Acta Derm Venereol (Stockh) 1995; 75: 177–179

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

Y. TENDLER[1], Y. SCHWARTZ[1], R. RESHEF[1], S.M. SHASHA[1], V. ROTTER[2] and T. SHKOLNIK[1]

[1]Clinical & Research Laboratories, Nahariya Regional Hospital, Nahariya and [2]Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel

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, PAbs 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.***

(Accepted October 28, 1994.)

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T. Shkolnik, Department Laboratories, Nahariya Regional Hospital, Nahariya 22100, Israel.

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 oncogenes such as E6 (a product of the 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 retinobalstoma 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 cycline-dependent kinases required for G, 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 oncogenes 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 2×2×1 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

 ISSN 0001-5555

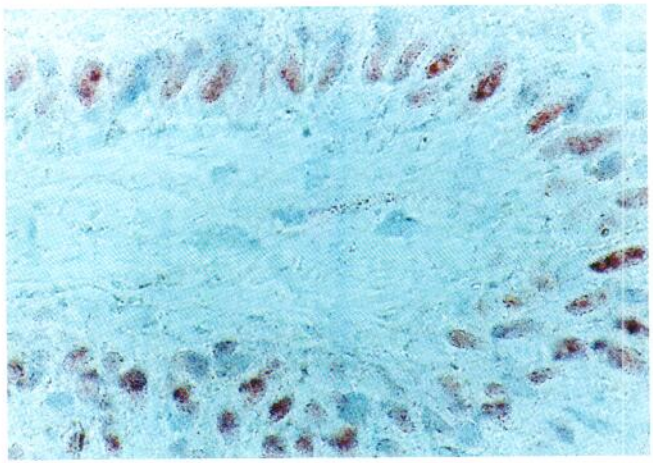

Fig. 1. Nuclear staining of positive p53 with PAb DO-1 (magnification ×200). The sample was depicted from one of the perineal lesions of patient No. 2.

prior to incubation overnight at 4°C with one of the appropriately diluted (1:200) anti-p53 monoclonal antibodies PAb 240 and DO-1.

PAb 240 is specific for the mutant form of p53 (14, 15). PAb DO-1 is a murine monoclonal antibody against recombinant human p53 fragment (16). The slides were washed in buffered wash solution (Immunostain) for 5 min. A biotinylated anti-mouse secondary antibody (Pharmatrade Ltd, Israel, manufactured by EURO/DOC Ltd, Witney OX8 6AN, U.K.) was then applied for 30 min. The slides were then incubated at room temperature in a humidity chamber, followed by washing in buffered wash solution for 5 min. A streptavidin complex was applied for 20 min at room temperature, again in a humidity chamber, and washed in buffered wash solution, for 5 min. The specimens were then incubated with a solution of aminoethylcarbazole (Pharmatrade Ltd, Israel, manufactured by EURO/DPC Ltd, Witney OX8 6AN U.K. for 3–5 min. The slides were then washed, visualized and photographed.

Viral gene detection by the hybri-cyte non-radioactive test

The hybri-cyte viral gene HPV detection kit (Hybri Cyte – *PBS-Orgenics*, 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-Orgenics France (Ref: 250006, 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 monoclonal 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 rinsed, mounted, and visualized by light microscope. In parallel to the samples, positive and negative control sections were tested.

RESULTS

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

DISCUSSION

Polyclonal and monoclonal antibodies raised against human p53 efficiently detect the mutant p53 protein in routine histopathology 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 vizualized with immunohistochemistry due to the short life of normal wild-type p53. Most human primary tumors which exhibit overexpression of p53, estimated by immunohistochemical staining, show that in these tumors the mutant form is homozygotically expressed due to a process of loss of heterozygocity (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 basalium collected from 12 patients an overexpression

Table I. *Condyloma acuminatum, clinical data*

Patient No.	MAb's		Age	Sex	Quantity/Localization of lesion
	DO-1	240			
1	+	–	18	f	3 vulva
2	+	–	29	f	4 perineum
3	+	–	45	f	2 vulva
4	+	–	34	f	2 vulva
5	+	–	51	f	2 vulva
6	+	–	20	f	2 vulva
7	+	–	38	f	2 perineum
8	+	–	37	f	2 vulva
9	+	–	31	m	1 penis
10	+	–	40	m	1 penis
11	+	–	26	m	1 penis
12	+	–	29	m	5 penis

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 homogeneously 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 effected 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.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Science and Arts – the State of Israel and approved by the ethics committee for animal research.

We would like to thank Mrs. Jacqueline Cohen for her excellent secretarial assistance, and Dr. Boris Zilman for his kind assistance.

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