HPV and p53 expression in epithelial ovarian carcinoma

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Summary

Objectives: Human papillomavirus is the causal factor for cervical cancer. However, the role of HPV infection in ovarian cancer is unclear. This study aimed to determine the presence of human papillomavirus (HPV) in ovarian cancer tissues along with the expression of tumor suppressor gene p53. We also investigated any possible association of HPV with p53 gene mutations in ovarian carcinoma.

Methods: Archived human ovarian cancer tissues (n = 40 cases of epithelial ovarian cancer) embedded in paraffin blocks were used. Controls were 32 non-malignant ovarian tumor tissue blocks. In situ hybridization (ISH) and immunohistochemistry (IHC) were used to detect the presence of HPV and p53 expression, respectively.

Results: Of the total, 37.5% (n = 15) of malignant and 28.1% (n = 9) of benign ovarian tumors were positive for HPV (OR: 1.5 CI: 0.5-4.1, p = 0.4). The difference was not statistically significant. However, p53 was detected in 72.5% (n = 29) of malignant cases compared to 37.5% (n = 12) of benign cases (OR: 4.3 CI: 1.6-11.9, p = 0.003). Furthermore, a positive correlation between HPV and p53 expressions in ovarian cancer tissue samples was detected (r = 0.47, p = 0.001).

Conclusions: HPV does not seem to be a major component in the development of ovarian carcinoma, nevertheless HPV positivity seems to contribute to the pathogenesis in at least some ovarian carcinoma cases by way of interaction with tumor suppressor p53.

Key words: HPV; p53; Ovarian carcinoma; Immunohistochemistry; In situ hybridization.

Introduction

Genital HPV infections are common among young women. The etiological role of high-risk HPV such as types 16 and 18 in preinvasive and invasive cervical cancer has been demonstrated by epidemiological evidence and molecular studies [1-3]. Upper genital tract sites have also been investigated for evidence of HPV. HPV DNA has generally been detected in endometrial cancers, although at a much lower frequency than in cervical cancer [4, 5]. However, reports on HPV DNA in ovarian cancer have been conflicting [5-8]. Cancer develops when the delicate balance between cell cycle progression and apoptosis is disrupted in multiple steps. p53 is a tumor suppressor gene located on chromosome 17p13, and a little over 50% of human tumors contain mutations in this gene [9]. p53 has two distinctive functions, including G1 arrest and apoptosis, particularly when cells are experiencing DNA damage. p53 induces G1 arrest by increasing transcription of cyclin-dependent kinase inhibitor p21 [10]. If the cell fails to repair the damage to its DNA then the normal wild type p53 induces apoptosis by activating the death gene bax and down regulating survival genes like bcl-2 [11].

HPV's role in cellular transformation is associated with the loss of normal tumor suppressor function of p53 and pRb gene products after inactivation by E6 and E7 viral proteins, respectively [12, 13]. There are only two other clinico-pathologic studies in the literature where the relationship between HPV and p53 in ovarian carcinoma was sought [14, 15]. We aimed to look at whether HPV infection plays a role in ovarian carcinogenesis and also investigate any possible association of HPV with p53 gene mutations, which are fairly common in epithelial ovarian carcinomas [16].

Materials and Methods

Immunohistochemistry

Several 4 μ-thick sections were obtained from each paraffin-embedded block and mounted on poly-L-lysine-coated slides. The sections in a citrate buffer (0.01 mol/l, pH 6) were heated in a microwave oven for 15 min at maximum power (700W), and then cooled at room temperature for 20 minutes. A standard 3-step immunoperoxidase ABC (avidin-biotin peroxidase complex) technique was used to detect p53 (Ab-5, Neomarkers, CA, USA). The expression of p53 was graded in a semiquantitative manner using a 0-2+ scale: (0) - no staining; (1+) - focal and mild staining of tumor cells; (2+) - diffuse and significant staining of tumor cells. One and two positives were considered as positively stained.

In situ hybridization method

Sections of 4 μm in thickness were cut and immersed sequentially in two changes of clearing solution (xylene or histoclear) for five minutes each, two changes of 99% alcohol and three changes of 95% alcohol for one minute each, and five rinses in deionized water. Tissues were boiled in Target Retrieval Solution (Dako, Denmark) at 95-99°C for 40 minutes followed by cooling at room temperature for 20 minutes, then four washes in deionized water, and immersion in Proteinase K solution (a 1:5000 dilution). Following digestion, sections were rinsed in four changes of deionized water, then immersed in 0.3% H_2O_2 in methanol for 20 minutes, and rinsed in five washes of deionized water. Slides are allowed to air dry for 15 minutes, then one

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drop of probe is applied to the tissue section, and a cover slip is applied to the section. The probe and HPV target DNA are denatured by placing the cover slipped slides on a flat heating block surface or pre-warmed oven at 92°C for five minutes. Following denaturation, slides are transferred to a pre-warmed humid chamber for hybridization at 37°C for 60 minutes. Following hybridization, cover slips are removed by immersing the slides in room temperature 1X TBST (Dako, Denmark). Slides were transferred to a fresh TBST bath before stringent washing at 48°C for 20-30 minutes followed by rinsing the slides in three changes of 1X TBST for one minute each. Detection of hybridized probe is then performed using the GenPoint Tyramide Signal Amplification System for Biotinylated Probes (Dako, Denmark).

Cases were considered positive for HPV DNA whenever specific dark purple nuclear staining occurred in tumor cells. The proportion of HPV DNA positive tumor cells was graded in a semiquantitative manner using a 0-2+ scale: (0) - no staining; (1+) - focal and mild staining of tumor cells; (2+) - diffuse and significant staining of tumor cells. One and two positives were considered as positively stained.

Statistical analysis

Statistical analysis was performed using the chi square test. Odds ratio (OR) and confidence interval (CI) were calculated. Spearman's rho correlation test was used to look at correlations between p53 expression and HPV.

Results

p53 was detected in 29 of 40 malignant ovarian tumors (72.5%) compared to 12 of 32 benign ovarian tumors (37.5%). The difference was statistically significant with Fisher's exact test (OR: 4.3 CI: 1.6-11.9, p = 0.003, Table 1), (Figures 1 and 2).

In 40 epithelial ovarian carcinoma cases 15 were HPV positive determined by in situ hybridization (ISH). The positive rate was 37.5%. In 32 benign ovarian tumors nine were positive for HPV determined by ISH. The positive rate was 28.2%. The difference was not statistically significant (OR: 1.5 CI: 0.5-4.1, p = 0.4, Table 1), (Figures 3 and 4).

Table 1. — *HPV infection and p53 expression in benign and malignant ovarian tumors.*

	Cancer		Benign	
p53	no.	%	no.	%
neg	11	27.5%	20	62.5%
pos	29	72.5%	12	37.5%
			p: 0.003, OR: 4.3, CI: 1.6 - 11.9	
HPV	no.	%	no.	%
neg	25	62.5%	23	71.9%
pos	15	37.5%	9	28.1%
			p: 0.4, OR: 1.5, CI: 0.5 - 4.1	

Table 2. — *Correlation between HPV and p53.*

HPV	p53 neg	+	++
neg	26	15	7
+	2	1	5
++	3	1	12

p: 0.001, r: 0.47 Spearman's rho.

Furthermore, analysis with Spearman's correlation test showed a positive correlation between HPV and p53 expression in ovarian cancer tissue samples (r = 0.47, p = 0.001, Table 2).

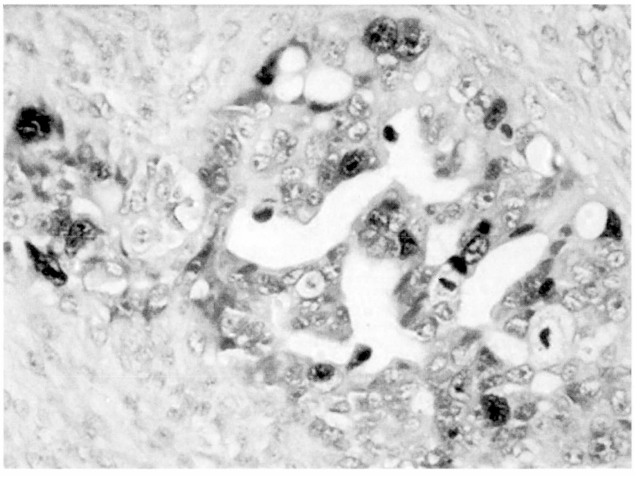
Discussion

Human papillomavirus plays a causal role in cervical cancer, and it has also been detected in vulvar and vaginal carcinomas [17]. However, the role of HPV in the pathogenesis of ovarian cancer is controversial. In fact today the pathogenesis of ovarian carcinoma, which is one of the most lethal malignancies of the female genital tract, is not yet clear. Since the first report by Kaufman *et al.*, on the presence of HPV infection in ovarian cancer, there has been considerable enthusiasm on research of any role that HPV plays in the development of ovarian carcinoma [18]. Today there are over 15 reports on the subject, and other than a few which showed presence of HPV infection in ovarian carcinomas, most of the reports were unable to show HPV infection [6, 14, 19]. Recently, Wu *et al.* [14] using IHC showed the presence of HPV-16 infection in 36% of their ovarian cancer study population whereas the rate was only 6.7% in normal ovarian tissues; with these results these authors suggested HPV infection might play a role in ovarian carcinogenesis. We also found 37.5% HPV positivity in ovarian carcinoma specimens; however, due to the 28.2% presence rate in benign ovarian tumors there was no statistically significant difference.

Mutations or loss of function in p53 genes have been frequently detected in ovarian carcinoma [20, 21]. In accordance with these studies we also found that 72.5% of our ovarian carcinoma cases were p53 positive. Since the normal p53 is not detectable by immunohistochemistry due to its short half-life, p53 overexpression is mostly considered to represent a mutant form that is unable to regulate cell cycle and apoptosis. In our study we found a high percentage of p53 immunoreactivity in ovarian cancer tissues indicating abnormal p53 function. In their excellent review of p53 expression and mutation in ovarian carcinomas, Kmet *et al.* noted that 17% of the 526 tumors (pooled data of 13 studies) were found to be positive for p53 overexpression by IHC but were negative for mutations detected by PCR and or direct sequencing [21]. It is obvious that not all of p53 over expression is necessarily secondary to mutations and there might be other mechanisms of abnormal p53 function. Studies on cell lines have shown that the inactivation of p53 through interaction and complex formation with HPV E6 oncoprotein is a very important feature in cervical cancer [22, 23]. While that is the case with cervical cancer it is not known if this is also true for ovarian carcinoma, however studies on ovarian cancer have mostly shown overexpression of p53 [20, 21].

Although analysis of our results on HPV presence in ovarian carcinoma showed no significant differences between malignant and benign ovarian tumors, we found HPV presence in 37.5% of ovarian carcinoma cases. In

Fig. 1



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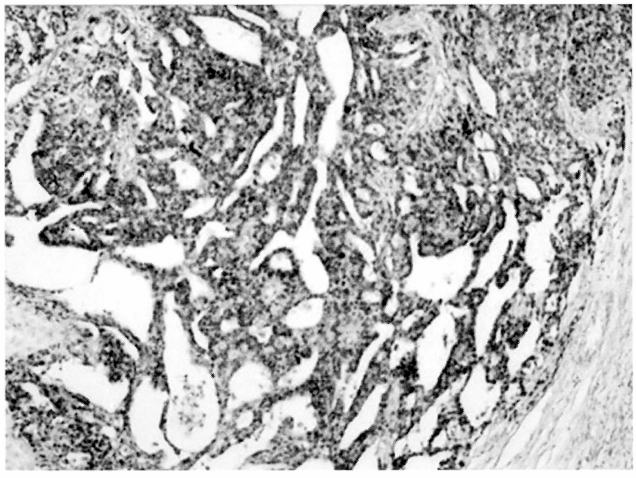
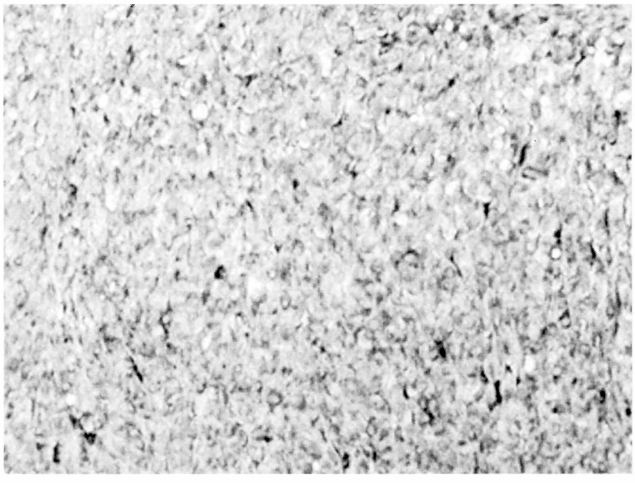


Fig. 3



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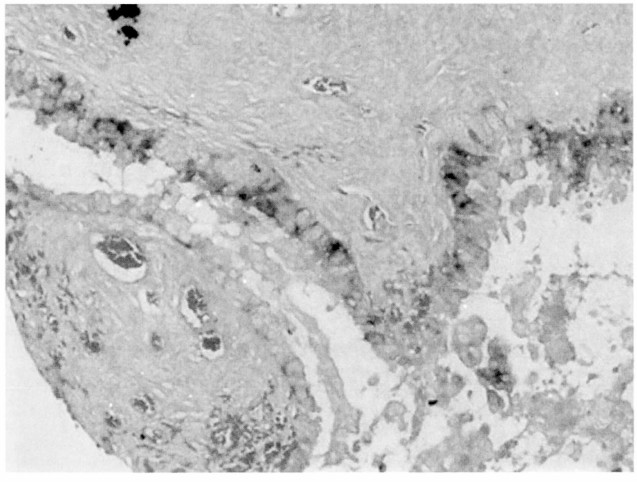

Figure 1. — Focal p53 staining in tumor cells is observed.
Figure 2. — Diffuse and intense p53 staining is noted in all tumor cells.
Figure 3. — Diffuse and intense HPV DNA positivity is observed in tumor cells.
Figure 4. — Significant HPV DNA positive mucinous cells lining cystic adenofibroma can be seen.

the present study a positive correlation was observed between HPV status and p53 over-expression. Hence, HPV seems to interact with p53 in at least some ovarian carcinoma cases. Pestell and co-workers have shown inactivation of p53 by the human HPV E6 gene in ovarian cancer cell line A2780 [16]. It is possible that in at least some of our specimens the over-expression of p53 might be due to complex formations with E6 protein of high-risk HPVs based on the positive correlation observed between HPV status and p53 expression. Of the two other studies in the literature looking at any correlation between HPV presence and p53 expression, Wu *et al.* [14] did not find any correlation, although they showed increased HPV 16 presence in ovarian carcinomas. On the contrary Li *et al.* in a recent study suggested that p53 codon 72 homozygous genotype is correlated with HPV-associated ovarian carcinoma [15].

In conclusion, although not a major contributor as in cervical cancer, HPV infection may still play a role in at least some cases of epithelial ovarian carcinomas by way of interacting with p53 tumor suppressor genes. However, the molecular interactions between HPV and p53 should be investigated in ovarian carcinomas as different mechanisms other than in cervical carcinomas might be present in other tumors including epithelial ovarian carcinomas.

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