNA-positive adenosquamous carcinoma, and all cases of the HPV DNA-positive squamous cell carcinoma. HPV DNA was confined to the areas of carcinoma and squamous cervical intraepithelial neoplasia (CIN) associated with carcinoma. Among 36 cases in which CIN was associated with adenocarcinoma (9 cases), adenosquamous carcinoma (19 cases), and squamous cell carcinoma (8 cases), the same type of HPV DNA was present in the carcinoma and the associated CIN that constituted 12 cases (3 adenocarcinomas, 5 adenosquamous carcinomas, and 4 squamous cell carcinomas). Two cases (one adenocarcinoma and one adenosquamous carcinoma) contained HPV DNA in the carcinoma but not in the associated CIN. The incidence of HPV DNA did not show a significant correlation with the existence of CIN or histological differentiation of carcinoma.

INTRODUCTION

The development of molecular biological methods has provided a means of detecting HPV DNA in genital premalignant and malignant lesions (1–5). Recent studies have shown that HPV DNA, especially HPV-16 and HPV-18 DNA, is present in 20 to 70% of invasive carcinoma of the uterine cervix (6–13). It has been believed that most carcinoma of the uterine cervix containing HPV DNA is squamous cell carcinoma, and little attention has been paid to other histological types of carcinoma of the uterine cervix.

Invasive carcinomas of the uterine cervix are mainly divided into three histological subtypes: squamous cell (epidermoid) carcinoma; adenocarcinoma; and adenosquamous carcinoma. Compared to squamous cell carcinoma, histogenesis of adenocarcinoma and adenosquamous carcinoma has not been studied extensively because carcinoma of the latter two histological types comprises 10 to 30%, a relatively small proportion of carcinoma of the uterine cervix (14). Unlike squamous cell carcinoma, adenocarcinoma has not been linked with sexual promiscuity which may facilitate transmission of HPV infection. However, it has been shown that 20 to 40% of adenocarcinoma and adenosquamous carcinoma coexists with CIN (15) and may contain HPV DNA (8, 10, 16). Also, the studies of cultured human cell lines that originated from adenocarcinoma and undifferentiated carcinoma of the uterine cervix have shown the presence of HPV genomes (6, 17).

The technique of in situ hybridization has two advantages for detecting HPV DNA compared to that of filter hybridization. (a) One can detect the precise localization of HPV DNA in tissues consisting of more than one type of cell with in situ hybridization. (b) In situ hybridization makes retrospective study of HPV DNA in paraffin-embedded tissues possible.

Applying in situ hybridization (12, 18, 19) with 3H-labeled HPV-2, HPV-6, HPV-16, and HPV-18 DNA probes of a high specific activity, 5 genome copies of homologous HPV DNA per cell can be detected. The presence of HPV DNA in adenocarcinoma and adenosquamous carcinoma was studied on tissues which had been embedded in paraffin and stored in our laboratory over the past 12 yr.

MATERIALS AND METHODS

Formalin-fixed, paraffin-embedded tissues of 40 cases of adenocarcinoma, 44 cases of adenosquamous carcinoma, and, as a control, those of 24 cases of squamous cell carcinoma were chosen from the file of routine surgical specimens of the University of Minnesota Hospitals and Clinics. The specimens were removed by cone biopsies or hysterectomies between 1974 and 1986. The cases were selected so that most cases used for this study were in International Federation of Gynecology and Obstetrics Stage Ib, because carcinoma, associated lesions, and normal tissue components can be seen in one tissue section. Carcinoma whose cell types could not be determined with certainty with a light microscope was examined, and its histological type was confirmed with an electron microscope. The cells possessing bundles of tonofilaments, frequently attached to macula adherens (desmosomes), were classified as squamous cell carcinoma. The cells containing secretory vacuoles and/or possessing microvilli projecting into intracellular spaces were considered to be adenosquamous carcinoma. Carcinoma composed of the above two types of cells was classified as adenosquamous carcinoma. Some adenosquamous carcinoma showed both ultrastructural markers in a single cell (adenosquamous carcinoma with ambiguous differentiation). Sections of 9 cases of adenocarcinoma, 19 cases of adenosquamous carcinoma, and 8 cases of squamous cell carcinoma containing CIN contiguous to invasive carcinoma were included in this study. The tissue blocks were chosen so that carcinoma, normal squamous epithelium, normal endocervical columnar epithelium, and in some cases, associated CIN would be present in the same section.

Originally tissue samples were fixed in buffered 10% formalin and embedded in paraffin for routine histological examination. As described previously (12, 18, 19), paraffin blocks of appropriate tissues were cut
Table 1  *Histological diagnoses of invasive carcinoma of the uterine cervix and detection of HPV DNA by in situ hybridization*

There was no statistically significant difference of HPV DNA positivity among histological subtypes of carcinoma (χ² test, 0.25 < P), whereas the difference of the HPV types among three major histological types of carcinoma was statistically significant (χ² test, P < 0.005).

<table>
<thead>
<tr>
<th>Type of HPV DNA</th>
<th>Histological subtypes</th>
<th>No. of cases</th>
<th>HPV DNA positive</th>
<th>Type of HPV DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HPV-16</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>40</td>
<td>17 (42.5)†</td>
<td>1</td>
<td>16*</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>30</td>
<td>15</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>44</td>
<td>16 (36.4)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Mixed adenosquamous carcinoma</td>
<td>28</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Glassy cell carcinoma</td>
<td>12</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>24</td>
<td>13 (54.2)</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Keratinizing</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Large cell nonkeratinizing</td>
<td>13</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Small cell nonkeratinizing</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>46 (42.6)</td>
<td>22</td>
<td>24</td>
</tr>
</tbody>
</table>

† Numbers in parentheses, percentage.

* Four adenocarcinomas with HPV-18 DNA also hybridized with HPV-2 DNA weakly.

Fig. 1. Presence of HPV-18 DNA in well-differentiated endocervical adenocarcinoma by *in situ* hybridization. The sections may be 100–500 μm apart in the same block because 10–20 sections were processed for different probes or for controls. As a consequence, the structure does not appear exactly identical. Original magnifications, × 255. A, section stained with H & E; B, section hybridized with HPV-18 DNA probe. Silver grains produced by the HPV-18 DNA probe appear aggregated in several foci over the nuclei. Hematoxylin counterstain.

into 5-μm sections, put on a drop of 1% Elmer's glue on microscope slides that were acid washed, treated with a Denhardt's solution overnight, and acetylated. Tissue sections were dried onto the slides at 45°C. The slides were incubated at 37°C overnight and at 60°C for 4 h.

Tissue sections were depaaffinized in 2 changes of xylene for 10 min each, in 3 changes of absolute ethanol for 5 min each, and air dried. Slides were put in 0.2 N HCl for 30 min at room temperature, washed briefly in distilled water, dipped in a 0.153 M triethanolamine hydrochloride solution (pH 7.4) for 15 min at room temperature, washed briefly in distilled water, incubated in 3× SSC for 30 min at 68°C, washed briefly in distilled water, and dipped for 5 min in a 0.05% digitonin solution (pH 7.4) containing 125 mM sucrose, 60 mM KCl, and 3 mM HEPES (pH 7.4). The slides were then washed briefly in distilled water, digested for 15 min at 37°C with 5 to 10 μg/ml of proteinase K in 20 mM Tris buffer (pH 7.4) containing 2 mM CaCl₂, washed twice in distilled water for 5 min each, and dehydrated in graded ethanol (70% twice, 95% once, for 5 min each). Tissue sections were incubated in a solution of 2× SSC containing 100 μg/ml of RNase A and 10 units/ml of RNase T₁ for 60 min at 37°C, washed twice in 2× SSC for 5 min each, and dipped briefly in distilled water. They were then refixed in 5% paraformaldehyde in 0.3 N NaOH in phosphate-buffered saline for 2 h at room temperature in the dark, washed twice in 2× SSC for 5 min each, dipped briefly in distilled water, and dehydrated in graded ethanol.

Molecularly cloned HPV-2, HPV-6, HPV-16, and HPV-18 DNAs were excised from their plasmid vectors, nick translated with [³²P]dCTP, [³²P]dATP, and [³²P]dTTP to the specific activities of 0.7 to 1.1 × 10⁶ dpm/μg DNA, and heat denatured. The probes were 90 to 160 nucleo-
HPV IN CERVICAL CARCINOMA

Table 2 Coexisting CIN associated with carcinoma of the uterine cervix and detection of HPV DNA by in situ hybridization

Table 2 Coexisting CIN associated with carcinoma of the uterine cervix and detection of HPV DNA by in situ hybridization

<table>
<thead>
<tr>
<th>Types of HPV DNA</th>
<th>Histological subtypes with/without CIN</th>
<th>No. of cases</th>
<th>HPV DNA positive</th>
<th>HPV-16</th>
<th>HPV-18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>40</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>With CIN</td>
<td>9</td>
<td>4 (44)*</td>
<td>1</td>
<td>3*</td>
</tr>
<tr>
<td></td>
<td>Without CIN</td>
<td>31</td>
<td>13 (42)</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Adenosquamous carcinoma</td>
<td>44</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>With CIN</td>
<td>19</td>
<td>6 (32)</td>
<td>4*</td>
<td>2*</td>
</tr>
<tr>
<td></td>
<td>Without CIN</td>
<td>25</td>
<td>10 (40)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Squamous cell carcinoma</td>
<td>24</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>With CIN</td>
<td>8</td>
<td>4 (50)</td>
<td>4*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Without CIN</td>
<td>16</td>
<td>9 (56)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.
* One case with HPV-16 DNA and two cases with HPV-18 DNA contained HPV DNA in the areas of carcinoma and CIN.
* Four cases with HPV-16 DNA and one case with HPV-18 DNA contained HPV DNA in the areas of carcinoma and CIN.
* Four cases with HPV-16 DNA contained HPV DNA in the areas of carcinoma and CIN.

Table 3 Relationship between histological differentiation of carcinoma of the uterine cervix and detection of HPV DNA by in situ hybridization

Table 3 Relationship between histological differentiation of carcinoma of the uterine cervix and detection of HPV DNA by in situ hybridization

<table>
<thead>
<tr>
<th>Histological differentiation</th>
<th>No. of cases</th>
<th>HPV DNA positive</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>40</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>13 (54)*</td>
<td>1 (11)</td>
<td>3 (43)</td>
<td></td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>14</td>
<td>2 (15)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>6</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>24</td>
<td>13</td>
<td>6 (43)</td>
<td>10 (34)</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>8</td>
<td>3 (50)</td>
<td>6 (43)</td>
<td>10 (34)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>10</td>
<td>4 (40)</td>
<td>6 (43)</td>
<td>10 (34)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>8</td>
<td>6 (75)</td>
<td>6 (43)</td>
<td>10 (34)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

Hybridization solution contained 50% formamide, 10% (w/v) dextran sulfate, 10 mM HEPES (pH 7.4), 0.6 M NaCl, 1 mM EDTA, 0.02% (w/v) Ficoll, 0.02% (w/v) bovine serum albumin, 0.02% (w/v) polyvinyl pyrrolidone, 200 μg/ml of depurinated salmon sperm DNA, 100 μg/ml of yeast tRNA, and 0.06 μg/ml of the 3H-labeled HPV DNA probe. The hybridization solution was pipetted onto the sections, which were then covered with HCI-treated and siliconized coverslips, and sealed with mineral oil. The slides were baked at 80°C for 15 min in a glass tray and quenched on ice for 10 min. The tissue sections were hybridized for 60 to 70 h at room temperature in the dark.

The oil on the slides was removed by three 5-min washes in CHCl3, and coverslips were removed in 50% formamide:0.6 M NaCl:10 mM Tris buffer (pH 7.4):1 mM EDTA. Tissue sections were washed for 3 days in 1500 ml of this formamide solution with pieces of nitrocellulose paper at room temperature in the dark. The slides were dehydrated in graded ethanol containing 0.3 M ammonium acetate to stabilize hybrids and air dried.

In the darkroom, the slides were dipped in Kodak NTB-2 nuclear track emulsion which was diluted with an equal volume of 0.6 M ammonium acetate and warmed to 42-45°C. The slides were then air dried.

Fig. 2. Presence of HPV-16 DNA in large cell nonkeratinizing squamous cell carcinoma by in situ hybridization. Original magnification, x 396. A, section stained with H & E; B, section hybridized with HPV-16 DNA probe. Silver grains produced by HPV-16 DNA probe were scattered over the nuclei. Hematoxylin counterstain. C, negative control section hybridized with pBR 322 DNA probe. Hematoxylin counterstain.
dried for 1 h, put into light-proof slide boxes containing a small amount of anhydrous CaCl₂, and stored at 4°C for 4 wk. The slides were developed in Kodak D-19 developer for 3 min at room temperature, dipped briefly in distilled water, fixed in Kodak fixer for 3 min, stained with Schmitt alum hematoxylin, and mounted with Glycergel (Dako) under coverslips. Silver grains on the nuclei were counted, and more than 5 grains per nucleus above background was considered to be positive. Reproducibility was confirmed with duplicate adjacent tissue sections. Repeated experiments were performed on equivocal cases. A negative control was made using paraffin-embedded tissue of endometrial carcinoma known to be HPV DNA negative by filter hybridization. In addition, 2 slides from each specimen in the study were processed with no heat denaturation, and 2 slides with a heterologous DNA probe (³H-labeled pBR322). HPV-6 DNA-positive condyloma acuminatum and tissues of carcinoma of the cervix proven to contain HPV-16 or HPV-18 DNA by filter hybridization were also used as positive controls with each batch. One of the sequential sections was prepared and stained with hematoxylin:eosin for histopathological examination. Under the above conditions, the method could detect 5 genome copies of homologous HPV DNA per cell as determined by parallel in situ and filter hybridizations on HPV DNA-containing cervical tissues and HeLa cells (12, 19). No cross-reactivity between types of HPV with a few exceptions has been observed; that is, HPV DNA other than those in the mixed probe could not be detected by this method (12).

RESULTS

HPV DNA was detected in 17 cases (42.5%) of adenocarcinoma, 16 cases (36.4%) of adenosquamous carcinoma, and 13 cases (54.2%) of squamous cell carcinoma using mixed probes of HPV-2, HPV-6, HPV-16, and HPV-18 DNA. Further detailed histological subtypes of the three major types of cervical carcinoma and the results of hybridization are shown in Table 1. Although 15 cases of mucinous adenocarcinoma and 2 cases of endometrioid adenocarcinoma contained HPV DNA, none of 7 cases of clear cell carcinoma (mesonephroid carcinoma) hybridized with HPV DNA. In adenosquamous carcinoma, 10 cases of mixed adenosquamous carcinoma, 3 cases of glassy cell carcinoma, and 3 cases of adenoid cystic carcinoma contained HPV DNA. HPV DNA was detected in 4 cases of keratinizing squamous cell carcinoma, 6 cases of large cell nonkeratinizing squamous cell carcinoma, and 3 cases of small cell nonkeratinizing squamous cell carcinoma. There was no statistically significant difference of HPV DNA positivity among the subtypes of carcinoma ($\chi^2$ test, 0.25 < $P$).

The types of HPV DNA in the HPV DNA-positive tissues were further analyzed using individual DNA probes of HPV-2, HPV-6, HPV-16, or HPV-18 under high stringency conditions. HPV-18 DNA was detected in all but one case of HPV DNA-positive adenosquamous carcinoma (Fig. 1). HPV-16 DNA was detected in 10 cases of HPV DNA-positive adenosquamous carcinoma. Eight of 16 cases (50%) of HPV DNA-positive adenosquamous carcinoma contained HPV-18 DNA. Eight cases of HPV DNA-positive adenosquamous carcinoma and all 13 cases of HPV DNA-positive squamous cell carcinoma contained HPV-16 DNA (Fig. 2). The proportions of the cases containing HPV-16 DNA and HPV-18 DNA in three histological types of carcinoma were statistically different ($\chi^2$ test, $P < 0.005$). Four cases of adenocarcinoma containing a large amount of HPV-18 DNA also hybridized weakly with HPV-2 DNA, which was later interpreted as cross-hybridization with HPV-18 DNA. No case contained more than one type of HPV except for those four cases of HPV-2/HPV-18 cross-hybridization (6).

The presence of HPV DNA in CIN adjacent to carcinoma is tabulated in Table 2. There was no case that showed HPV DNA only in the area of CIN. Among 36 cases of carcinoma associated with CIN (9 cases of adenocarcinoma, 19 cases of adenosquamous carcinoma, and 8 cases of squamous carcinoma), 12 cases (3 cases of adenocarcinoma, 5 cases of aden-
osquamous carcinoma, and 4 cases of squamous cell carcinoma) contained the same type of HPV DNA in the area of carcinoma and CIN, whereas 2 cases (1 case of adenocarcinoma and 1 case of adenosquamous carcinoma) contained HPV DNA in the areas of carcinoma but not in the area of CIN. The prevalence of HPV DNA in carcinoma with CIN and in carcinoma without CIN was not significantly different ($\chi^2$ test, 0.75 < $P$).

There were some differences in localization of HPV-18 DNA and HPV-16 DNA. HPV-18 DNA was detected in all areas of carcinoma, but this was not always the case with HPV-16 DNA. The grains of HPV-18 DNA tended to aggregate in several foci over the nuclei, whereas those of HPV-16 DNA tended to be scattered over the nuclei (Figs. 1 and 2). The existence of HPV-18 DNA in CIN associated with carcinoma seemed to be contiguous to the carcinoma and limited to a small area near the carcinoma. HPV-16 DNA in CIN associated with carcinoma was sometimes detected in an area distant from the invasive carcinoma.

The correlation between histological differentiation and incidence of HPV DNA was analyzed (Table 3). There was no significant difference in the prevalence of HPV DNA among three different grades of differentiation in 3 major histological types of cervical carcinoma ($\chi^2$ test, 0.25 < $P$). In some cases, a single type of HPV DNA was detected in areas of both well-differentiated carcinoma and poorly differentiated carcinoma (Fig. 3).

**DISCUSSION**

Dürst et al. (4) first isolated and molecularly cloned HPV-16 DNA from the tissue of cervical carcinoma. HPV-16 DNA was present in 46.3% (19 of 41) of cervical carcinoma from different geographical regions as well as in a few cases of CIN, vulvar carcinoma, penile carcinoma, and condyloa acuminatum. Boschard et al. molecularly cloned HPV-18 DNA from cervical carcinoma and detected HPV-18 DNA in 22.4% (11 of 49) of cervical carcinoma, 1 case of penile carcinoma, and in 3 established cell lines (HeLa, KB, and C4-I) that originated from human cervical carcinoma. No HPV-18 DNA was identified in CIN, vulvar carcinoma, condyloa acuminatum, or normal tissue (6).

Yee et al. reported the presence of HPV-18 DNA in four established cell lines (ME180, MS571, C-10 and C-4II, HeLa) and HPV-16 DNA in two cell lines (SiHa, CaSki) (17). All those cell lines had originated from human cervical carcinoma. Thus, it appears that HPV-18 DNA was the most prevalent type of HPV DNA found in established cell lines that originated from cervical carcinoma. On the other hand, HPV-18 DNA has been detected in 5 to 25% of clinical specimens of cervical carcinoma, whereas HPV-16 DNA is the most common type of HPV DNA being detected in 20 to 70% of cervical carcinoma (4, 6, 13). Although histological types of cervical carcinoma studied were not usually elaborated in those reports, the prevalence of HPV-16 and HPV-18 DNA in established cell lines and clinical specimens may be related to the carcinoma cell types.

Squamous cell carcinoma, thus far, is the most common type of carcinoma of the uterine cervix. Adenocarcinoma and adenosquamous carcinoma constituted about 6% of the primary cervical carcinoma in most of the statistics until a decade ago (20—24). Now, these types of cervical carcinoma are observed in 20 to 30% of cervical carcinoma (14, 25—27). Fukushima et al. reported a case of adenocarcinoma and a case of undifferentiated carcinoma containing HPV DNA in 1985 (8). Smotkin et al. reported that 1 of 2 adenocarcinomas contained HPV-16 DNA, 4 of 5 adenosquamous carcinomas contained HPV-18 DNA, 1 case of glassy cell carcinoma, and 3 of 5 squamous cell carcinomas contained HPV-16 DNA by filter hybridization (16). They raised a possibility that this HPV-16 DNA detected in 1 case of adenocarcinoma might not be derived from the adenocarcinoma but from the adjacent benign or neoplastic squamous epithelium. In our study, HPV-18 DNA was detected in the majority of HPV-positive adenocarcinomas and in one-half of HPV-positive adenosquamous carcinomas. HPV-16 DNA was the predominant type in squamous cell carcinoma, and it also was observed in one-half of HPV-positive adenosquamous carcinomas. HPV-18 DNA and HPV-16 DNA seemed to exist separately and have different target cells. We could not distinguish the histological differences between adenocarcinoma or adenosquamous carcinoma containing HPV-16 DNA and those containing HPV-18 DNA, however.

Adenocarcinoma of the uterine cervix is believed to arise from the columnar epithelium of the uterine endocervix through a precursor stage of adenocarcinoma in situ (28—30), whereas squamous cell carcinoma originates from “reserve cells” of the endocervical cells through the stage of squamous metaplasia, condyloa acuminatum, and CIN. Assuming that HPV-18 DNA is the agent responsible for transforming the endocervical cells to adenocarcinoma, one might assume that there is a subclinical stage of benign HPV infection of the endocervical cells comparable to condyloa acuminatum of the uterine cervix as the initial benign lesion, some of which evolve to squamous cell carcinoma of the uterine cervix through the stage of CIN (11, 31, 32). Such benign HPV infection of the columnar cells of the endocervix has not been yet identified. Our findings, however, suggest that infection of the endocervical cells by HPV may be nearly as frequent as that of squamous cells of the uterine cervix. It is interesting that the proportion of adenocarcinoma and adenosquamous carcinoma of the uterine cervix in cervical carcinoma started to rise during the 1970s in parallel to the increased incidence of benign HPV infection of the external genitalia (14). The causative relationship between adenocarcinoma of the uterine cervix and HPV, particularly HPV-18, must be studied further.

Three main pathways of histogenesis of adenosquamous carcinoma have been assumed in the past: adenocarcinoma which arose from columnar cells may turn into adenosquamous carcinoma through metaplasia; primitive “reserve cells” of columnar epithelium may become neoplastic and differentiate into two cell types due to its bipotential nature of differentiation; and pure adenocarcinoma and pure squamous cell carcinoma may collide with each other and form adenosquamous carcinoma (26, 27). In our study, HPV-18 DNA or HPV-16 DNA was detected essentially in all of the histological subtypes of adenosquamous carcinoma, i.e., mixed adenosquamous carcinoma, glassy cell carcinoma, and adenosquamous carcinoma, and squamous cell carcinoma can be seen in a single cell (adenosquamous carcinoma of ambiguous differentiation).

It must be emphasized that in situ hybridization does not detect HPV DNA other than those used in the mixed probes...
nor HPV DNA at less than 5 copies per cell. The prevalences of HPV DNA in three types of cervical carcinoma do not include the presence of HPV-31, HPV-33, HPV-35, and others found in cervical carcinoma at low rates of prevalence. Considering this fact, the presence of HPV in adenocarcinoma and adenosquamous carcinoma may be higher than the prevalence demonstrated in this study.

ACKNOWLEDGMENTS

The authors thank Nancy Swanson for typing this manuscript and Jean Engbring for assistance with photography.

REFERENCES

Human Papillomavirus Types and Localization in Adenocarcinoma and Adenosquamous Carcinoma of the Uterine Cervix: A Study by in Situ DNA Hybridization

Toru Tase, Takashi Okagaki, Barbara A. Clark, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/4/993

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.