The presence of HPV 16, 18 and p53 immunohistochemical staining in tumor tissue of Israeli Jewish women with cervical and vulvar neoplasia

J. Menczer, *M. D.*; **Y. Fintsi**[1], *M.D.*; **S. Arbel-Alon,** *M.D.*; **L. Tell**[1], *M.Sc.*; **E. Friedman**[2], *M.D. Ph.D.*; **A. Jackman**[3], *M. Sc.*; **L. Sherman**[3], *Ph. D.*

Departments of Obstetrics and Gynecology, and [1]Pathology, E. Wolfson Medical Center, Holon; [2]Susanne-Levi-Gertner Oncogenetic Unit, Sheba Medical Center, Tel-Hashomer; [3]Department of Human Microbiology, Sackler Faculty of Medicine, Tel-Aviv University (Israel)

Summary

The incidence of cervical neoplasia in Israeli Jewish women is persistently lower, while that of vulvar carcinoma is comparable to that in other populations. The aim of the present investigation was to assess the prevalence of HPV and of immunohistochemically detected mutant p53 in Israeli Jewish women with cervical and vulvar neoplasia compared with other populations.

Tissue sections from formalin-fixed paraffin-embedded blocks of ten patients with CIN III, 29 with invasive squamous cell carcinoma, three with adenocarcinoma and 14 with invasive vulvar carcinoma, were examined for the presence of HPV 16 and HPV 18 DNA by PCR amplification, and for mutant p53 protein by immunohistochemical staining. HPV negative cases were re-examined with a sensitive primer.

HPV DNA was detected in eight patients with CIN III and in 23 patients with invasive squamous carcinoma. In the remaining cervical squamous neoplasia tissue analysis with the sensitive primer could not be done. HPV DNA was also detected in two patients with adenocarcinoma and in nine (64.2%) patients with vulvar carcinoma.

Positive p53 immunohistochemical staining was found only in one CIN III patient, in six (20.7%) squamous carcinoma and in 11 (78.6%) vulvar carcinoma patients. Of the p53 immunohistochemical staining positive tissues, two with cervical carcinoma and six with vulvar carcinoma were also HPV-positive.

The prevalence of HPV and of positive p53 immunohistochemical staining in our series of Israeli Jewish women with cervical and vulvar neoplasia is similar to that in other populations, suggesting that the etiological factors are probably also alike.

Key words: Jewish women; HPV 16, 189; p53; Cervical neoplasia; Vulvar carcinoma.

Introduction

Two mechanisms involving the p53 suppressor gene are associated with the etiology of cervical neoplasia. Oncogenic human papillomavirus (HPV) E6 protein binds to the p53 protein and inactivates it by degradation [1, 2] thus impeding its tumor suppressor activity. Another mechanism of inactivation of the p53 protein product suppressor activity are mutations in this gene. While oncogenic HPV subtypes have been shown to be present in about 90% of cervical carcinoma tissue samples (3), mutations of p53 are infrequent [4, 5]. The most common subtypes of HPV associated with cervical neoplasia are HPV 16 and HPV 18 [3, 6, 7]. In contrast to the normal, or wild type p53, the half-life of the mutant p53 protein is prolonged [8], it accumulates in the nucleus and can be detected by immunohistochemical staining techniques in paraffin-embedded tissue [9]. Currently there is evidence that the HPV virus may also play an etiological role in some vulvar neoplasias [10-13]. An association between mutant p53 and vulvar cancer has also been reported [10, 13-17].

The incidence of carcinoma of the uterine cervix is persistently very low (5.5 per 100,000) among Israeli Jewish women compared to other populations [18].

On the basis of the "organ system" and multicentric origin concept of malignancies of the lower genital tract [19-22], we have previously compared some aspects of squamous invasive cervical and vulvar carcinoma in Israeli Jewish women [23]. While the incidence of cervical cancer was very low, the incidence of vulvar carcinoma was comparable to that in white women in the US.

The present investigation was performed to assess if the prevalence of HPV in Israeli Jewish women with cervical and vulvar neoplasia and of immunohistochemically detected mutant p53 is similar to that found in other populations.

Materials and Methods

Non-selected formalin-fixed paraffin blocks of 42 Israeli Jewish patients with histologically confirmed cervical neoplasia and 14 with invasive vulvar carcinoma, were located. The histological slides of all patients were reviewed by a single pathologist (Y. F.), the histologic diagnosis was reconfirmed and tissue sections from the blocks in the area of the neoplasm were prepared.

All tissue sections were examined for the presence of HPV 16 and HPV 18 DNA by PCR amplification and for mutant p53 protein by immunohistochemical staining.

Revised manuscript accepted for publication August 26, 1999

PCR amplification

DNA was extracted from 10 μm sections by deparaffination and digestion with proteinase K in a separate laboratory (E. F.) PCR amplification was carried out as previously described [24]. Briefly, 8 μl DNA were amplified in a 100 μl PCR reaction mixture containing 35pmole of the 5' (sense) and 3' (antisense) primers, 1.0mM of each of the dNTPs and 2 U Taq polymerase (TakaRa, SHUZO Co., Ltd, Japan). The reaction was subjected to 28 PCR amplification cycles, beginning with DNA denaturation at 94°C for 1 minute, followed by primer annealing at 55°C for 1 minute and elongation at 72°C for 1 minute. The final elongation step was prolonged for 8 minutes. In cases where a second round of amplification was performed, 2% of the first mix was amplified using the same reaction parameters.

HPV type specific primers used for amplification of HPV 16 DNA were: 16E6s-101 5' - GCA ATG TTT CAG GAC CCA CA - 3' and 16E7as-820 5' - GTG TGC CCA TTA ACA GGT CT-3'. For reamplification primers were: 16E6s-200 5' - TGC AAG CAA CAG TTA CTG CG-3' and 16E7as-570 5' - TCC ATG CAT GAT TAC AGC TG-3'. For amplification and reamplification of HPV 18 DNA primers used were: 18E6s-141 5' - AAG CTA CCT GAT CTG TGC AC-3' and 18E7as-606 5' - GCC TTA GGT CCA TG CAT ACT - 3'.

Products were analyzed by electrophoresis on 1.4% agarose gels stained with ethidium bromide. Plasmids carrying HPV 16 and 18 genomic DNA served as positive controls for PCR amplification. Negative samples in the first round of amplification were reamplified. Samples that remained negative underwent an additional analysis after DNA purification with sensitive primers that are able to identify and amplify short DNA segments containing only 100 bases. In this analysis the type specific primers used for HPV 16 DNA were 16Es-101 described above and 16E6as-225 5'-TCACGTCGCAG-TAACTGTTG-3' and for HPV 18 DNA 18E6-501 5' CACTA-TAGAGGCCAGTGCCA-3' and 18E7as-606 described above.

Immunohistochemical staining method

A labeled streptovidin-biotin detection method, also known as streptovidin-biotin amplification (Histostain-SP Kit, Zymed Laboratories SO. San-Francisco, CA) was employed.

Tissue sections were deparaffinised in xylene and rehydrated with graded alcohols. Microwave pretreatment was performed, to enhance the immunoreactivity, by incubating the slides in citrate buffer for 25 minutes in 92°C.

After heating, the slides were cooled 10-20 minutes at room temperature. Endogenous peroxidase activity was then quenched by bathing slides in 3% H_2O_2 for 5 minutes at room temperature.

After rinsing 5 minutes with PBS, slides were treated with serum blocking solution - 10% non-immune serum for 10 minutes at room temperature and the serum was then blotted off.

Next, mouse anti-p53 clone: Pab 1801 (Zymed Laboratories SO. San-Francisco, CA) at a dilution of 1:50 was applied to the slides and incubated overnight at 4°C, followed by incubation with abiotinylated second antibody and with streptavidin-peroxidase, both for 10 minutes each at room temperature. Between incubations, the sections were washed in PBS for 5 minutes.

The sections were then treated with chromogen amynoethyl-carbazide, rinsed in distilled water and immediately counter-stained with hematoxylin and mounted with coverslips. Colon adenocarcinoma was used as a positive control.

Strong and/or widespread nuclear staining was considered positive.

Results

Of the total group of patients, 10 had cervical intraepithelial neoplasia grade III (CIN III) 29 had cervical invasive squamous cell carcinoma, three had cervical adenocarcinoma and 14 had invasive squamous cell carcinoma of the vulva. Selected characteristics of these patients are presented in Table 1. The median age of patients with CIN III and cervical invasive squamous carcinoma was 42 and 53 years, respectively. The most frequent place of birth was Europe, most patients were married and of those with invasive carcinoma most were diagnosed at an advanced stage. All the patients with vulvar tumors had invasive keratinizing squamous cell carcinoma and were older than 55.

Among the 10 CIN III tissue samples, eight contained HPV (seven HPV 16 and one HPV 18). Among the 29 cervical invasive squamous cell carcinoma samples, 21 contained HPV 16 and two HPV 18. In the remaining two CIN III and six cervical invasive squamous carcinoma tissue samples, analysis with the sensitive primer pairs could not be performed because of insufficient DNA extract. Of the positive HPV samples, four with CIN III and 11 with invasive squamous carcinoma HPV DNA were detected only after analysis with the sensitive primer was done. Thus all the tissue samples that were also analysed with the sensitive primer pairs, were HPV DNA positive.

Two of the three adenocarcinoma samples also contained HPV 16. None of the adenocarcinoma tissue samples contained HPV 18.

Positive p53 immunostaining was found in six (20.7%) of the invasive squamous carcinoma tissue samples, and two of these also contained HPV 16. Staining was also positive in one CIN III tissue sample but in none of the adenocarcinoma samples. Nine (64.2%) of the vulvar carcinoma tissue samples were HPV DNA positive (eight HPV 16 and one HPV 18). Eleven (78.6%) of the vulvar carcinoma patients had positive immunohistochemical staining and six of them were also HPV DNA positive.

Table 1. — *Selected characteristics of the study group patients.*

	Type of tumor			
		Cervical carcinoma		Vulvar carcinoma
Characteristics	CIN III (n=10)	Invasive squamous (n=29)	Adenocarcinoma (n=3)	Invasive squamous (n=14)
Age				
median	42	53	43	75
range	24-84	29-86	40-44	55-87
Place of birth				
Israel	5	7	–	–
Europe	1	16	1	9
Other	4	6	2	5
Marital status				
Single	3	4	–	–
Married	5	18	3	3
Other	2	7	–	11
Stage				
I	–	16	3	10
II-III	–	13	–	4

Discussion

A low HPV prevalence rate (36%) was previously reported from Israel in a series of 22 cervical carcinoma patients, using the southern blot hybridization technique [25]. Our study of Israeli Jewish women with CIN III and invasive squamous cervical cancer, using a sensitive PCR technique indicates that, as in other populations, the prevalence of HPV 16, 18, is very high [3]. HPV 16 was also detected in two of our three adenocarcinoma tissue samples and is consistent with current data showing that this tumor may also be HPV associated [26, 27]. In the general Israeli population, the prevalence of HPV seems to be low [28]. We have previously attributed the low incidence of cervical neoplasia in Israeli women to the low pool of herpes simplex virus type 2 in the general Israeli population [29]. This may be true for HPV as well. For many years it has been predicted that the incidence of invasive cervical carcinoma in Israeli Jewish women will increase [30, 31] due to the change in sexual habits. Such an increase has hitherto not occurred and the incidence remains low [18].

Similar to cervical carcinoma the predominant oncogenic type in vulvar carcinoma is HPV 16 [10-12, 32-35], but other oncogenic types including HPV 18, 33, 45 [35] have also been reported. We examined only for HPV 16 and HPV 18 DNA. The prevalence of HPV-associated invasive vulvar carcinoma in our small vulvar carcinoma series (64.2%), is somewhat high. Using the PCR technique, it ranges in most series from 18% to 40% [10-13]. The prevalence is reported to be histology and age-related, being highest in nonkeratinizing and warty carcinomas [12, 13, 32, 33] and higher in younger than in older patients [11, 12, 32]. All our vulvar carcinoma patients had keratinizing squamous carcinoma, but were older than 55 years and all those with HPV positive tumor tissue specimens were from patients in their eighth and ninth decade. The high prevalence of HPV positivity in our series of patients with vulvar and cervical neoplasia may be attributed to the use of very sensitive primers that are able to detect even small HPV DNA segments.

Positive immunohistochemical p53 staining was observed in 20.2% of our cases with cervical invasive squamous cell carcinoma. This percentage is in the 20% to 74% range of positive staining reported in other studies [36-41]. None of our adenocarcinoma tissue samples had positive p53 staining. However, positive staining was reported in larger series of adenocarcinoma [26, 27].

A high prevalence (33%-75%) of positive p53 immunohistochemical staining in vulvar carcinoma was reported in several studies [10, 13-17]. A slightly higher rate (78.6%) was found in our study.

In cervical as well as in vulvar carcinoma positive immunohistochemical staining does not necessarily indicate mutations [42, 43]. In cervical carcinoma the prevalence of positive immunohistochemical staining is high, yet the actual rate of mutations is very low [4, 5]. In vulvar carcinoma, using the PCR method, mutations were found in five out of eight (62%) tissue cultures of carcinoma cell lines [44] and in 31% to 45% of paraffin-embedded tumor tissue samples [45, 46]. Thus, in contrast to cervical cancer, in vulvar carcinoma mutations are a frequent event and there seems to be a better correlation between the rate of positive immunohistochemical staining and the rate of actual mutations.

Currently it is known that association of HPV and p53 in cervical cancer is not mutually exclusive and the concomitant presence of HPV and positive immunohistochemical p53 staining observed by us has also been reported by others [47]. In vulvar carcinoma Pilloti *et al.* [46] found no p53 mutations in two HPV 16 positive vulvar tumors, whereas among seven HPV negative tumors, four contained p53 mutations. On the other hand, Tervahauta *et al.* [14] found immunohistochemically weakly positive p53 staining in one, and moderately positive staining in two out of five fresh vulvar carcinoma tissue samples. Similarly Lee *et al.* [48] found p53 mutations in one of 12 (18%) HPV positive vulvar carcinoma tissue samples and in four of nine (44%) HPV negative tissue samples. Two of our HPV 16 DNA positive vulvar tumor tissue samples were also p53 positive. It thus seems that in vulvar carcinoma p53 and HPV are also not mutually exclusive, although mutations are apparently more prevalent in HPV negative tumors.

In spite of the change in sexual habits and the association between vulvar and cervical neoplasia with sexually transmitted HPV, the incidence of cervical cancer in Israel is persistently low, while the incidence of vulvar carcinoma has decreased [49]. The exact reason for these trends are obscure.

The prevalence of HPV and of positive p53 immunohistochemical staining in our series of Israeli Jewish women with cervical and vulvar neoplasia are similar to those in other populations, and seem to indicate that the etiological factors are probably also alike.

References

[1] Werness B. A., Levine A. J., Howley P. M.: "Association of human papilloma virus types 16 and 18 E6 proteins with p53". *Science*, 1990, *248*, 76.

[2] Scheffner M., Munger K., Byrne J. C., Howley P. M.: "The E6 oncoprotein encoded by human papilloma virus types 16 and 18 promotes the degradation of p53". *Cell.*, 1990, *63*, 1129.

[3] Bosch F. X., Manos M., Munoz N., Sherman M., Jansen A. M., Peto J., Schiffman M. H. *et al.*: "Prevalence of human papillomavirus in cervical cancer: a worldwide perspective". *J. Natl. Cancer Inst.*, 1995, *87*, 796.

[4] Fujita M., Inoue M., Tanizawa O., Iwamoto S., Enomoto T.: "Alterations of the p53 gene in human primary cervical carcinoma with and without human papilloma virus infection". *Cancer Research*, 1992, *52*, 5323.

[5] Busby-Earle R. M. C., Steel C. M., Bird C. C.: "Cervical carcinoma: low frequency of allele loss at loci implicated in other common malignancies". *Brit. J. Cancer*, 1993, *67*, 71.

[6] Matsukura M., Sugase M.: "Identification of genital human papillomaviruses in cervical biopsy specimens: segregation of specific virus types in specific clinicopathological lesions". *Int. J. Cancer*, 1995, *61*, 13.

[7] Lorincz A. T., Reid R., Jenson A. B., Greenberg M. D., Lancaster W., Kurman R. J.: "Human papillomavirus infection of the cervix: relative risk association of 15 common anogenital types". *Obstet. Gynecol.*, 1992, *72*, 328.

[8] Finlay C. A., Hinds P. W., Tan T. H., Eliyahu D., Oren M., Levine A. J.: "Activating mutations for transformation by p53 produce a gene that forms hsc70 - p53 complex with an altered half life". *Mol. Cell. Biol.*, 1988, *8*, 531.

[9] Kerns B. M., Jordan P. A., Moore M. H., Humphrey P. A., Berchuk A., Kohler M. F., Bast R. C. *et al.*: "p53 overexpression in formalin-fixed paraffin-embedded tissue detected by immunohistochemistry". *J. Histochem. Cytochem.*, 1988, *40*, 1047.

[10] Kagie M. J., Kenter G. G., Tollennaar R. A., Hermans J., Trimbos J. B., Fleuren G. J.: "p53 protein overexpression is common and independent of human papillomavirus infection in squamous cell carcinoma of the vulva". *Cancer*, 1997, *80*, 1228.

[11] Kagie M. J., Kenter G. G., Zommerdijk-Nooijen Y., Hermans J., Schuuring E., Timmers P. J., Trimbos J. B., Fleuren G. J.: "Human papillomavirus infection in squamous cell carcinoma of the vulva, in various synchronous epithelial changes and in normal vulvar skin". *Gynecol. Oncol.*, 1997, *67*, 178.

[12] Toki T., Kurman R. J., Park J. S., Kessis T., Daniel R. W., Shah K. V.: "Probable non-papillomavirus etiology of squamous cell carcinoma of the vulva in older women. A clinicopathologic study using in situ hybridization and polymerase chain reaction". *Int. J. Gynecol. Pathol.*, 1991, *10*, 107.

[13] Pilotti S., D'Amato L., Della Torre G., Donghi R., Longoni A., Giarola M., Sampietro G. *et al.*: "Papillomavirus, p53 alterations, and primary carcinoma of the vulva". *Diagn. Mol. Pathol.*, 1995, *4*, 239.

[14] Tervahauta A. I., Syrjanen S. M., Vayrynen M., Saastamoinen J., Syrijanen K. J.: "Expression of p53 protein related to the presence of human papillomavirus (HPV) DNA in genital carcinomas and precancer lesions". *Anticancer Res.*, 1993, *13*, 1107.

[15] Milde Langosch K., Albrecht K., Joram S., Schlechte H., Giessing M., Loning T.: "Presence and persistence of HPV infection and p53 in cancer of the cervix uteri and the vulva". *Int. J. Cancer*, 1995, *36*, 639.

[16] Gordinier M. E., Steinhoff M. M., Hogan J. W., Peipert J. F., Gajewski W. H., Falkenberry S. S., Granai C. O.: "S-phase fraction, p53, and HER-2/neu status as predictors of nodal metastasis in early vulvar cancer". *Gynecol. Oncol.*, 1997, *67*, 200.

[17] McConnell D. T., Miller I. D., Parkin D. E., Murray G. I.: "p53 protein expression in a population-based series of primary vulval squamous cell carcinoma and immediate adjacent field change". *Gynecol. Oncol.*, 1997, *67*, 248.

[18] Barchana M., Andreev H., Alon R.: "Israel Cancer Registry". Cancer in Israel 1994 Ministry of Health. Jerusalem 1997.

[19] Marcus S. L.: "Multiple squamous cell carcinoma involving the cervix, vagina, and vulva. The theory of multicentric origin". *Am. J. Obstet. Gynecol.*, 1960, *80*, 802.

[20] Rutledge F., Smith J. P., Franklin E. W.: "Carcinoma of the vulva". *Am. J. Obstet. Gynecol.*, 1970, *106*, 1117.

[21] Jimerson G. K., Merill J. A.: "Multicentric squamous malignancy involving both cervix and vulva". *Cancer*, 1970, *26*, 150.

[22] Franklin E. W., Rutledge F. D.: "Epidemiology of epidermoid carcinoma of the vulva". *Obstet. Gynecol.*, 1970, *39*, 165.

[23] Menczer J., Voliovitch Y., Modan B., Modan M., Steinitz R.: "Some epidemiologic aspects of carcinoma of the vulva in Israel". *Am. J. Obstet. Gynecol.*, 1982, *143*, 893.

[24] Sherman L., Malloul N., Golan I., Durst M., Baram A.: "Expression and splicing patterns of human papillomavirus type-16 mRNAs in pre-cancerous lesions and carcinomas of the cervix in human keratinocytes immortalized by HPV 16, and in cell lines established from cervical cancers". *Int. J. Cancer*, 1992, *50*, 356.

[25] Mitrani-Rosenbaum S., Gal D., Friedman M., Kitron N., Tsviel R., Mordel N., Anteby S. O.: "Papillomaviruses in lesions of the lower genital tract in Israeli patients". *Eur. J. Cancer Clin. Oncol.*, 1988, *24*, 725.

[26] Uchiyama M., Iwasaka T., Matsuo N., Hachisugo T., Mori M., Sugimori H.: "Correlation between human papilloma virus and p53 gene overexpression in adenocarcinoma of the uterine cervix". *Gynecol. Oncol.*, 1997, *65*, 23.

[27] Parker M. F., Arroyo G. F., Geradts J., Sbichi A. L., Park R. C., Taylor R. R., Birrer M. J.: "Molecular characterization of adenocarcinoma of the cervix". *Gynecol. Oncol.*, 1997, *64*, 242.

[28] Isacsohn M., Dolberg L., Gottschalk Sabag S., Mitrani-Rosenbaum S., Nubani N., Diamant Y. Z., Goldsmidt R.: "The inter-relationship of herpes virus, papilloma 16/18 virus infection and pap smear pathology in Israeli women". *Isr. J. Med. Sci*, 1994, *30*, 383.

[29] Menczer J., Leventon-Kriss S., Modan M., Oelsner G., Gerichter C. B.: "Antibodies to herpes simplex virus in Jewish women with cervical cancer and in healthy Jewish women of Israel". *J. Natl. Cancer Inst.*, 1975, *55*, 3.

[30] Baram A., Schachter A.: "Cervical carcinoma: disease of the future for Jewish women". *Lancet*, 1982, *i*, 747.

[31] Glezerman M., Piura B., Insler V.: "Cervical cancer in Jewish women". *Am. J. Obstet. Gynecol.*, 1989, *161*, 1186.

[32] Hording U., Junge J., Daugaard S., Lundvall F., Poulsen H., Bock J. E.: "Vulvar squamous cell carcinoma and papillomaviruses: indication for two different etiologies". *Gynecol. Oncol.*, 1994, *52*, 241.

[33] Nuovo G. J., Delvenne P., MacConnell P., Chalas E., Neto C., Mann W. J.: "Correlation of histology and detection of human papillomavirus DNA in vulvar cancer". *Gynecol. Oncol.*, 1991, *43*, 275.

[34] Monk B. J., Burger R. A., Lin F., Parham G., Vasilev S. A., Wilczynski S. P.: "Prognostic significance of human papillomavirus DNA in vulvar carcinoma". *Obstet. Gynecol.*, 1995, *85*, 709.

[35] Iwasawa A., Nieminen P., Lehtinen M., Paavonen J.: "Human papillomavirus in squamous cell carcinoma of the vulva by polymerase chain reaction". *Obstet. Gynecol.*, 1997, *89*, 81.

[36] Bosari S., Roncalli M., Viale G., Bossi P., Coggi G.: "p53 immunoreactivity in inflammatory and neoplastic diseases of the uterine cervix". *J. Pathol.*, 1993, *169*, 425.

[37] Oka K., Nakano T., Arai T.: "p53CMI expression is not associated with prognosis in uterine cervical carcinoma". *Cancer*, 1993, *72*, 160.

[38] Gitsch G., Kainz C., Joura E., Breitenecker G.: "Mutant p53 product in patients with stage III cervical cancer". *Anticancer Res.*, 1992, *12*, 2241.

[39] Kainz C., Kohlberger P., Gitsch G., Sliutz G., Breitenecker G., Reinthaller A.: "Mutant p53 in patients with invasive cervical cancer stages IB to IIB". *Gynecol. Oncol.*, 1995, *57*, 212.

[40] Holm R., Skomedal H., Helland P., Borresen A., Nesland J. M.: "Immunohistochemical analysis of p53 protein overexpression in normal, premalignant and malignant tissues of the cervix". *J. Pathol.*, 1995, *169*, 21.

[41] Mital K. R., Lin O., Chan W., Goswami S., Demopoulus R. I.: "Cervical squamous dysplasias and carcinomas with immunodetectable p53 frequently contain HPV". *Gynecol. Oncol.*, 1995, *58*, 289.

[42] Schneider J., Rubio M. P., Rodriguez-Escudero F. J., Seizinger B. R., Castresane J. S.: "Identification of p53 mutations by means of single strand conformation polymorphism analysis in gynaecological tumors: comparison with results of immunohistochemistry". *Eur. J. Cancer*, 1994, *30A*, 504.

[43] MacGeoch C., Barnes D. M., Newton J. A., Mohamed S., Hodgson S. V., Ng M., Bishop D. T., Spurr N. K.: p53 protein detected by immunohistochemical staining is not always mutant". *Dis. Markers*, 1993, *11*, 239.

[44] Hietanen S. H., Kurvinen K., Syrjanen K., Grenman S., Carey T., McClatchey K., Syrjanen S.: "Mutation of tumor suppressor gene p53 is frequently found in vulvar carcinoma cells". *Am. J. Obstet. Gynecol.*, 1995, *173*, 1477.

[45] Sliutz G., Schmidt W., Tempfer C., Speiser P., Gitsch G., Eder S. *et al.*: "Detection of p53 point mutations in primary human vulvar cancer by PCR and temperature gradient gel electrophoresis". *Gynecol. Oncol.*, 1997, *64*, 93,

[46] Pilotti S., Donghi R., D'Amato L. *et al.*: "Papillomavirus, p53 alteration and primary carcinoma of the vulva". *Eur. J. Cancer*, 1993, *29A*, 924.
[47] Schiffman M. H.: "Recent progress in defining the epidemiology of human papillomavirus infection and cervical neoplasia". *J. Natl. Cancer Inst.*, 1992, *84*, 394.
[48] Lee Y. Y., Wilczynski S. P., Chumakov A., Chih D., Koeffler H. P.: "Carcinoma of the vulva: HPV and p53 mutations". *Oncogene*, 1994, *9*, 1655.
[49] Menczer J., Barchana M., Andreev H., Arbel-Alon S., Modan B.: "Selected epidemiological time trends of vulvar carcinoma in Israel". *Int. J. Gynecol. Cancer*, 1999, *9*, 24.

Address reprint requests to:
Prof. JOSEPH MENCZER, M. D.
Director, Gynecologic Oncology
E. Wolfson Medical Center,
Holon 58100 (Israel)