Association Between Human Papillomavirus Type 18 Variants and Histopathology of Cervical Cancer

Marcela Lizano, Jaime Berumen, Miriam C. Guido, Leonora Casas, Alejandro García-Carrancá*

Adenocarcinoma, adenosquamous carcinoma, and small-cell carcinoma of the uterine cervix are reported to be low in incidence but clinically important. They usually exhibit a more aggressive biologic behavior and have a poorer prognosis than squamous cell carcinomas at similar stages (1–3). Although human papillomavirus type 16 (HPV16) is associated predominantly with squamous cell carcinomas and HPV18 is associated predominantly with adeno- carcinomas and adenosquamous carcinomas (4), differences in prognosis among these groups of cancers are largely still not understood. On the other hand, few reports have evaluated the role of HPV type in the development of small-cell carcinomas [e.g., (5)]. DNA sequence variations among different isolates of HPV16 and HPV18 have been found in various geographic locations and ethnic groups (6–11). To date, these variations have not been shown to correlate with pathologic features (12), and it is not clear whether HPV genetic-intratype variability could account for

Notes

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References

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the wide spectrum of pathology found in the associated lesions.

To determine whether certain HPV variants from known virus types could be associated with particular histologic forms of cervical carcinoma, we analyzed the HPV-variant status in a sample of tumors from Mexican patients. Fifty-three frozen biopsy specimens were obtained from a random sample of a consecutive group of patients who sought medical care during the period from 1992 through 1993 at the Instituto Nacional de Cancerología in Mexico City. These samples consisted of 13 adenocarcinomas, 13 adenosquamous carcinomas, and 27 squamous cell carcinomas of the uterine cervix. Another group of 24 paraffin-embedded biopsy specimens obtained from the pathology files of the same institute was also selected for this study; these specimens were collected between 1985 and 1994, and all were obtained from lesions diagnosed as small-cell carcinoma of the uterine cervix. This study was approved by an institutional review board. All diagnoses were based on the pathology records that accompanied each specimen, and these records included a histologic determination made according to proposed criteria (13).

We assessed the presence of HPV DNA in these specimens, and we attempted to define the virus types by modifying a previously described single-strand conformation polymorphism–polymerase chain reaction (SSCP–PCR) assay (14). Although the SSCP–PCR patterns generated from many of the specimens were clearly identifiable as originating from HPV type 16, 18, or 31, other patterns suggested that other virus types or variants of known types were present (Fig. 1, A). To confirm these results and to determine the origins of the variant electrophoretic patterns, PCR products from the HPV L1 gene (15) and the LCR (i.e., long control region) (7,16) were directly sequenced for those specimens that tested positive for viral DNA (e.g., Fig. 1, B). This analysis revealed the presence of HPV types 45 and 58 (Table 1, A) and showed the existence of variants of HPV types 16 (11 cases), 18 (eight cases), and 45 (three cases) in these tumors (Table 1, B).

A variant of HPV16 that was observed frequently in this Mexican population (Fig. 1, A; compare 16 ref [i.e., prototype reference] lanes with 16 var [variant] lanes) showed the same nucleotide changes as two variants already described, i.e., Bb-2 and IND-8, which belong to a group of Brazilian and Indian variants, respectively, that are considered to be representatives of a novel branch of HPV16 diversity (17). This variant has been grouped together with other variants into the Asian-American branch (10). One of the

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Fig. 1. Variants of human papillomavirus (HPV) types 16, 18, and 45 in cervical tumors from a Mexican population and alteration of the DNA sequence of an L1 gene fragment. A sensitive single-strand conformation polymorphism–polymerase chain reaction (SSCP–PCR) assay allowed us to type HPV-positive samples by comparing the electrophoretic migration patterns of L1 gene amplification products. A) Samples that were shown to have amplifiable DNA (i.e., positive for β-globin gene PCR products) were analyzed for HPV DNA by use of GP5/GP6 consensus primers (26) that allow amplification of a 140-base-pair fragment of the HPV L1 gene. DNA samples from plasmids or cell lines known to contain specific viral sequences were also amplified, and the products were used to generate “control” electrophoretic patterns for different HPV types. The SSCP–PCR analysis was performed essentially as described previously (14). Electrophoresis in 6% polyacrylamide gels was carried out at 10 W for approximately 4 hours at 4 °C. ds = double-stranded DNA; ref and var = prototype-reference clones and variant clones, respectively, for the indicated HPV types (confirmed by DNA sequence analysis). Note that neither control nor reference DNA was available for HPV45; characterization of the HPV45 variant was based on DNA sequence analysis. Pairs of samples that had previously yielded similar patterns were loaded side by side onto the gel for a better comparison. B) DNA sequences of specific PCR products generated from the HPV L1 gene by use of primers MY09/MY11 (15) are shown for variant (var) or reference (ref) viral DNAs. Nucleotide position numbers are indicated to the left, whereas nucleotide changes (ref → var) are indicated to the right of each sequence panel.
Table 1. Distribution of different human papillomavirus (HPV) types and variants in carcinomas of uterine cervix of different histologic origin

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>HPV16</th>
<th>HPV18</th>
<th>HPV31</th>
<th>HPV45</th>
<th>HPV58</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma (n = 27)</td>
<td>13</td>
<td>6</td>
<td>3</td>
<td>—</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>Adenosquamous carcinoma (n = 13)</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>Adenocarcinoma (n = 13)</td>
<td>4</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>Small-cell carcinoma (n = 24)</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>13§</td>
</tr>
</tbody>
</table>

A) HPV types according to histologic type of carcinoma

B) Distribution of HPV16 and HPV18 reference (ref) clones and variants (var) according to histologic type of carcinoma

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>HPV16</th>
<th>HPV18</th>
</tr>
</thead>
<tbody>
<tr>
<td>ref var</td>
<td>ref var-1 var-2</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Small-cell carcinoma</td>
<td>—</td>
<td>10</td>
</tr>
</tbody>
</table>

*The presence of different HPV types and their variants was analyzed in a sample of tumors isolated from a homogeneous population of Mexican origin who belonged to a low socioeconomic class and who exhibited no variations in race and ethnicity. HPV DNA was detected by amplification of a conserved region of the HPV L1 gene, using primers GP5/GP6 (26) and the polymerase chain reaction.

†56 of 77 carcinomas were positive for HPV.

‡The histologic pattern of small-cell carcinoma was defined by the cytologic features of the tumor cells as well as by their grouping pattern. The cells were small, with scanty cytoplasm and uniform round-to-oval nuclei, with no prominent nucleoli.

§DNA from three HPV-positive, but HPV18-negative, samples was not available for further typing.

The most important finding in this study was the apparently exclusive association between HPV18 var-2 and squamous cell carcinoma, in contrast to the reference clone, which was found to be associated with all other histologic types except this one (Table 1, B). The other HPV18 variant (var-1) was observed in all histologic types except squamous cell carcinoma. Finally, the HPV45 variant identified in this study was present exclusively in adenosquamous carcinomas (Table 1, A) and was represented in about one fourth (three [27%] of 11) of all HPV-positive tumors of this histologic type.

The association of particular HPV variants with specific histologic types of cervical cancer suggests either that different cellular factors influence viral ability to transform cells or that specific variants exhibit an intrinsic growth advantage over other HPV isolates in certain types of cells. It has been suggested that viral diversity correlates with the ethnic characteristics of populations rather than with geography (19). In Mexico, mixed ethnic groups are enriched mostly by American-Indian (Amérindian) rather than by Caucasian or African components (20). It is of particular interest that, besides the reference clone, the only HPV16 variant detected in this Mexican population has been found previously in Brazil (IND-8), i.e., in a population of Amerindian origin (17). Yamada et al. (10) detected the same variant along with many other HPV16 variants in a population in the United States with a high Amerindian component, suggesting that this variant may be frequent among certain populations in America.

Although patients infected with the HPV16 variant seemed to be younger...
(seven of 11 women were ≤40 years of age) than those harboring the reference clone (eight of 10 women were >40 years of age), no significant associations were established. Nevertheless, in a preliminary study that included 120 patients with cervical carcinoma from a Mexican population, we observed that this variant was present in the tumors from younger women, in contrast to the reference clone, which was found in older women (Berumen J, Casas L, Lizano M, Guido MC, García-Carrancá A: unpublished data). These observations suggest that the HPV16 variant found in the Mexican population and apparently characteristic of Amerindian women may represent a higher risk variant. This possibility could explain, in part, the high incidence of cervical carcinoma observed among Latin-American women (21, 22). Another possibility is that the variant is of recent origin and was not around when the older women were sexually active, although four individuals (with adenosquamous carcinomas) of 11 who had the variant were older than 40 years of age (41, 43, 44, and 55 years of age, respectively).

The fact that one of the HPV18 variants (var-2) was found exclusively in squamous cell carcinomas suggests that this isolate may be associated with less aggressive behavior, since this group of tumors has a relatively better prognosis than adenoscarcinomas or adenosquamous carcinomas. Indeed, when we analyzed a group of small-cell cervical carcinomas (accounting for <1% of cervical neoplasias and with the worst prognosis) for the presence of HPV DNA, we found that all HPV18-positive tumors contained the reference clone. Since there were no HPV18 variants in this group, it is possible that the variants detected in our study may represent less aggressive isolates. An HPV18 variant with an apparently less aggressive behavior has already been described in precancerous lesions (23). It is tempting to speculate that this variant could represent, together with var-2 (and probably with var-1), a subgroup of HPV18 variants with a different biologic behavior.

Because certain HPV isolates and the histopathologic characteristics of tumors appear to be associated, it will be important to determine whether these associations reflect true functional differences among viral variants that affect the relative risk of progression of precancerous lesions. This information could also be relevant for some immunization strategies, since some amino acid (aa) positions, including aa 379 and aa 415 for HPV16 and aa 399 for HPV18, are part of identified L1 linear epitopes (9,24,25). Finally, it will be important to assess the prevalence of each variant in the normal population to determine its associated relative risk in the development of cervical cancer.

References

(23) Hecht JL, Kadish AS, Jiang G, Burk RD. Genetic characterization of the human papillomavirus (HPV) 18 E2 gene in clinical specimens suggests the presence of a subtype


Notes

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