Histological Types of Carcinoma of the Uterine Cervix and the Detectability of Human Papillomavirus DNA

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ABSTRACT

Using the Southern DNA hybridization technique, tissues from 17 cases of invasive carcinoma of the uterine cervix, including nine cases of squamous cell carcinoma, four cases of adenocarcinoma, one case of adenosquamous carcinoma, and three cases of undifferentiated carcinoma, were examined for the presence of human papillomavirus (HPV) DNA. None of the studied cases had histologically confirmed association of condyloma acuminatum or cervical intraepithelial neoplasia in the vicinity. HPV DNA was detected in two of 17 cases under low stringency conditions. One lesion was undifferentiated carcinoma, and another was squamous cell carcinoma. Hybridization under high stringency conditions with a variety of HPV DNA probes indicated the presence of HPV-16 in these two lesions. The other HPV-positive lesion was adenocarcinoma, demonstrating weak hybridizations with HPV-2 and HPV-16 DNA probes only under high stringency conditions. Altogether, three of 17 cases (17.6%) contained HPV DNA. This observation contrasts the rate of HPV DNA present in 15 of 18 cases (83.3%) of the tissues of cervical intraepithelial neoplasia. Our data suggest that HPV was not consistently detected in invasive squamous cell carcinoma, despite the frequent association of HPV with its supposed precursor lesions of cervical intraepithelial neoplasia.

INTRODUCTION

It is generally believed that a significant proportion of squamous cell carcinoma of the uterine cervix is preceded by prernoplastic precursors, dysplasia and carcinoma in situ, categorically designated as CIN3 (1). Recent studies using DNA hybridization techniques demonstrated that HPV DNA was present in a large proportion of CIN (2–7). However, the presence of HPV DNA in invasive carcinoma of the uterine cervix is not unequivocal; HPV DNA was found in 6.5 to 72.2% of the tissues of cervical intraepithelial neoplasia by other investigators (5, 7–11).

If a majority of squamous cell carcinomas develop from CIN, one would expect that squamous cell carcinoma would contain HPV DNA at a high rate, whereas other types of cancer of the uterine cervix might not. This work has been carried out to examine the presence of HPV in cervical carcinoma of various histological types, perhaps of different etiologies, and to shed further light on this important issue.

MATERIALS AND METHODS

The tissue samples were obtained from 17 patients with invasive carcinoma of the uterine cervix which included squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, and undifferentiated carcinoma. Clinically and histologically, none of these cases had coexisting condyloma acuminatum or CIN in the vicinity. Separately, 18 cases of CIN were studied for comparison with invasive carcinoma of the uterine cervix. The diagnoses of the cervical lesions were confirmed by histopathological examinations on specimens obtained by biopsy under direct vision. The results of 5 cases of CIN were included in our previous publications (6, 7). Verrucous carcinoma (Ackerman) of the cervixovagina, which we previously reported, was excluded from this series, because verrucous carcinoma (Ackerman) may not represent true carcinoma in its biological behavior (12).

The tissue samples for DNA hybridization were frozen immediately after biopsy, using liquid nitrogen, and stored at −70°C until processed for hybridization. For DNA hybridization, the frozen tissues were thawed and minced. Viral and cellular DNA was extracted from tissue samples as described previously (7, 13). The DNA was electrophoresed through 1% agarose gel and transferred to nitrocellulose filters as described previously (14, 15). The filters were dried at 80°C under vacuum for 2 h and prehybridized at 37°C for 24 h in a hybridization solution which contained 500 µg of depurinated salmon sperm DNA per ml, 0.1% Denhardt’s solution (16), 20 mM sodium phosphate of pH 6.8, 1 mM sodium chloride, 0.1% SDS, and formamide (30% for low stringency and 50% for high stringency conditions) (17). A fresh hybridization solution containing nick-translated 32P-HPV DNA probes (5-ng probe per ml solution and 10% dextran sulfate (14)) was added to the filters and incubated at 37°C for 20 h. The HPV DNA probes used in these conditions were those of HPV-1 through HPV-6, and in some cases, HPV-16 and HPV-EV.

Filters were washed 3 times in 2x SSC, 0.1% SDS, and 0.1% sodium pyrophosphate at room temperature and for an additional 4 h in SSC (3x SSC for low stringency and 0.4x SSC for high stringency conditions, 0.1% SDS, and 0.1% sodium pyrophosphate at 50°C). These filters were dried and autoradiographed on Kodak X-Omat X-ray film at −70°C with an intensifying screen. We then tested for HPV type-specific DNA by hybridization under high stringency conditions.

Initially, low stringency hybridization conditions, which allow as much as 33% base pair mismatch during DNA-DNA hybridization (thus allowing all papillomavirus DNAs to hybridize to one another) (18), were used to screen the tissues for the presence of any HPV DNA (Fig. 1A). Then, the tissue DNA was hybridized under stringent conditions, which do not permit cross-hybridization between different types of HPV, to determine the specific type(s) of HPV found (Fig. 1B). This assay system is quite sensitive, since we are able to detect as few as one copy of viral genome per 20 diploid cells with a homologous HPV probe and as few as one copy per 3 cells with a heterologous HPV probe (Table 1).

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2 To whom requests for reprints should be addressed, at Box 131, Mayo Memorial Bldg., University of Minnesota, 420 Delaware Street, S.E. Minneapolis, MN 55455.
3 The abbreviations used are: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; SDS, sodium dodecyl sulfate; HPV-EV, a variant of human papillomavirus isolated from epidermodysplasia verruciformis by Green et al (11); SSC, standard saline citrate (0.3 M NaCl; 0.03 M sodium citrate, pH 7.0).
RESULTS

HPV DNA was detected in one case of undifferentiated carcinoma of the cervix and one case of squamous cell carcinoma of the cervix under low stringency conditions. The extract from these tissues hybridized with the HPV-16 probe under high stringency conditions. DNA from one case of adenocarcinoma hybridized weakly with the HPV-2 and HPV-16 probes under high stringency conditions. The remaining 13 cases did not hybridize with the HPV DNA probe under low stringency or high stringency conditions. The results are summarized in Table 1.

In contrast, 15 of 18 (83.3%) cases of CIN hybridized with the HPV-6 or HPV-EV DNA probe under low stringency conditions. Under high stringency conditions, DNA from 7 cases of CIN hybridized with the HPV-6 probe; DNA from 6 cases of CIN hybridized with the HPV-16 probe. DNA from 6 cases hybridized with more than one probe. Four cases which hybridized with the HPV-6 probe or HPV-EV probe under low stringency conditions did not hybridize with any of the probes used under high stringency conditions, thus indicating the presence of an HPV type other than those tested for in this study. The results are shown in Table 2.

The number of cases which were positive for HPV DNA with various HPV probes under high and low stringency conditions is subdivided by the histological type of cervical lesion and summarized in Table 3.

DISCUSSION

Using DNA hybridization procedures, Green et al. (11) detected HPV-related DNA in 2 of 31 (6.5%) cervical carcinomas using HPV-EV DNA as a probe. Gissmann et al. (10) showed that HPV-11 DNA or related sequences were demonstrated in 4 of 19 (21.1%) malignant tumors of the cervix, which included carcinoma in situ. Lancaster et al. (5) observed that one of 6 (16.7%) cervical carcinomas contained HPV hybridizing with an HPV-11 probe under high stringency conditions. More recently, Dürst et al. (8) reported that HPV DNA was found in 72.2% of cervical cancer tissues obtained from German patients and in 43.5% of cervical cancer tissues from Kenyan and Brazilian patients, in which HPV-16 DNA was the most common type of HPV. Details of the histological types of the cervical cancers in their series were not elucidated.

In our study, HPV DNA could not be detected in a majority of 17 cases of invasive carcinoma of the uterine cervix under low stringency conditions using several HPV DNA probes including HPV-6 and HPV-16. Only one case of undifferentiated carcinoma

<table>
<thead>
<tr>
<th>Case</th>
<th>Sensitivity (DNA copies/cell)</th>
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<tbody>
<tr>
<td>No.</td>
<td>Patient</td>
</tr>
<tr>
<td>1.</td>
<td>Z. P.</td>
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<tr>
<td>2.</td>
<td>J. C.</td>
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<tr>
<td>3.</td>
<td>L. L.</td>
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<td>4.</td>
<td>B. L.</td>
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<td>5.</td>
<td>S. R.</td>
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<td>6.</td>
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<td>B. B.</td>
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<td>8.</td>
<td>S. D.</td>
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<td>10.</td>
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<td>15.</td>
<td>A. J.</td>
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<tr>
<td>16.</td>
<td>P. R.</td>
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* ND, not done due to insufficient specimen.
and one case of squamous cell carcinoma exhibited positive hybridization with HPV-16 under high stringency conditions. One case of adenocarcinoma showed weak hybridization with HPV-2 and HPV-16 probes, which was detected only under high stringency conditions. HPV-6 was the probe used under low stringency conditions. Detection of heterologous types of HPV is slightly less sensitive, and since the copy number of HPV-2 and HPV-16 was very low in this tissue, it could only be detected with appropriate probes as was observed under high stringency conditions. HPV-2 has previously been found in association with Bowen’s disease (19), and thus, its oncogenic potential should not be underestimated. The prevalence of HPV DNA in invasive carcinoma of the cervix in our study appears lower than those from previous observations reported from outside the United States thus far. However, our results generally match with the prevalence of HPV genomes in cervical carcinomas reported from institutions in the United States (5, 11).

The sensitivity of the methods we used was as high as, if not higher than, those used by other investigators (Table 1). Therefore, the relatively low prevalence of HPV DNA in invasive carcinoma of the cervix we observed was not attributable to the method used. The presence of HPV DNA in a high percentage of CIN (83.3%) in our study would support this notion.

It is commonly believed that invasive squamous cell carcinoma of the uterine cervix develops from CIN. The histogenesis of other types of invasive carcinoma of the cervix is regarded to be different from that of squamous cell carcinoma. Inasmuch as HPV DNA was associated with CIN at a high rate in our study, HPV may be a causative agent of squamous cell carcinoma of the uterine cervix. One would therefore expect that a significant number of invasive squamous cell carcinomas would contain HPV genomes. Our results were contrary to this expectation.

The cases of cervical carcinoma at our institution included a high proportion (31.0%) of adenocarcinoma and adenosquamous carcinoma, an observation similar to that reported from other institutions in the United States (9, 20). It was possible that some squamous cell carcinoma developed from adenocarcinoma or adenosquamous carcinoma and thus may not have been preceded by CIN. Therefore, a low prevalence of HPV DNA in invasive squamous cell carcinoma of the cervix may be attributable to the geographical difference in the proportion of HPV-induced squamous cell carcinoma of the uterine cervix as reflected in epidemiological data of various histological types of cervical carcinoma in our area. Yet, this does not seem to be clear from our results, as some positive results were found at a low frequency in each of the histological types studied.

Recently, Crum et al. (21) reported that HPV-16 may be more prevalent in CIN containing atypical mitoses; therefore, CIN containing HPV-16 DNA might have a higher potential of invasion than CIN containing HPV-6. While our study here shows HPV-16 in all levels of CIN, other data4 on neoplasms of female genitalia, including vagina, vulva, and cervix, showed a slightly higher preponderance of HPV-16 in a high grade of premalignant conditions, although it is somewhat premature to speculate on the significance of this observation.

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