P27 expression in normal epithelium and condylomas of the vulva in HIV positive and negative women

F. Zucchi, *M. D.*; **J. Focchi,** *M. D., Ph. D.*; **I. D. C. G. Silva,** *M. D., Ph. D.*; **E. C. Baracat,** *M. D., Ph. D.*
J. N. Stávale, *M. D., Ph. D.*; **J. C. L. Ribalta,** *M. D., Ph. D.*

Department of Gynecology, Federal University of São Paulo (Brazil)

Summary

Purpose: The objective of the present study was to evaluate the expression of the p27 protein in the normal epithelium and vulvar condylomas in human immunodeficiency (HIV) positive and negative patients.

Methods: Eight samples of normal vulvar epithelium were evaluated (Group A), ten of the HIV negative vulvar condyloma patients (Group B) and another eight of the vulvar condyloma HIV positive patients (Group C). The DNA of human papillomavirus (HPV) was identified by means of polymerase chain reaction (PCR). Immunohistochemistry was the method used to evaluate the expression of p27 using monoclonal mouse antibody (Monoclonal Mouse, anti-human p27, Clone Sx 53 G8). The immunoexpression was evaluated at a magnification of 400x, counting a minimum of 1,000 cells per slide.

Results: The results obtained were the following: a) comparing groups A and B and groups A and C there was a significant difference in relation to the expression of the p27 protein which was 63.32% in group A and only 13.35% and 18.89% in groups B and C, respectively; b) comparing groups B and C among them, there was no significant difference.

Conclusion: We concluded that in normal vulvar tissue the p27 protein is present in a large number of cells and that in vulval condylomas its expression is very much lowered both in HIV positive and negative cases.

Key words: Condylomas; Human papillomavirus; p27.

Introduction

Vulvar condyloma is the most common sexually transmitted disease of viral origin in the sexually active population [1, 2, 3]. It is caused by HPV (human papillomavirus) and at this location, the most often found types are 6 and 11 [4, 5, 6]. Immunologically affected individuals seem to maintain the persistent form of HPV DNA, so acuminated condylomas and the subclinical forms of this infection are frequently found in patients with acquired immunodeficiency virus (HIV) [7].

Normally cells with damaged DNA activate the inhibitor signs of the cell cycle and its duplication is prohibited, making this cycle stationary in G1, providing enough time for the DNA repair system to be activated. An important regulatory mechanism of the cell cycle is represented by cyclin-dependent kinase inhibitors (CKI) [8, 9]. These inhibitors are proteins originating from tumor suppressor genes which are activated as a defense and cellular repair mechanism.

The cyclin-dependent kinases are divided into two groups: the Kip/Cip family which includes p21, p27 and p57 and the so-called Ink4 inhibitors represented by p16, p18 and p19.

The p27 protein inhibits the cyclins E.CDK2, A.CDK2 and D2 CDK4 forbidding the cell to enter in to phase S.

Toyoshima and Hunter [10] were the first to isolate p27 in rat fibroblasts in 1994. The same year Poliak *et al.* [11] were able to clone this protein and describe its function as being an inhibitor of cyclin-dependent kinases thus arresting the cells in contact with growth factor TGF beta in phase G1 of the cell cycle and not being able to progress to phase S. Lloyd *et al.* [12] studied other functions of p27 [13, 14, 15].

Studies have shown that the human papillomavirus can control the activity of p27 [16, 17].

The HPV oncoprotein E7 can interfere alone in cyclin A and block p27 and p57 [18, 19].

Many studies referring to p27 protein expression show an important lowering of the above in more aggressive tumors [20].

Recently some studies have concluded that the expression of p27 was significantly smaller in intraepithelial cervical neoplasms when compared to normal tissue and did not depend on the degree of cellular differentiation or the subtype of the infectant HPV [21, 22].

The p27 is a tumor suppressing gene which relates to other genes and molecular level cell defense. Thus any study that further elucidates its action, especially in early HPV induced lesions, is considered very important. This is why we proposed to study it in vulvar lesions.

Materials and Methods

The present study was carried out at the Pathology of the Lower Genital Tract and Colposcopy Section of the Gynecology Department at the Federal University of São Paulo, Paulista School of Medicine.

Samples of vulvar condylomas were obtained under local anesthesia by using a Gaylor Medina forceps, and samples of vulvar tissue were obtained by means of colpoperineoplasty.

Revised manuscript accepted for publication October 15, 2001

Part of the samples were used for the extraction of viral DNA through PCR for identification and typing of HPV. Another part was fixed in 10% formaldehyde, dehydrated with ethilic alcohol, diaphanized with xylene and embedded in paraffin. Following this, the pieces were cut using a microtome and three histological cuts were made, two for immuno-histochemical studies and the third for histopathological evaluation.

The histopathological study confirmed eight samples of normal vulvar tissue (Group A) and 18 vulvar condylomas, ten in HIV negative patients (Group B) and eight in HIV positive patients (Group C).

Identification of the p27 protein was done by an immunohistochemical method using monoclonal antibodies by immunization of laboratory animals with human antigens (Monoclonal Mouse p27, Clone Sx 53 G8) in the dilution of 1:25-1:50. After this, secondary antibodies following the Strepto-Avidine Biotin-Peroxydase (Strepto ABC, Dako), method, at the dilution of 1:500. After proper treatment the epithelial cells containing protein p27 acquire a dark brownish color.

Immunoexpression of protein p27 was evaluated using digital image analysis consisting of an optic microscope coupled to a videocamera. The immunoexpression was evaluated at a magnification of 400x, counting a minimum of 1,000 cells per slide and only the nuclei that were dark brown were considered positive. The percentages of p27 in the epithelium were obtained from the relationship of positive and negative cells.

Results

Table 1 shows that a mean of 63.32% was obtained for group A; 13.35% for group B and 18.89% for C with a standard error of 5.11% 2.66% and 5.22% for A, B and C, respectively.

When comparing groups A and B and A and C there were significant differences; when comparing groups B and C, no significant difference was noted. We also noted that the expression of p27 was much lower in cells of the 18 condylomas in relation to cells of normal vulvar epithelium (16.12% vs. 63.63%) and that there was no important difference when comparing HIV positive and negative patients.

Figures 1 and 2 show that the expression of protein p27 was higher and more uniform in the basal layer and in the peripheral area of the papillae both in normal tissue as well as in HPV infected tissue.

Figures 3 and 4 show that when comparing the intermediary layers of the epithelium, p27 was much higher in normal tissue than in tissue infected by HPV.

Table 1. — *Distribution of the expression of p27 protein (%) in the evaluated groups.*

Case no.	p27 (%)		
	A	B	C
01	57.95	14.47	12.10
02	61.37	5.11	20.49
03	83.27	2.45	11.63
04	77.63	5.79	37.82
05	71.60	26.10	8.22
06	37.40	9.99	4.79
07	65.48	27.49	10.89
08	54.29	13.67	45.14
09	—	17.17	—
10	—	11.23	—
Mean	63.32	13.35	18.89
SE	5.11	2.66	5.22

SE = standard error

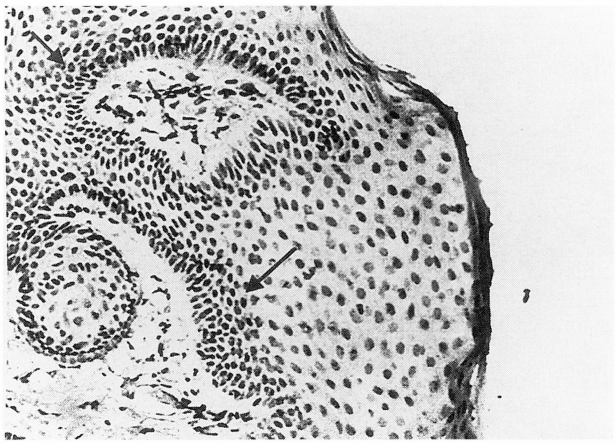

Figure 1. — Fragment of the normal vulva, magnification 100x. Note larger regularity of colored cells in the basal layer and in the dermal papillae.

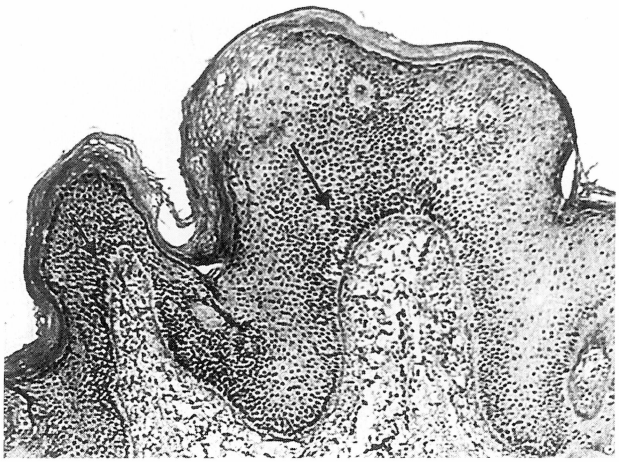

Figure 2. — Condyloma of an HIV+ patient, magnification 100x. Note larger number of colored cells with p27 in the basal layer and papillae.

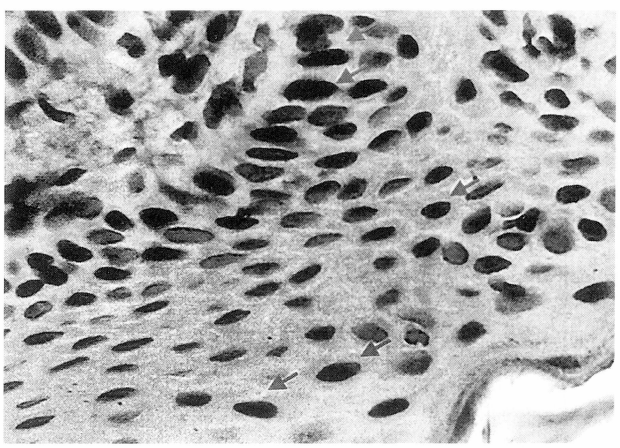

Figure 3. — Fragment of normal vulvar tissue, magnification 400x. Note larger quantity of colored cells with p27.

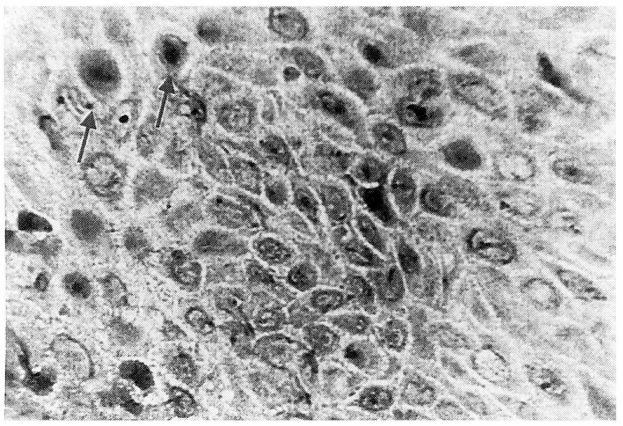

Figure 4. — Vulvar condyloma, magnification 400x. Note rare colored cells with p27.

Discussion

It is clearly defined that HPV is a crucial factor in the genesis of lower genital tract carcinomas and that its proteins E6 and E7 are potentially oncogenic. These proteins interfere in the control of the cell cycle leading to disordered proliferation.

The oncogenes were first identified in viruses capable of inducing transformation; later it was demonstrated that cell genes correspond to viral genes involved in the normal function of cells [24].

HPV infection is a fundamental but not sufficient step in determining carcinogenesis needing the presence of molecular co-factors and immunological alterations of the host. In intraepithelial neoplasias, HPV DNA is found in the episome form in high frequency. In invasive cancer, HPV DNA 16 and 18 are frequently integrated to the host genome.

The E6 protein of HPV promotes the ligation of ubiquitins to p53, leading to its degradation and as a consequence there is no p27 production which is dependent on p53 [23].

The E7 protein of HPV interferes with the retinoblastoma protein (pRB) leading to an abnormal proliferation because it prohibits the action of inhibitors of the cyclin-dependent kinases of the Ink4 family.

We also observed marked lowering of p27 expression in vulvar warts. This fact leads us to believe that this protein has its expression affected long before the neoplastic transformation installs itself, thus being an early phenomenon in carcionogenesis.

Similar to the studies of Troncone *et al.* [21, 22] of the cervix, our study did not show a correlation with the type of HPV when evaluating the expression of protein p27 in genital warts.

This finding alone demonstrates that even low risk viruses are capable of lowering the expression of this tumor suppressor gene to levels comparable to those observed in invasive neoplasias.

References

[1] Barret T. J., Silbar J. D., Mc Ginley: "Genital warts: a veneral disease". *JAMA*, 1954, *154*, 333.

[2] Dunn A. E., Ogilvie M. M.: "Intranuclear virus particles in human genital warts tissue: observations on the ultrastructure of the epidermal layer". *J. Ultrastruct.*, 1968, *22*, 282.

[3] Oriel J. D., Almedia J. D.: "Demonstration of virus particles in human genital warts". *Br. J. Vem. Dis.*, 1971, *46*, 1.

[4] Monoharan V., Sommerville J. M.: "Benign squamous papillomatosis: case report". *Genitourin. Med.*, 1987, *63*, 393.

[5] Reid M. N., Laimins L. A.: "Human papillomavirus - related disease of the vulva". *Clin. Obst. Ginecol.*, 1991, *34*, 630.

[6] Siegel J. F., Mellinger B. C.: "Human papillomavirus in the male patient". *Urol. Clin. North America*, 1992, *19 (1)*, 83.

[7] Surentheran T., Harwood C. A., Spik P. J., Sinclair A. L., Leigh I. M., Proby C. M. *et al.*: "Detection and typing of human papillomaviruses in mucosal and cutaneous biopsies from immunossupressed and immunocompetent patients with epidermodysplasia verruciformis: a unified diagnostic approach". *J. Clin. Pathol.*, 1998, *51*, 606.

[8] Granã X., Reddy E. P.: "Cell cycle control in mammalian cells: role of cyclin dependent kinases (Cdks), growth suppressor genes and cyclin dependent kinases inhibitor (Ckis)", *Oncogene*, 1995, *11*, 211.

[9] Sherr C. J., Roberts J. M.: "Inhibitors of mammalian G1 cyclin-dependent kinases". *Gene Day*, 1995, *9*, 1149.

[10] Toyoshima H., Hunter T.: "p27 a novel inhibitor of G1 cyclin-Cdk protein kinase activity, is related to p21". *Cell*, 1994, *78*, 67.

[11] Polyak K., Lee M. H., Erdjumente-Bromage H., Roberts J. M., Tempst P., Massaque J.: "Cloning of p27 (Kip 1), a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals". *Cell*, 1994, *78*, 59.

[12] Lloyd R. V., Lori A. E., Long J., Elzbieta K., Xiang Q., John C. C., Bernd N. S.: "p27: A multifactorial cyclin-dependent-kinase inhibitor with prognostic significance in human cancers". *Am. J. Pathol.*, 1999, *154*, 313.

[13] Chen J., Willingham T., Shuford M., Nisen P.: "Tumor suppression in inhibition of aneuploid cell accumulation in human brain tumor cells by ectopic overexpression of cyclin-dependent kinase inhibitor p27". *J. Clin. Invest.*, 1996, *97*, 1983.

[14] Nakayama K., Ishida M., Shirane M., Inomata A., Inoue T., Shishido N., Nakayama K. I.: "Mice lacking p27 display increased body size, multiple organ hyperplasia, retinal dysplasia and pituitary tumors". *Cell*, 1996, *85*, 707.

[15] Ophascharoensuk V., Fero M. L., Hughes J., Roberts J. M., Shankland S. J.: "The cyclin-dependent kinase inhibitor p27 safeguards agaist inflamatory injury". *Nature Med.*, 1998, *4*, 575.

[16] Mal A., Poon R. Y., Howe P. H., Toyoshima H., Hunter T., Harter M. L.: "Inactivation of 27 by the viral E1A oncoprotein in TGF beta treated cells". *Nature*, 1996, *380*, 262.

[17] Ruech M. N., Laimins L. A.: "Human papillomavirus oncproteins alter differentiation-dependent cell cycle exit on suspension in semisolid medium". *Virology*, 1998, *250 (1)*, 19.

[18] Bem-Bassat H., Rosenbaum-Mitrani S., Hartsztark Z., Kleinberger-Doron N., Plowman G., Levitzki R. *et al.*: "Inhibitors of epidermal growth factor receptor kinase an of cyclin-dependent kinase 2 activation induce growth factor arrest, differentiation, and apoptosis of human papillomavirus 16-immortalized human keratinocites". *Cancer Res.*, 1997, *57 (17)*, 3741.

[19] Crish J. F., Bone F., Balasubrabanian S., Zaim T. M., Wagner T., Yun J. *et al.*: "Suprabasal expression of the human papillomavirus type 16 oncoprotein alters expression of cell cycle regulatory proteins". *Carcinogenesis*, 2000, *21 (5)*, 1031.

[20] Steeg P. S., Abram J. S.: "Cancer prognostics: past, present and p27". *Nature Med.*, 1997, *3*, 606.

[21] Troncone G., Vetrani A., De Rosa G., Gerbasio D., Palombini L.: "Cyclin dependent kinase inhibitor p7 expression in normal and neoplastic cervical ephitelium". *J. Clin. Pathol.*, 1999, *52*, 880.

[22] Zehbe I., Ratsch A., Alunni-Fabroni M.: "Overriding of cyclin-dependent kinase inhibitor by high and low risk human papillomavirus types: evidence for an in vivo role in cervical lesions". *Oncogene*, 1999, *18*, 2202.

[23] Miwa K., Miyamoto S., Kato H.: "The role of p53 inactivation in human cervical cell carcinoma development". *Oncogene*, 1995, *10*, 219.

[24] Weinberg R. A.: "The action of oncogenes in the cytoplasm and nucleus". *Science*, 1985, *230*, 770.

Address reprint requests to:
F. ZUCCHI M.D.
Molecular Gynecology Lab.
Federal Univ. of Sao Paulo
Rua Pedro de Toledo 781, 4th fl.
Sao Paulo, SP 04023-060 (Brazil)