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Analysis and Clinical Implications of *p53* Gene Mutations and Human Papillomavirus Type 16 and 18 Infection in Primary Adenocarcinoma of the Uterine Cervix

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Mutant *p53* is frequently detected in endometrial and ovarian carcinoma, but it is rare in cervical cancers. Previous reports focused on cervical squamous cell carcinoma, whereas cervical adenocarcinoma was given little attention. We searched for *p53* gene mutations in 74 primary cervical adenocarcinomas with known human papillomavirus (HPV) status. Our aim was to evaluate the prevalence of *p53* mutations and to investigate their possible role as an independent prognostic factor. We found mutations in 13.5% with a high rate of G:C → A:T transitions as observed in endometrial adenocarcinoma. As *p53* mutations are more frequently detected in malignancies of high grade, high stage, and large size, this molecular event seems to play a role in the progression rather than in the induction of cervical adenocarcinoma. In our series, patients with HPV-negative tumors and patients with mutated neoplasms, irrespective of HPV infection, had a shorter survival. Yet the absence of HPV infection and presence of *p53* mutations are not independent risk factors for tumor-related death after adjustment for clinicopathological confounders. The only significant and independent predictors of survival are age of patient, stage of disease, tumor grade, and presence of lymph node metastases. *(Am J Pathol 1998, 152:1057–1063)*

Alterations of the *p53* tumor suppressor gene by base substitutions, deletions, insertions, and allelic losses or rearrangements have been observed in a variety of human tumors and are the genetic lesions that are most commonly associated with human malignancy. The analysis of the 10 most frequent tumor types worldwide has shown that *p53* mutations are present in 40 to 45% of cases.[1] This suggests that the alterations in the *p53* gene, which is involved in many crucial cellular functions, including cell cycle regulation, DNA repair, cellular differentiation, and apoptosis, constitute a critical step in human carcinogenesis. In some tumors, such as esophageal or testicular cancers, the finding of *p53* mutations in preneoplastic lesions suggests their early involvement in carcinogenesis. In other tumors, such as breast and bladder cancers or melanoma, their occurrence mainly in high-grade and/or advanced neoplasms suggests that they are implied in tumor progression rather than in induction.

Evidence from *in vitro* experiments, and from analysis of human tissues, indicates that the loss of p53 protein function, through gene alterations or through binding with specific cellular or viral proteins, may also play a role in the pathogenesis of genital carcinomas. Mutant *p53* is frequently detected in endometrial and ovarian carcinoma, particularly in high-grade and high-stage tumors, but it is rare in cervical cancers.[2]

Most cervical carcinomas harbor human papillomavirus (HPV) DNA of high-risk type and express E6 and E7 viral oncoproteins. HPV-16 and -18 E6 gene product binds specifically to wild-type p53 protein, an event that leads to p53 functional inactivation and rapid degradation via the ubiquitin pathway.[3,4] In addition, overexpression of high-risk E6 protein from eukaryotic expression vectors inhibits *p53*-mediated transcriptional activation.[5–7] Thus, co-expression of high-risk E6 and of wild-type p53 protein could result in the same phenotypic effect as that induced by *p53* gene mutations and could explain the generally low prevalence of *p53* mutations in cervical cancer. Early reports postulated that *p53* mutations were confined to HPV-negative cervical carcinomas and tumor-derived cell lines.[8] However, in subsequent

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studies, mutant *p53* was also detected in HPV-positive cases.[9–12]

Most previous reports focused on cervical squamous-cell carcinoma, whereas cervical adenocarcinoma was given little attention.[11,13] We analyzed a series of 74 primary cervical adenocarcinomas for HPV infection and for the presence of *p53* mutations within the highly conserved region corresponding to exons 5 to 8. The screening for *p53* mutations was performed by denaturing gradient gel electrophoresis (DGGE) and sequencing. Our aim was first to evaluate the prevalence of *p53* mutations and second, by analyzing their correlation with HPV status and with several clinicopathological parameters, to investigate their possible role as an independent prognostic factor.

Materials and Methods

Samples

Seventy-four primary cervical adenocarcinomas were retrieved from our files. The age of patients ranged from 26 to 84 years (mean, 54.7 ± 13.8 SD). The tumors were classified and graded in accordance with standard criteria.[14] They included 49 (66.2%) mucinous tumors (43 endocervical type and 6 intestinal type). Nine endocervical-type tumors also exhibited limited areas of endometrioid differentiation, and one had a focal clear cell pattern. The remaining 25 (33.8%) cases were nonmucinous tumors (20 endometrioid and 5 clear cell). Irrespective of their histological type, 10.8% of cases were well differentiated, 60.8% moderately differentiated, and 28.4% poorly differentiated tumors.

The clinical status was known for all patients, and average follow-up was 45.3 months (95% confidence interval (CI), 10.0 to 189.6). Tumors were staged in accordance with the International Federation of Gynecologic Oncology (FIGO, 1989). Fifty cases were stage Ib, 6 stage IIa, 10 stage IIb, 3 stage III, and 5 stage IV. Lymph node dissections were available for 53 (71.6%) patients.

All tumor samples were from routine formalin-fixed, paraffin-embedded material. Tissue blocks had at least 85% of neoplastic cells; to meet this requirement, tumor areas were dissected when necessary from the surrounding normal tissues. The tumor sections selected for the study did not contain benign, atypical, or malignant squamous epithelium.

p53 *Molecular Analysis*

DNA Extraction

One to three sections (depending on sample size) of formalin-fixed, paraffin-embedded tumor tissue were incubated at 58°C overnight in 200 μl of extraction buffer (50 mmol/L KCl, 10 mmol/L Tris/HCl, pH 7.5, 2.5 mmol/L $MgCl_2$, 0.1 mg/ml gelatin, 0.45% NonidetP-40, 0.45% Tween-20, and 0.5 mg/ml proteinase K). The solution was heated at 95°C for 15 minutes to inactivate proteinase K and subsequently centrifuged. Five to ten microliters of digested material was used directly for enzymatic amplification with polymerase chain reaction (PCR).

PCR Amplification

We analyzed exons 5 to 8 of the *p53* gene, where 90% of mutations are known to occur in human tumors. Oligonucleotide primers used for amplification were as follows: exon 5a, 5′-TTCCTCTTCCGCAGTACTC-3′ and 5′-GC clamp TGGCGCGGACGCGGGTGCCG-3′; exon 5b, 5′-GC clamp TTCCACACCCCCGCCCGGCA-3′ and 5′-CTGGGGACCCTGGGCAA-3′; exon 6, 5′-GC clamp GAGACGACAGGGCTGGTT-3′ and 5′-CCACTGACAACCACCCTT-3′; exon 7, 5′-GC clamp TGGCTCTGACTGTACCACC-3′ and 5′-CAAGTGGCTCCTGACCTGGA-3′; and exon 8, 5′-GC clamp ATCCTGAGTAGTGGTAATCT-3′ and 5′-TACCTCGCTTAGTGCTCCCT-3′. The sequence of the GC clamp was 5′-CGCCCGCCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCC-3′.

Exon 5 was amplified in two fragments, 5a and 5b, as described by Borresen et al.[15] One member of each primer pair contained a 5′ 40-bp GC-rich sequence (GC clamp). GC-clamped primers were used to obtain suitable amplified products for DGGE analysis. Amplification was performed on 5 to 10 μl of DNA, using 12.5 pmol of each primer, 1 U of thermostable DNA polymerase, and 200 μmol/L each of dNTPs in a volume of 50 μl of buffer (50 mmol/L KCl, 10 mmol/L Tris/HCl, pH 8.4, and 0.2 mg/ml bovine serum albumin). Thirty to forty cycles of amplification were performed in a programmable thermal cycler (MJ Research, Watertown, MA). Cycling parameters were 1 minute at 94°C, 1.5 minutes at 53°C for exons 5a, 5b, and 6, at 56°C for exon 7, and at 58°C for exon 8, and 2 minutes at 72°C. To avoid contamination by PCR product carryover and false positive results, we analyzed PCR products in a different laboratory; we also used aliquotted reagents, aerosol-free tips and all other recommended precautions including a careful cleaning of the microtome before sectioning, the use of disposable microtome blades, handling of the sections with clean forceps, and carrying out all procedures wearing gloves.

DGGE Analysis

The PCR products obtained with the GC-clamped ampliprimers were analyzed by DGGE. The introduction of GC clamp into the amplified fragments brings the probability of detection of any base change within the fragment close to 100%.[16–18] In our conditions, the DGGE method allowed us to identify mutations in 4 to 5% of the cells utilized for DNA extraction.[19] The gel apparatus was essentially as described by Myers et al.[20] Specific DGGE conditions for each exons are described in Table 1.

All DNAs showing variant DGGE patterns were re-amplified and re-analyzed in independent experiments, all of which confirmed the previous results. We then sequenced samples with variant patterns to identify the kind of mutation.

Table 1. DGGE Conditions

Exon	PCR product (bp)	Polyacrylamide (%)	Denaturant range (%)
5a	158	8	60–65
5b	210	6	65–80
6	270	6	50–70
7	168	8	55–75
8	191	8	50–75

DNA Sequencing

DNA was eluted from DGGE homoduplex bands by the incubation of gel fragments overnight at 37°C in 80 μl of elution buffer (0.5 mol/L NH_4 acetate, 10 mmol/L Mg acetate, 1 mmol/L EDTA, pH 8, and 0.1% SDS). After centrifugation, the supernatant was used for PCR reaction with specific primers without GC clamps. The PCR product was cloned into the plasmid pCR vector (TA cloning kit, Invitrogen, San Diego, CA) in accordance with the supplier's instructions. We performed the sequence by means of the dideoxy procedure,[21] using the ^{T7}Sequencing kit (Pharmacia Biotech, Sollentuna, Sweden) and following the manufacturer's instructions. All samples sharing the same DGGE pattern proved to carry the same mutation.

HPV DNA Analysis

DNA extraction, primers, and PCR amplification procedures for the detection of HPV-16 and -18 DNA were described in our previous study in which choice of primers and strategy of amplification are also described.[22] To avoid the possibility of negative cases being infected with other HPV types, we further amplified negative cases with consensus primers MY09/MY11.[23] Negative samples and DNAs extracted from formalin-fixed, paraffin-embedded cervical condylomas infected by HPV were used as controls in such cases.

Statistical Analysis

We used the Mann-Whitney test to compare continuous variables. Pearson χ^2 test and Fisher exact test were used for the comparison of categorical variables, where appropriate. The Mantel-Haenszel test for linear association was used to test for linear trend across ordered categories. Kaplan-Meier product-limit estimates and log-rank test were used to evaluate the univariate effect of variables on overall survival. The Cox proportional hazard model was used to compute both univariate and multivariate odds ratios and 95% confidence interval of tumor-related death. Statistical analysis was performed with SPSS for Windows (version 6.1).

Results

p53 *Gene*

PCR-based DGGE analysis of *p53* exons 5 to 8 revealed a variant pattern in 14 tumors. In all of these cases, both normal and mutated homoduplexes were present together with two heteroduplex bands. A four-band pattern is to be expected, both for mutated homozygous and heterozygous tumor samples, when the tissue sections utilized for DNA extraction are contaminated by non-neoplastic cells, which contribute the normal allele sequence. All samples showing a variant DGGE pattern were demonstrated, by DNA sequencing, to carry a gene mutation. In six cases, sequence analysis revealed a common polymorphism, which was due to a silent nucleotide substitution in exon 6. Some of the observed electrophoretic patterns and DNA sequences are shown in Figure 1. Two cases, in addition to silent mutations, also showed a functional mutation. The overall prevalence of somatic *p53* mutations in our series was 13.5% (10/74). Localization and type of nucleotide and amino acid variations in mutated cases are shown in Table 2, together with selected clinicopathological parameters. Nine mutations were single base changes; seven of these led to an amino acid substitution, with one resulting in a stop codon and one affecting a splicing site. One mutation was a single base insertion that gave a frame shift and a premature stop codon. Of nine single-base mutations, five were located at known hot spots (codons 175 and 273), and seven were G:C→A:T base transitions, five of which were located at CpG sites.

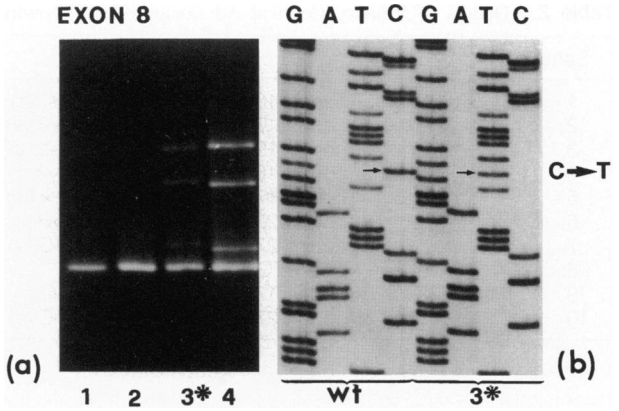

Figure 1. a: DGGE analysis of amplified fragments of exon 8 from normal samples (lanes 1 and 2) from mutated samples (lanes 3 and 4). b: Wild-type and mutated sequences of exon 8. Sample 3* (sample 7 in Table 2) shows a CGT→TGT (Arg→Cys) at codon 273.

Table 3 reports the main clinical and pathological features of the patients on the basis of the *p53* gene status. The mean age of the patients with the mutation did not differ significantly from that of women with the wild-type gene. Patients with *p53* mutations were more likely to be at an advanced stage than those without mutations (χ^2 for trend = 14.6; $P = 0.00014$). Moreover, high-grade tumors and large size neoplasms tended to be more frequently mutated (χ^2 for trend = 3.32; $P = 0.068$ and $P = 0.03$ by Fisher exact test). Most of the mutated tumors were of mucinous histotype, although the data did not reach statistical significance. Findings regarding lymph nodes metastases and HPV infection did not differ significantly between the two groups. On the other hand, the mean survival time was significantly shorter in patients with mutated tumors (25.8 months; 95% CI = 13.9 to 37.8)

Table 2. Details of Primary Cervical Adenocarcinoma Showing *p53* Gene Mutations

Sample	*p53* mutations	Age	Stage	Histotype	Grade
1	Exon 5 (codon 166, A insertion, frameshift)	60	2a	Endocervical	G3
2	Exon 5 (codon 175, CGC→CAC, Arg→His)	72	2a	Endocervical	G1
3	Exon 6 (codon 192, CAG→TAG, Gln→stop)	51	3b	Endocervical	G3
4	Exon 5 (codon 161, GCC→GAC, Ala→Asp)	54	4a	Endocervical	G3
5	Exon 5 (codon 175, CGC→CAC, Arg→His)	71	2b	Intestinal	G3
6	Exon 8 (codon 273, CGT→CAT, Arg→His)	65	4b	Endocervical	G2
7	Exon 8 (codon 273, CGT→TGT, Arg→Cys)	32	1b	Endocervical	G3
8	I base intron 6 (GTC→ATC, splicing)	39	1b	Endometrioid	G2
9	Exon 5 (codon 179, CAT→AAT, His→Asn)	63	2a	Intestinal	G2
10	Exon 8 (codon 273, CGT→TGT, Arg→Cys)	69	4	Clear cell	G3

than in those with the wild-type gene (109.9 months; 95% CI = 82.7 to 137.3; $P = 0.0097$ by log-rank test).

HPV-16 and -18 Infection

HPV-16 and/or -18 DNA sequences were detected by PCR amplification in 56 (75.7%) tumors of our series. HPV-16 was found in 15 (20.3%) cases, HPV-18 in 24 (32.4%) cases, and both HPVs in 17 (23%) cases. Amplification of negative cases with consensus primers MY09/MY11 failed to yield any additional tumor with HPV DNA sequences.

Table 3. Clinicopathological Features According to *p53* Gene Status

	Number (%) of cases		
	p53 mutated gene (n = 10)	*p53* wild-type gene (n = 64)	*P* value
Stage			
Ib	2 (20)	48 (75)	
II	4 (40)	12 (18.7)	
III–IV	4 (40)	4 (6.2)	0.00014
Histotype			
Endocervical*	6	37	
Intestinal*	2 (80)*	4 (64.1)*	
Endometrioid†	1	19	
Clear cell†	1 (20)†	4 (35.9)†	0.4
Grade			
G1	1 (10)	7 (10.9)	
G2	3 (30)	42 (65.6)	
G3	6 (60)	15 (23.4)	0.068
Cervix diameter			
<4 cm	3 (30)	44 (68.7)	
>4 cm	7 (70)	20 (31.2)	0.03
Lymph nodes‡			
Positive	1 (10)	6 (9.4)	
Negative	4 (40)	42 (65.6)	0.52
Missing	5 (50)	16 (25)	0.24
HPV			
Positive	9 (90)	47 (73.4)	
Negative	1 (10)	17 (26.6)	0.43

For the *p53* mutated gene, mean age was 57.6 ± 13.6 (SD) years; mean survival time was 25.8 ± 6.11 (SE) months. For the *p53* wild-type gene, mean age was 54.2 ± 13.9 months ($P = 0.43$ *versus* mutated gene); mean survival time was 109.9 ± 13.9 months ($P = 0.0097$ *versus* mutated gene).

*Mucin-secreting histotype.

†Nonmucinous histotype.

‡Calculated for 45 cases (13 with mutations and 32 with wild-type gene).

The mean age of patients with HPV infection was 51.9 ± 13 years; it was 63.3 ± 13.1 years in the HPV-negative group ($P = 0.002$).

HPV oncogenic sequences were found in 46 of 49 (93.9%) mucinous tumors and in 10 of 25 (40%) nonmucinous neoplasms ($P = 0.00000$), and 85% (17/21) of poorly differentiated tumors (G3), 76.1% (34/45) of moderately differentiated tumors (G2), and 62.5% (5/8) of well differentiated tumors (G1) were HPV positive (χ^2 for trend = 0.54; $P = 0.33$). Nine (16.1%) HPV-positive cases and one HPV-negative (5.5%) tumor carried mutant *p53*. Five mutated cases were infected by HPV-18, two by HPV-16, and two by both oncogenic viruses.

In univariate analysis, patients with HPV-positive tumors had a better overall survival (124.5 months; 95% CI = 95.7 to 153.9) than did those with HPV-negative tumors (54.2 months; 95% CI = 22.9 to 85.5; $P = 0.0045$ by log-rank test).

Survival Analysis

The crude and adjusted odds ratios of tumor-related death are reported in Table 4. The prognostic variables

Table 4. Crude and Adjusted Odds Ratios of Tumor-Related Death

	Crude odds ratios (95% CI)	Adjusted odds ratios (95% CI)
Age*	1.05 (1.02–1.07)	1.05 (1.01–1.09)
Stage		
FIGO Ib	Reference	Reference
FIGO II	3.18 (1.36–7.42)	1.05 (0.36–3.01)
FIGO III/IV	11.9 (4.61–30.93)	3.80 (1.21–11.83)
Mucin-secreting tumor	0.47 (0.24–0.93)	0.55 (0.18–1.64)
Grading		
Grade 1	Reference	Reference
Grade 2	0.79 (0.26–2.38)	3.01 (0.83–11.5)
Grade 3	1.94 (0.63–5.96)	5.62 (1.29–24.4)
Cervix diameter >4 cm	2.16 (1.08–4.32)	1.37 (0.55–3.45)
Lymph nodes		
Negative	Reference	Reference
Positive	6.36 (2.36–17.2)	4.69 (1.49–14.8)
Missing	4.16 (1.88–9.20)	4.22 (1.56–11.44)
p53 mutation	2.93 (1.24–6.92)	1.53 (0.46–5.11)
HPV infection	0.38 (0.19–0.77)	1.1 (0.37–3.24)

*For each year of increment.

evaluated were age of patient, stage of disease, mucin secretion, differentiation grade, size of tumor, lymph node metastases, *p53* gene mutation, and HPV infection. In univariate analysis, mucin secretion and HPV infection were associated with an increased likelihood of survival (odds ratio = 0.47 and CI = 0.24 to 0.93 and odds ratio = 0.38 and CI = 0.19 to 0.77, respectively), whereas *p53* mutations were associated with an increased risk of death (odds ratio = 2.93; CI = 1.24 to 6.92). However, after adjustment for potential confounder (age, stage of disease, tumor grade, and lymph node metastases), neither mucin secretion nor HPV infection nor *p53* gene mutation could be related to survival. Age, stage, grade, and lymph node status were the only significant and independent predictors of survival.

Discussion

Epidemiological and clinicopathological data suggest that cervical squamous cell carcinoma (SC) and primary cervical adenocarcinoma (AC), as well as varying in biological behavior, may differ in etiopathological mechanisms.[24] Cervical AC has a lower incidence than does SC, and as for risk factors, including the age of patients affected, it is more similar to endometrial adenocarcinoma than to cervical SC.[24]

HPV types 16 and 18 have also been found to be associated with adenocarcinoma, and although earlier reports suggested a minor etiopathological role of the virus, recent investigations, performed by PCR in extensive series, support the importance of the infection in the development of AC.[22,25–28] In our series, the prevalence of HPV infection was 75.7%, a figure that is in the range of most other PCR-based studies on cervical AC[22,25–28] but lower than the rate determined for cervical SC.[29]

K-*ras* mutations, which are infrequent in SC,[30] are often detected in AC, and in a previous study we found that their overall frequency in AC is approximately the same as that reported for endometrial adenocarcinoma.[31] Furthermore, most of the mutations we found in our series were conversion from glycine to aspartic acid at codon 12, as commonly observed in endometrial adenocarcinoma. This observation, in association with other genetic similarities, might constitute evidence that endometrial and cervical adenocarcinoma share etiological characteristics.

Several studies have addressed the question of *p53* mutations in cervical neoplasia, reporting a prevalence that varies from 7 to 13%.[2] Crook suggested that only HPV-negative tumors have *p53* gene mutation,[8] but subsequent studies also identified mutant *p53* in HPV-positive cases.[9–12] When data from the literature are pooled, the overall rate of *p53* mutations is approximately 3.1% in HPV-positive and 19.5% in HPV-negative tumors (Table 5). As for primary AC, Jiko et al[13] analyzed 25 cases, and more recently Milde-Langosh et al[11] reported a series of 51 cervical cancers, 26 of which were adenocarcinomas. To the best of our knowledge, the present series is the largest so far analyzed.

Table 5. Frequency of *p53* Mutations in Cervical Carcinomas

Tissue type	HPV		Reference
	Positive	Negative	
Tumor	0/25	3/3	8
Tumor	0/15	0/5	32
Tumor	2/29	0/7	9
Tumor	1/30	2/16	33
Tumor	2/88	0/4	15
Tumor	2/7	1/1	34
Tumor	1/42	2/21	10
Tumor	1/11	6/12	13
Tumor	2/41	2/10	11
Tumor	2/133	0/3	12
Total	13/421 (3.1%)	16/82 (19.5%)	

We found *p53* somatic mutations in 10 (13.5%) cases. The possibility that a significant proportion of mutated sequences could have escaped detection can be excluded, as our method is sensitive enough to detect mutated sequences in 4 to 5% of the cells. Most mutated tumors were high grade, high stage, and large size, as also reported for endometrial adenocarcinoma.[35] Our results are in keeping with the figures reported by Milde-Langosh,[11] who identified mutations in 7.7% of cases of AC. The higher frequency of mutations (22.2%) reported by Jiko et al[13] might be related to the high prevalence of HPV-negative and advanced tumors in their series. But the influence of genetic and environmental factors on the Japanese cohort cannot be excluded. Besides the high rate of *p53* mutation, the Japanese cohort also shows a very low frequency of K-*ras* mutation (4%), which is in contrast with our own findings.

The mutation most frequently found in our series was the G:C→A:T base transition, which comprised approximately 50% of the *p53* mutations in the various tumors.[36] Five of the seven G:C→A:T transitions occurred at CpG dinucleotides located at one of the two codons 273 and 175. The cytosin of the CpG dinucleotide is prone to spontaneous deamination, which results in a mismatch of the T:G base pair, which can then be converted to T:A by DNA repair. This mechanism may at least partially explain the mutation pattern of many cervical cancers. However, as both transition and transversion have been described in cervical cancers, it is conceivable that exogenous agents, along with endogenous mechanisms, play a role in the carcinogenetic process.[10]

The high rate of G:C→A:T transitions in our series highlighted another genetic similarity between cervical and endometrial adenocarcinoma. In fact, in endometrial adenocarcinoma, G:C→A:T transitions are the *p53* mutations most frequently identified.[2]

In the present series, patients with HPV-negative tumors had a worse overall survival than those with HPV-positive cancers. Previously, others had suggested that poor prognosis in patients with HPV-negative cancers reflects the higher prevalence of *p53* mutations.[8] According to our results, this hypothesis might not be true for cervical AC, as we found *p53* mutations in nine (16.1%) HPV-positive cases and in only one (5.5%) HPV-negative tumor. On the other hand, the presence of *p53* mutations

is associated with a significantly shorter mean survival time irrespective of the presence of HPV. Therefore, both the absence of HPV infection and the presence of *p53* mutations would seem to be independent contributors to a poor prognosis. However, after adjustment for potential confounder, the only significant and independent predictors of survival are age of patient, stage of disease, tumor grade, and presence of lymph node metastases.

In conclusion, the type of *p53* mutations that we found in cervical AC further supports the hypothesis of affinities between this tumor and endometrial adenocarcinoma and gives credit to the possibility that, as well as sharing epidemiological and clinicopathological features, the two tumors follow similar genetic pathways. As *p53* mutations are more frequently detected in malignancies of high grade, high stage, and large size, this molecular event seems to play a role in the progression rather than in the induction of cervical AC. The poor prognosis in HPV-negative cervical AC is not related to the presence of *p53* mutations, which in our series were more often detected in HPV-positive cases. On the other hand, irrespective of HPV infection, the presence of *p53* mutations is associated with a significantly shorter mean survival time. However, the absence of HPV infection and presence of *p53* mutations are not independent risk factors for tumor-related death after adjustment for clinicopathological confounders.

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