Immunohistochemical Detection of p53 Protein Expression in HPV-induced Condyloma Acuminatum

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Immunohistochemical peroxidase staining for p53 protein was performed on 22 condyloma acuminatum tissue samples from patients infected with human papillomavirus (HPV). The purpose of our study was to understand the benign character of this syndrome.

The patients studied were infected by HPV type 6 and 11. Two monoclonal antibodies, PAb5 DO-1 and 240, were used to detect the p53 protein.

Overexpression of wild-type p53 was found in the nuclei of the basal cell layers. In healthy tissues and non-infected patients no p53 protein expression was detected.

We would like to speculate that infection with HPVs and their viral protein E7, which is implicated in disruption of normal growth, may regulate the induction of wild-type p53 overexpression, as is known for DNA-damaging agents such as UV or X-radiation. Key word: wild-type p53 overexpression.

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p53 protein is a nuclear phosphoprotein that interacts with large T antigen, the oncogene product of the simian virus 40 (1). It has further been shown that p53 forms physical complexes with oncoproteins such as E6 (a product of human papillomavirus, HPV) (2).

Approximately 20 different types of HPVs may infect the epithelium of the genital mucosa. The resultant lesions range from benign condylomas to premalignant and invasive carcinomas (3).

DNA typing of HPV has indicated a correlation between certain types of HPV and the pathological diagnosis of the lesion.

Thus, HPV 6 and HPV 11 account for the majority of benign condylomas (4).

Studies of human cell transformation have shown that HPV 6 and HPV 11 DNA do not immortalize primary human epithelial cells (5). Patients infected with HPV 6 and 11 have benign condylomas, and with the exception of rare cases of verrucous cancers of the vulva, are thought to be at low risk for developing invasive cancers (4).

The genome of the papillomaviruses is relatively small, containing approximately 8,000 nucleotides that are known to encode eight specific protein products (3).

Two of these viral proteins, E6 and E7, have been implicated in the disruption of normal growth regulation that occurs in HPV-infected cells. The two oncoproteins — E6 and E7 play a major role in the pathophysiology of HPV-induced disorders.

E7 reacts with retinoblastoma protein (6), and E6 proteins degrade specifically with a regulatory protein the p53 (7, 8).

When the E6 protein originates from a high-risk HPV (such as HPV 16 or 18), it binds to p53 and abrogates its activity. On the other hand, when E6 protein is derived from a low-risk HPV (such as HPV 6 or 11), although it binds to p53 with significantly lower affinity than do the high-risk proteins, there is no abrogation of the p53 activity (9).

p53 overexpression promotes the transcription of WAF 1/ CIP 1 (10, 11), which is involved in growth arrest through inhibition of cyclin-dependent kinases required for G1 to S transition.

Interaction of p53 with the large T antigen was shown to induce the inactivation of p53 because of conformational modification. The p53 protein was also found to form complexes with the E1b protein of the adenoviruses. It has further been shown, as mentioned above, that p53 forms a physical complex with oncoproteins such as E6 (the product of the HPV virus) (7, 8).

The mutant p53 proteins found in human tumor cells have lost their growth-suppressive function, thus enhancing neoplastic transforming properties. There seems to be a selection for high-level expression of the mutant protein in the tumor (12).

To further evaluate the molecular mechanism which accounts for the accumulation of one of these forms in cells, we have focused our study on condyloma acuminatum — a benign common papilloma of viral origin, usually occurring on the mucous membrane or skin of the external genitals. To this end we stained tissues of the benign HPV — (HPV 6 and 11) induced genital disease for p53 levels. The patterns of expression of p53 were detected immunohistochemically.

MATERIAL AND METHODS

Patients and tissues

The patients were evaluated for condyloma acuminatum in the Colposcopy Outpatient Clinic, and the Department of Plastic Surgery, and underwent HPV typing in addition to their routine evaluation. Biopsies were done on female and male patients who had lesions clinically consistent with the diagnosis of condyloma acuminatum. All biopsies were obtained from primary genital warts.

Genital biopsy specimens were generally 2 x 2 x 1 mm. The fragments were immediately frozen in liquid nitrogen and stored at -80°C to be cut and prepared for histology and immunohistochemistry. Histologic specimens were classified according to Richart (13), and the histologic assessment was performed by one pathologist.

Immunohistochemistry

Staining was performed on frozen sections (4–5 μm). The frozen biopsies were cut, air-dried, and fixed in acetone for 1 min at 4°C, rehydrated, then incubated in PBS with 1% fetal calf serum for 10 min

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means of the hybri-cyte detection kit were mainly positive for HPV 11 and for HPV 6.

All cases of condyloma acuminatum in this study (12 cases) tested by ISH were positive only for HPV types 11 and 6 (7/12
HPV 11 and 5/12 HPV 6). In a former study of our group (17),
38 cervical samples with a histology of CIN 3 and CIN 2 showed 39% HPV 16-positive and 36% HPV 18-positive.

All samples were positive for p53 (DO-1) staining, both in
HPV 6 and HPV 11 infection; the major expression of p53
protein was detected in the basal layer of the epithelium (Fig. 1).
No staining of normal healthy genital mucosa was observed.

Table I shows the detection of p53 by the PAAb DO-1, but no
staining was evident when the mutant specific PAAb 240 antibody
was used on the same sections. Positive tissue controls included
a well characterized prostatic adenocarcinoma which had
stained with the antibody PAAb 240. Appropriate negative controls
consisted of substitution of the primary monoclonal antibody
with 10% in fetal calf serum in PBS. The absence of PAAb 240 staining is indicative for the wild-type character of the p53
overexpression. Fig. 1 shows preferentially nuclear staining by
PAAb DO-1.

DISCUSSION
Polyclonal and monoclonal antibodies raised against human
p53 efficiently detect the mutant p53 protein in routine histo-
pathology samples (15, 18). In this way, high levels of p53
mutant protein have been identified in many tumor types and all
major tumor cell lineages (18, 19). However, p53 protein, which
is a normal constituent of the cell, cannot as a rule be visualized
with immunohistochemistry due to the short life of normal
wild-type p53. Most human primary tumors which exhibit over-
expression of p53, estimated by immunohistochemical staining,
show that in these tumors the mutant form is homozygously
duplicated due to a process of loss of heterozygosity (18, 20).
Nuclear localization of wild-type p53 protein was suggested to be
fundamental to the manifestation of the suppressor activity of
this protein (20).

In the present study, performed on benign tissues of HPV-
infected condyloma acuminatum, we found in the 22 samples of
stratum basaleum collected from 12 patients an overexpression

Table I. Condyloma acuminatum, clinical data

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>MAbs’s</th>
<th>Age</th>
<th>Sex</th>
<th>Quantity/Localization of lesion</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>DO-1</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>18</td>
<td>f</td>
<td>3 vulva</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>29</td>
<td>f</td>
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<td>+</td>
<td>45</td>
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<td>34</td>
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<td>5</td>
<td>+</td>
<td>51</td>
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<td>6</td>
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<tr>
<td>7</td>
<td>+</td>
<td>38</td>
<td>f</td>
<td>2 perineum</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>37</td>
<td>f</td>
<td>2 vulva</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>31</td>
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</tr>
<tr>
<td>10</td>
<td>+</td>
<td>40</td>
<td>m</td>
<td>1 penis</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>26</td>
<td>m</td>
<td>1 penis</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>29</td>
<td>m</td>
<td>5 penis</td>
</tr>
</tbody>
</table>

RESULTS
Analyses of the cases of condyloma acuminatum tested by

Viral gene detection by the hybri-cyte non-radioactive test
The hybri-cyte viral gene HPV detection kit (Hybri Cyte – PBS-
Organics, Yavne, Israel 70650), manufactured by Parc de l’Innovation B.P.
209 – Illkirch Cedex 67405 France) detects and types HPV types 6, 11,
16 and 18 (17).

The test involves a colorimetric in situ method of hybridization,
which detects the presence of nucleic acids in target DNA (from
tissues), using non-radioactive labeled probes. The commercial probes
for in situ hybridization (ISH) are labeled by PBS-Organics France
(Ref: 250050, 0011, 0016, 0018) (17). Paraffin-embedded sections of 5
µm thickness were attached to coated microscopic slides.

After deparaffinization, the sections were digested with proteinase K
to enhance the accessibility of the target DNA hybridized to labeled
DNA probes.
The target DNA and labeled probes were simultaneously denatured
by heating and then hybridized. Following the post-hybridization
washes, hybridized DNA was visualized immunologically in a series of
reactions, described in details elsewhere (17), involving mouse mono-
clonal antibodies to modified DNA, alkaline phosphatase conjugated
to goat antibodies to mouse IgG, and chromogenic dye substance.

Dephosphorylation of the enzyme substrate resulted in the formation
of a purple precipitate at the site of hybridization. Treated slides were
inked, mounted, and visualized by light microscopy. In parallel to the
samples, positive and negative control sections were tested.
of wild-type p53 protein. The condyloma acuminatum-infected patients in our study were infected with HPV types 6 and 11 (see also a previous study of our group [17]). The overexpression of p53 detected by immunohistochemical staining in all our samples was found only in the basal stratum of condyloma acuminatum. These layers are known for a uniformly strong labelling of E7 gene transcription. On the other hand it was stated by Iftner et al. [21] that HPV E6 protein signals were homogenously but weakly expressed in the cytoplasm of basal cells. In some of Iftner’s cases weak signals were also discernible in suprabasal cells. E7 protein does not bind to p53 protein. Elevated levels of wild-type p53 protein were also found by Clark et al. [22] in recurrent benign laryngeal papillomas, harbouring HPV 6 and 11. Barbosa et al. [9] found a weak transformation activity of the E6 and E7 genes encoded by low-risk HPVs that bind with low affinity and no abrogation to p53 protein. It seems therefore that in the case of condyloma acuminatum the unusual overexpression of wild-type p53 was related to the HPV infection.

It is known [23-25] that overexpression of p53 protein can be observed when cells are affected by UV-radiation, X-radiation, chemotherapy and other DNA-damaging agents. An immediate cause of DNA damage is also the HPV DNA E7 oncogene, whose active transcription was detected in the basal stratum (21). The findings of Crook et al. [26], which showed an inverse relation between cervical carcinomas caused by HPV and mutant p53, support our hypothesis. They suggested that the gradual loss of p53 in cervical carcinoma was a result of E6 protein binding to p53, thereby abolishing its function. Crook’s findings suggest that p53 proteins are degraded by mediation of the E6 protein binding in vivo, abrogating the p53-mediated uncontrolled cell proliferation after DNA damage.

HPV infection and p53 gene mutation are not mutually exclusive, and HPV-negative carcinomas may arise via p53-independent pathways, as stated by Kessis et al. [27]. In our findings, however, when taken together, it is tempting to speculate that the benign character of condyloma acuminatum is related to the overexpression of wild-type p53 in the basal layer.

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