J Clin Pathol 1998;51:754–760

Immunohistochemical expression of mdm2 and p21WAF1 in invasive cervical cancer: correlation with p53 protein and high risk HPV infection

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Accepted for publication 16 April 1998

Abstract

***Aim*—To investigate the immunocytochemical staining pattern of mdm2 and p21^{WAF1} proteins in invasive cervical cancer and to determine its relation with the expression of p53 and with the high risk HPV infection.**

***Methods*—Immunocytochemistry for p53, mdm2, and p21^{WAF1} was performed in 31 paraffin embedded sections of invasive cervical cancer. The results were assessed by image analysis, evaluating for each protein the optical density of the immunostained area, scored as percentage of the total nuclear area. The presence of high risk human papillomavirus (HPV) infection was detected by using the polymerase chain reaction.**

***Results*—Immunostaining for both mdm2 and p21^{WAF1} was correlated with p53 expression; however, the correlation between p53 and mdm2 ($R = 0.49$; $p < 0.01$) was more significant than between p53 and p21^{WAF1} ($R = 0.31$; $p < 0.05$); the less stringent correlation between p53 and p21^{WAF1} might reflect the p53 independent mechanisms of p21^{WAF1} induction. Similar average levels of p53, mdm2, and p21^{WAF1} immunostaining were found in the presence or absence of high risk HPV-DNA, without significant differences between the two groups.**

***Conclusions*—These data suggest that mdm2 and p21^{WAF1} proteins are expressed in invasive cervical cancer and that their immunocytochemical staining pattern is not abrogated by the presence of high risk HPV genomic sequences.**

(*J Clin Pathol* 1998;51:754–760)

Keywords: cervical cancer; p53; mdm2; p21/WAF1/Cip1; human papilloma virus

The p53 tumour suppressor gene encodes a transcriptional factor central in the regulation of cell growth, DNA repair, and apoptosis induction. Its activity requires the induction of several target genes, including mdm2 and p21^{WAF1}. The inactivation of p53 is believed to play a major role in the carcinogenesis of the uterine cervix.[1] Two different mechanisms may explain the loss of p53 function in cervical cancer—a somatic gene mutation which leads to an inactive form,[1] and the enhanced protein degradation promoted by the E6 oncoprotein of the human papilloma virus types (HPV) 16 and 18.[2] In contrast to many other human tumour forms, p53 mutations are only rarely detected in cervical cancer.[3-5] Therefore it is the E6 mediated pathway that may be responsible, in the vast majority of the cases, for the inactivation of the p53 protein.

This issue has been widely addressed by histopathologists, and several reports have correlated the expression of the p53 protein as shown by immunohistochemistry with the presence or absence of HPV genomic sequences.[6-10] Because the majority of cervical carcinomas harbour high risk HPV-DNA,[11 12] in which the E6 gene is retained and transcribed, p53 immunoreactivity was expected to be demonstrable only in the HPV negative minority. Instead, it has been shown using heating techniques for antigen retrieval that p53 immunostaining is a common finding, regardless of the HPV-DNA status.[8]

The p53 protein therefore accumulates in cervical neoplastic cells, but little is known about its functional status. More than eliminating the cellular levels of the protein, the viral oncoprotein might act by binding to p53 and neutralising its transcriptional activity. The p53 transcriptional activity in HPV infected cells has been assessed mostly by in vitro studies.[13-15] The results obtained have not been entirely consistent, and there is still uncertainty over whether the p53 mediated induction of mdm2 and p21^{WAF1} is disrupted in HPV infected cultured cells.[15] A recent immunohistochemical study of cervical dysplastic lesions showed a high expression of p21^{WAF1}, and this finding was unrelated to the HPV infection[16]; this analysis extended to six cases of invasive cancer which also showed increased p21^{WAF1} expression, but the presence of HPV-DNA was not assessed.

Table 1 Summary of oligonucleotides used as primers for PCR reactions and probes for Southern analysis

Type	Primer/probe	Sequence	Source
β globin	PC04 primer	5'-CAA CTT CAT CCA CGT TCA CC-3'	Greer *et al* [18]
β globin	KM29 primer	5'-GGT TGG CCA ATC TAC TCC CAG G-3'	
HPV 16-E6	H-1 primer	5'-ATT AGT GAC TAT AGA CAT TA-3'	Fujita *et al* [19]
	H-2 primer	5'-GGC TTT TGA CAG TTA ATA CA-3'	
	H-4 probe	5'-ATG GAA CAA CAT TAG AAC AGC AAT ACA ACA AAC CGT TGT G	
HPV 18-E6	HPV AR18-1	5'-AAA CTA ACT AAC ACT GCG TTA TAC A-3'	Arends *et al* [20]
	HPV AR18-2	5'-ATG GCA CTG GCC TCT ATA GT-3'	
	HPV AR18-3 probe	5'-CCT GCG GTG CCA GAA ACC GTT GAA TCC AGC	
HPV-L1	GP5+ primer	5'-TTTGTTACTGTGGTAGATACTAC-3'	de Roda Husman *et al* [21]
	GP6+ primer	5'-GAAAAATAAACTGTAAATCATATTC-3'	

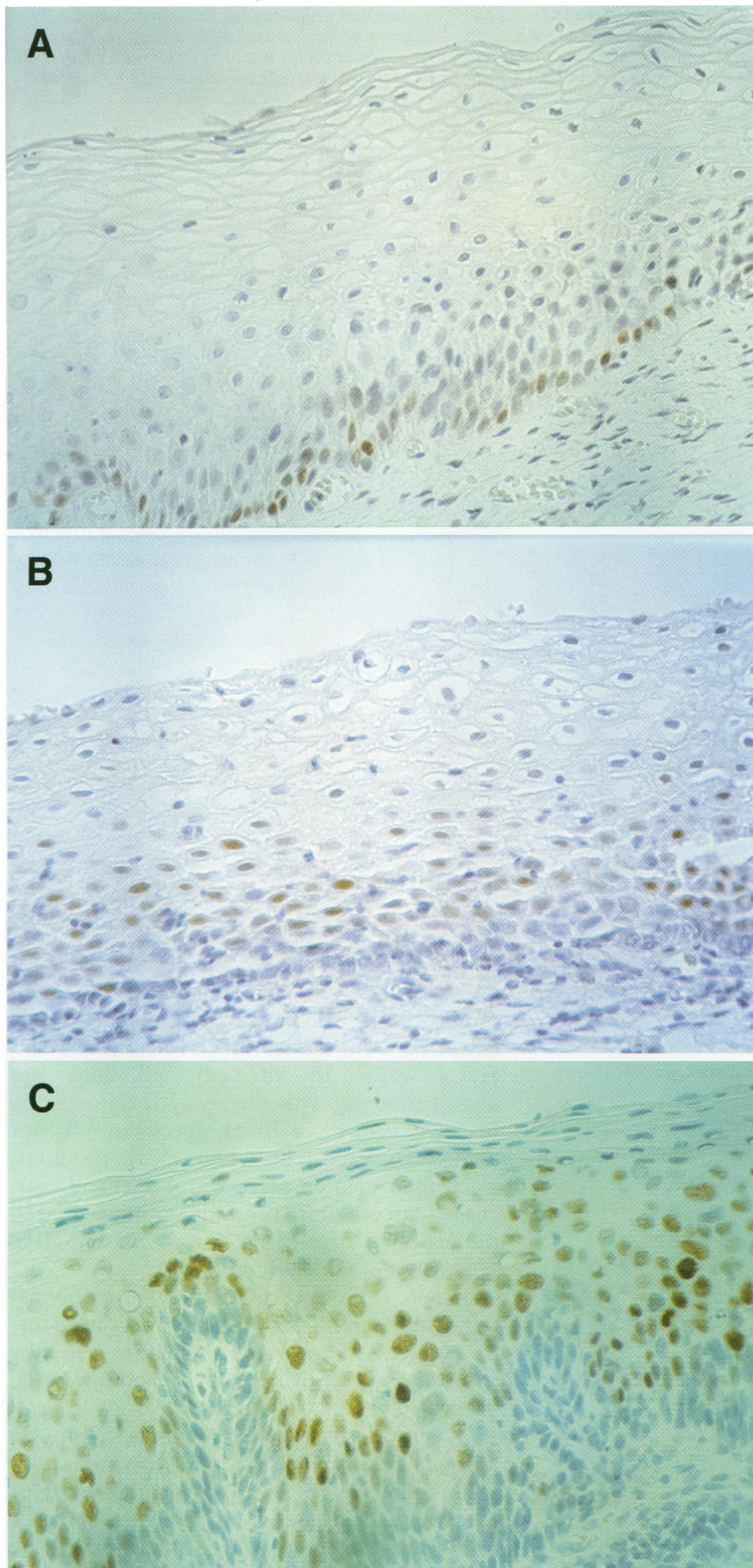

Figure 1 Immunohistochemical analysis of p53 and its target proteins in non-neoplastic cervical epithelium (case 95-728). The p53 antibody stained basal cell nuclei (A), while the suprabasal layers gave a signal with the mdm2 (B) and p21^{WAF1} (C) antibodies.

In this present study we analysed the expression of mdm2 and p21^{WAF1} proteins in a larger series of 31 cases of invasive cervical carcinoma. The immunocytochemical staining pattern of mdm2 and p21^{WAF1} was compared with the expression of p53 protein and with high risk HPV infection. Our results show that the immunocytochemical expression of mdm2 and p21^{WAF1} occurs in invasive cervical cancer and that this finding is not dependent on the absence of high risk HPV genomic sequences.

Methods

TISSUE SAMPLES

Paraffin embedded sections from 31 invasive squamous cell carcinomas of the cervix were obtained from the tissue bank of the pathology department of the Virgen de la Salud Hospital. As a source of non-neoplastic tissue, cervical sections were also obtained from five samples of hysterectomy performed for other reasons.

ANTIBODIES

Proteins were detected in paraffin tissue sections as follows: p53 protein with the monoclonal antibody D07 (Novocastra) generated against recombinant p53 protein, and specific for both human wild and mutant forms of the p53 protein; mdm2 protein by mdm2 mouse monoclonal antibody (Ab-1; Oncogene Science) raised against an epitope in the amino terminal region of human mdm2 protein; and p21^{WAF1} by p21 mouse monoclonal antibody (Ab-1; Oncogene Science) raised against recombinant human p21^{WAF1} protein.

To assess the antigenic preservation of the material examined, each case was initially stained with the PCNA antibody (Novocastra), which is known to yield staining in the proliferating cervical epithelium.

IMMUNOSTAINING TECHNIQUES

A pressure cooker heating step in a solution of sodium citrate was performed before incubation with antibodies. Xylene-dewaxed and alcohol-rehydrated paraffin sections were placed in Coplin jars filled with a 0.01 M trisodium citrate solution, and heated for three minutes in a conventional pressure cooker. After heating, slides were rinsed in cool running water for five minutes. They were then quickly washed in Tris buffered saline (TBS), pH 7.4, and incubated with the specific antibody.

Following incubation with the primary antibody, immunodetection was performed with biotinylated antimouse immunoglobulins, followed by peroxidase labelled streptavidine (LSAB-Dako) and diaminobencidine chromogen as substrate, using the Techmate 500 (Dako).

Incubation omitting the specific antibody, as well as with unrelated antibodies, was used as control.

QUANTITATIVE STUDY

Quantitative immunohistochemical investigation with a computerised analyser system (CAS 200, Becton Dickinson) was used to score individual nuclei of the tumour cells for the expression of the p53, mdm2, and p21^{WAF1} proteins. Nuclear boundary optical density and the antibody threshold were adjusted for each case examined. Five or more fields representative of each section were analysed, until a minimum of 500 cells had been studied. In each single case, the total optical density of the immunostained area against that of the nuclear area was evalu-

Table 2 Immunohistochemical evaluation of p53, mdm2, and p21^{WAF1} in five non-neoplastic cervical samples and in 31 cases of invasive squamous cell carcinoma of the cervix (ISCC). The immunostaining was assessed by image analysis evaluating, for each protein, the optical density of the immunostained area scored as percentage of the total nuclear area. In the neoplasm examined, the results were correlated with the PCR detection of the human papillomavirus (HPV) infection

Case	Histology	p53 (%)	mdm2 (%)	p21^{WAF1} (%)	HPV-DNA
(a) 95-3669	Normal	5	3	4	—
(b) 95-1886	Normal	7	5	9	—
(c) 95-487	Normal	3	0	4	—
(d) 95-728	Normal	8	6	11	—
(e) 95-276	Normal	4	7	5	—
(1) 93-2220	ISCC	40	10	44	16
(2) 92-786	ISCC	30	18	53	16
(3) 94-4576	ISCC	25	5	24	16
(4) 93-8314	ISCC	27	0	5	18
(5) 94-6728	ISCC	48	22	38	16
(6) 94-5361	ISCC	33	6	28	16
(7) 93-10545	ISCC	16	6	27	31
(8) 91-6012	ISCC	23	13	28	Negative
(9) 91-1399	ISCC	35	8	20	16
(10) 91-2899	ISCC	14	11	38	16
(11) 93-8619	ISCC	25	5	42	31
(12) 93-9144	ISCC	27	18	12	16
(13) 95-2943	ISCC	22	5	5	31
(14) 91-6440	ISCC	17	7	27	Negative
(15) 94-6165	ISCC	23	5	17	Failure of β globin amplification
(16) 91-5277	ISCC	85	22	38	Negative
(17) 92-7166	ISCC	0	0	5	35
(18) 95-3471	ISCC	30	25	35	16
(19) 92-3374	ISCC	33	15	60	Failure of β globin amplification
(20) 91-2243	ISCC	18	19	51	33
(21) 92-10031	ISCC	24	12	22	18
(22) 93-7729	ISCC	24	15	35	16
(23) 94-9177	ISCC	36	5	38	Negative
(24) 94-6770	ISCC	39	25	44	Failure of β globin amplification
(25) 95-7893	ISCC	9	8	40	16
(26) 91-6007	ISCC	40	12	51	Failure of β globin amplification
(27) 94-8335	ISCC	8	5	40	Negative
(28) 89-3481	ISCC	5	18	25	16
(29) 87-5819	ISCC	14	0	5	Negative
(30) 88-1631	ISCC	9	0	30	16
(31) 88-1057	ISCC	7	0	42	16

ated; in addition, the distribution of the protein expression was also considered, and the proportion of positive cells was assessed in each case.

HPV-DNA DETECTION BY POLYMERASE CHAIN REACTION

Xylene dewaxed and alcohol rehydrated paraffin sections of invasive cervical cancer were removed from glass slides and placed in 1.5 ml Eppendorf tubes; 100 µl of digestion buffer (50 mM Tris (pH 8.5), 1 mM EDTA, 0.5% Tween 20) containing 200 µg/ml of proteinase K were then added. Digestion was carried out at 55°C overnight; the samples were then heated to 98°C for 8–10 minutes to inactivate the proteinase K.[17] The supernatant was collected and stored at −20°C for later use in polymerase chain reaction (PCR) reactions. The suitability of the DNA for PCR studies was evaluated by amplifying a portion of the β1 globin gene by PCR in each case. Cases in which no β1 globin amplification occurred were excluded from further study.

High risk HPV-DNA was detected by PCR using specific primers for HPV types 16 and 18. The primer sequences used are summarised in table 1.[18-20]

All PCR reactions were carried out in a total volume of 50 µl. The reaction mixture contained PCR buffer (Perkin-Elmer), 1.5 mM $MgCl_2$, deoxyribonucleoside triphosphate (Pharmacia), 500 nM primers, and 2.5 units Taq polymerase (Perkin-Elmer).[17] Samples were amplified on a Perkin-Elmer programmable thermal controller; the mixture was denatured initially for four minutes at 94°C, followed by 35 cycles of amplification (94°C for one minute; 55°C for one minute; 72°C for one minute), and then incubation at 4°C for 10 minutes. As positive controls, DNA previously extracted from Caski (HPV 16 infected) and HeLa (HPV 18 infected) cell lines was amplified along with the study specimens. As negative control, a blank specimen was included in each run. The PCR products, electrophoresed on a 2% agarose gel and visualised with ethidium bromide staining, showed the expected sizes (β1 globin = 205 bp; HPV 16 = 109 bp; HPV 18 = 143 bp).

The PCR products obtained after amplification with the HPV 16 and 18 primers were further processed by Southern blotting and by hybridisation with specific oligonucleotide probes (table 1).[19 20] Aliquots (10 µl) of each PCR product were electrophoresed on 2% agarose gels, transferred onto Hybound N$^+$ nylon membranes (Amersham), hybridised with the specific ^{32}P end labelled oligonucleotide probes, and washed as described.[19 20] Autoradiography of the filters was performed with intensifying screens at −70°C with Fuji Rx films.

To ensure the detection of other high risk types in addition to type 16 and 18, the cases negative after amplification with the HPV 16 and 18 specific primers were subjected to a further PCR system using the recently described GP5+/GP6+ primers (table 1).[21] These are specific for a highly conserved region of the L1 gene which allows detection of a wide range of HPV types.[22] The reactions were carried out

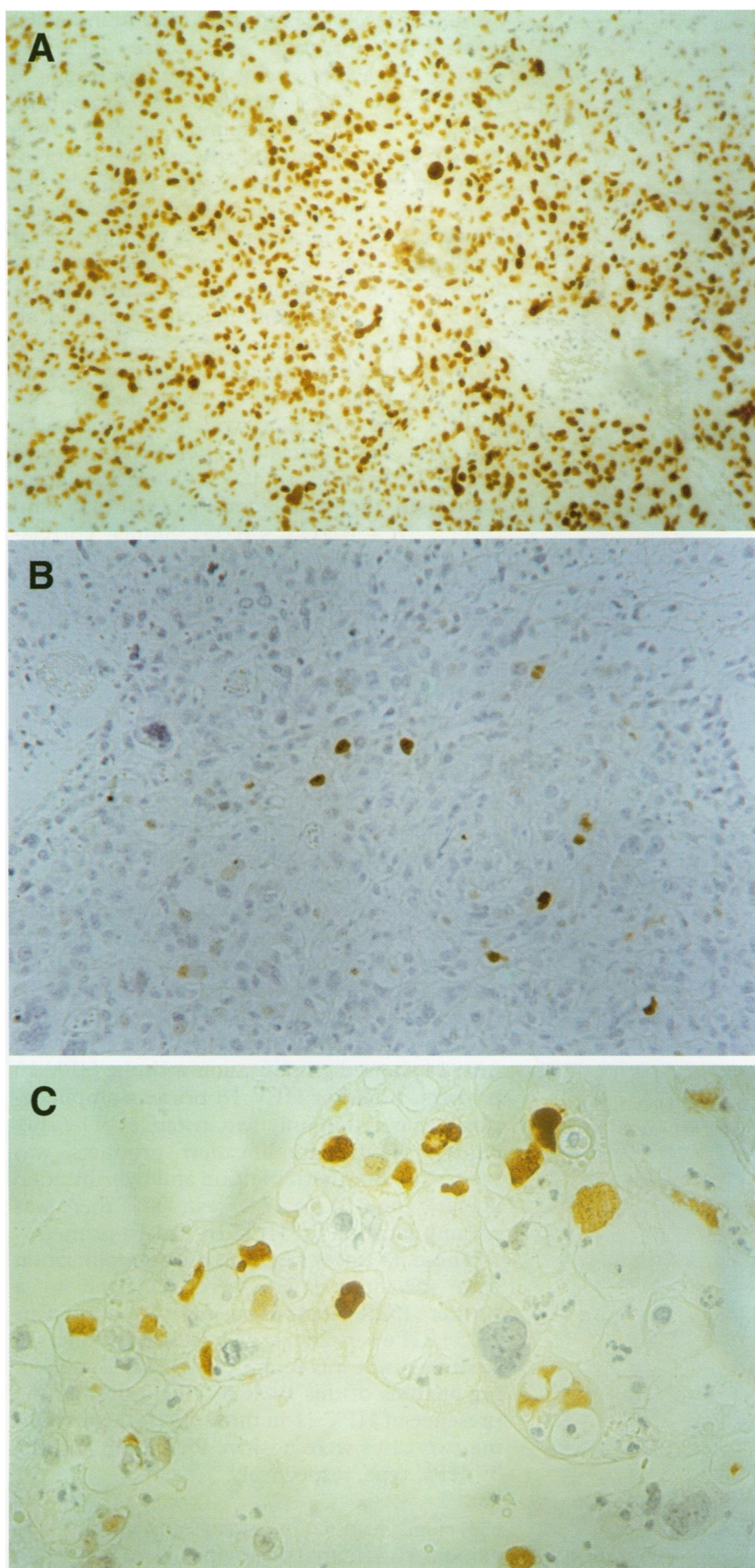

Figure 2 Immunohistochemical analysis of p53, mdm2, and p21^{WAF1} protein expression in neoplastic cervical epithelium in case 91-5277. This was the case with the highest expression of p53 (A). Staining for mdm2 (B) and p21^{WAF1} (C) was also observed in this case.

in a total volume of 50 µl containing 50 mM KCl, 10 mM Tris HCl (pH 8.3), 200 µM of each deoxyribonucleoside triphosphate (Pharmacia), 3.5 mm $MgCl_2$, 1 unit Taq polymerase (Perkin-Elmer), and 50 pmol of each primer.[22] A four minute denaturation step at 94°C was followed by 40 cycles of amplification (94°C for one minute; 40°C for two minutes; 72°C for 1.5 minutes), followed by incubation at 4°C for 10 minutes.[22] The GP5+/GP6+ PCR products, electrophoresed on a 2% agarose gel and visualised with ethidium bromide staining, showed the expected 150 bp sizes. In these cases, HPV typing was performed by sequencing, and the nucleotide sequences were determined from the GP5+/GP6+ PCR products (pCR fragments), using the dideoxy chain termination method.[23] We used standard protocols of the manufacturer for Taq DNA polymerase initiated cycle sequencing reactions with fluorescence labelled dideoxynucleotid terminators (Applied Biosystems). The sequencing reactions were analysed using a 377 automated DNA sequencer (Applied Biosystems) and compared to the HPV type specific L1 nucleotide sequences flanked by the GP5/GP6 region.[22]

STATISTICAL ANALYSIS

Data were analysed by using the SPSS software for Windows 6.1, 1994. The association between variables was verified by the Spearman's correlation coefficient for continuous variables. The differences in the expression of p53, mdm2, and p21^{WAF1} between the high risk HPV positive and negative groups were verified by the non-parametric Mann–Whitney U test.

Results

All the samples examined showed a positive nuclear signal after PCNA staining (data not shown), which ensured their suitability for the assessment of the staining pattern of p53, mdm2, and p21^{WAF1}.

p53, mdm2, AND p21^{WAF1} IN NON-NEOPLASTIC CERVICAL EPITHELIUM

We processed sections of non-neoplastic cervical tissue, obtained from five hysterectomies, to study the expression of p53, mdm2, and p21^{WAF1} in the absence of cancer. After staining, a case was evaluated by an image analyser to quantify the integrated optical density for each antibody. In the absence of neoplasia, the average value was 5% for p53, 4% for mdm2, and 7% for p21^{WAF1}. Only the p53 antibody stained basal cell nuclei (fig 1A), while the suprabasal layers gave a signal with the mdm2 and p21^{WAF1} antibodies (fig 1B and 1C).

p53, mdm2, AND p21^{WAF1} IMMUNOSTAINING IN INVASIVE CERVICAL CANCER

Tissue sections obtained from the same paraffin embedded blocks used to determine the presence of HPV-DNA were processed by immunohistochemistry for the expression of the p53, mdm2, and p21^{WAF1} proteins in 31 cases of invasive cervical cancer. As mentioned in Methods, the expression of these proteins was assessed in each case by measuring both the value of the integrated optical density of the protein and the percentage of cells showing a positive signal. In our series of cases the average value of the integrated optical density was 25% for p53, 10% for mdm2, and 31% for p21^{WAF1}; the pattern of expression of these proteins is given in table 2.

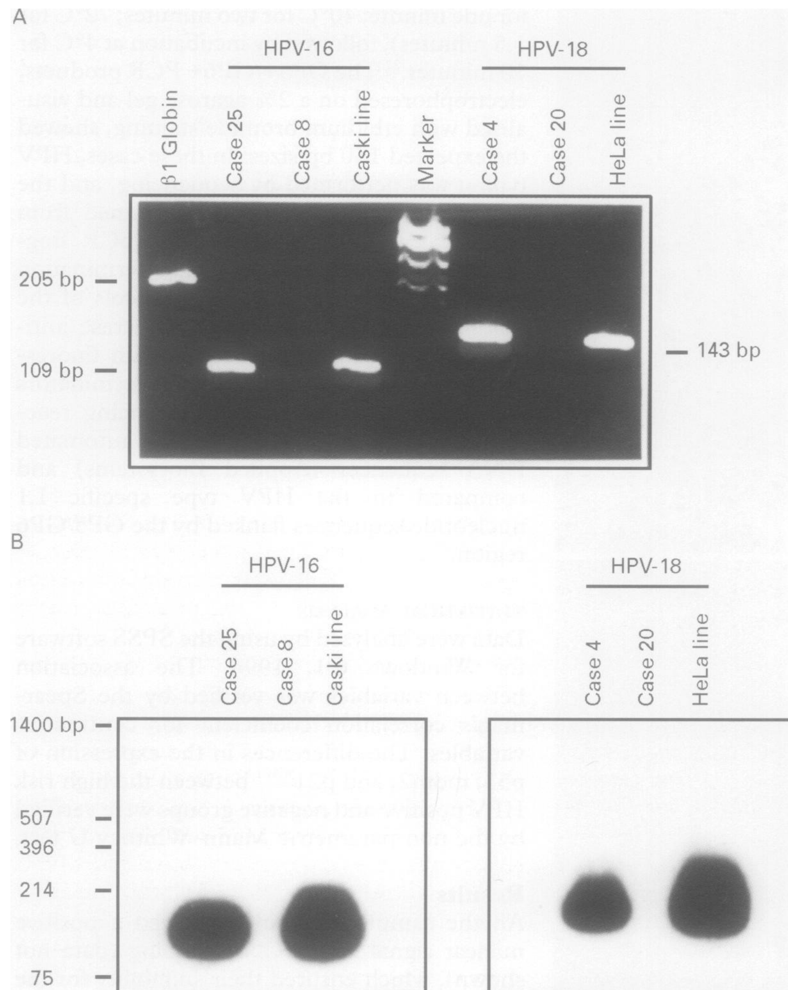

Figure 3 (A) Detection of PCR products amplified from β1 globin, HPV 16, and 18 sequences in DNA from invasive squamous cell cervical carcinomas. Bands of expected size are shown after amplification with β1 globin (lane 1), HPV 16 (case 25), and HPV 18 (case 4). Examples of negative amplification are shown for HPV 16 (case 8) and for HPV 18 (case 20). DNA extracted from Caski and HeLa cell lines was used as respective control for the amplification with HPV 16 and HPV 18 primers. (B) The PCR products obtained after amplification with the HPV 16 and 18 primers were electrophoresed, blotted, and hybridised with the specific oligonucleotide probes. The specificity of the amplification for type 16 (case 25) and for type 18 (case 4) was confirmed. As marker of molecular weight, pUc18 DNA digested with Hinf I was used.

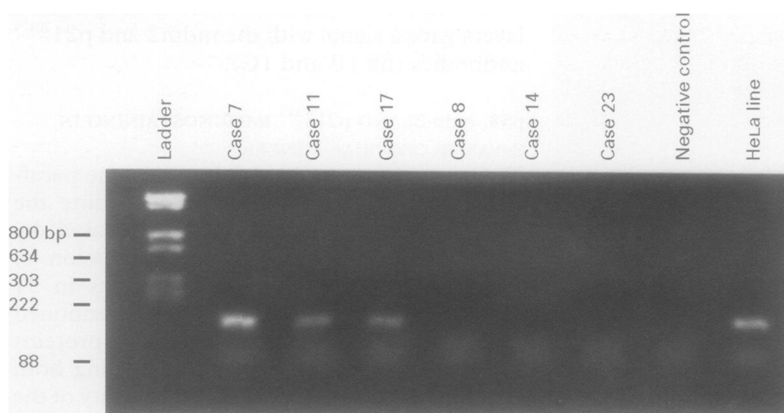

Figure 4 Detection of PCR products amplified from HPV sequences in DNA from invasive squamous cell cervical carcinomas, employing the GP5+/GP6+ consensus primers. Bands of expected size are shown after amplification in cases 7, 11, and 17. Examples of negative amplification are shown for in cases 8, 14, and 23. As a negative control, a blank specimen was processed, while HeLa cell line DNA was also amplified along with the study specimens. As a marker of molecular weight, pBR 322 DNA digested with Ava II and Ava II/Eco RI(Biorad) was used.

Similar levels of expression were found when considering for each single case (data not shown) the average value of the percentage of positive cells (25% for p53, 12% for mdm2, and 35% for $p21^{WAF1}$). Staining for $p21^{WAF1}$ was seen in all the cases, while there was no expression for p53 in one case or for mdm2 in five cases. In invasive tumours, the pattern of staining of p53, mdm2, and $p21^{WAF1}$ seen in normal epithelium was lost and we often found a heterogeneous staining pattern, with a variable fraction of positive tumour cells located at all levels of the epithelium (fig 2, A, B, and C). Considering the integrated optical density data, immunostaining for both mdm2 and $p21^{WAF1}$ was correlated with p53 expression; however, the correlation between p53 and mdm2 ($R = 0.49$; $p < 0.01$) was more significant than the correlation between p53 and $p21^{WAF1}$ ($R = 0.31$; $p < 0.05$). Similar correlations were obtained when the data relative to the percentage of the stained cells were considered (p53-mdm2: $R = 0.42$; $p < 0.05$; p53-$p21^{WAF1}$: $R = 0.37$; $p < 0.05$).

HPV-DNA DETECTION BY PCR

We used the PCR method to assess the presence of HPV-DNA sequences in the same paraffin embedded blocks investigated for p53, mdm2, and $p21^{WAF1}$ expression. As mentioned in Methods, a preliminary amplification of a portion of the β1 globin gene was carried out to ensure that the samples were suitable for the study. In four cases, there was failure of amplification of the constitutive gene; the remaining 27 cases—which showed appropriate amplification of the β1 globin gene—were selected for the reaction with 16 and 18 HPV type primers. Sixteen cases (59%) gave a positive result (fig 3A); 14 showed amplification with the HPV 16 primers, while for HPV 18 primers amplification was noted in only two instances. The specificity of the amplification products was verified by Southern blotting and hybridisation analysis (fig 3B). In 11 cases (41%) there was no amplification for HPV 16 and 18 primers.

These 11 cases negative after amplification with specific primers were subjected to a further PCR analysis using the consensus GP5+/GP6+ primers, and in five instances a positive result was found (fig 4). The sequencing analysis of this PCR products showed the presence of HPV 31 in three cases; the remaining two cases were positive for the 33 and the 35 HPV type, respectively.

p53, mdm2, AND $p21^{WAF1}$ EXPRESSION WITH REGARD TO THE HPV INFECTION

To investigate whether the expression of p53, mdm2, and $p21^{WAF1}$ was influenced by the presence of high risk HPV-DNA sequences, we correlated the average levels of integrated optical density expression of these proteins with the PCR results. We only considered the results obtained in the 27 cases that showed suitable amplifiable extracted DNA (table 2). The p53 protein showed slightly different average levels of expression in the presence (22.3%) and in the absence (30.5%) of amplification of the HPV 16 and 18 primers. The average value of

Table 3 p53, mdm2, and p21 expression in the HPV positive and negative groups

	*p53**	*mdm2**	*p21**
(A) Average values of integrated optical density			
HPV-DNA+	22.3 (12.1)	10 (7.7)	29.6 (14.4)
HPV-DNA−	30.5 (28.4)	8.7 (7.8)	29.3 (13.1)
(B) Average percentage of positive cells			
HPV DNA+	23.4 (15.2)	12.6 (9.0)	34.1 (17.0)
HPV DNA−	29.1 (26.2)	10.0 (7.7)	31.1 (11.6)

Values are mean (SD).
*HPV+ *v* HPV−, NS.

mdm2 was 10% in the HPV positive group and 8.7% in the HPV negative group; p21^{WAF1} expression was the same (29%) in presence of high risk HPV-DNA and in its absence. These differences in the expression of p53, mdm2, and p21^{WAF1} between the HPV positive and HPV negative groups were not statistically significant (table 3A), nor did we find significant differences when the data on the percentage of positive cells were considered (table 3B).

Discussion

Our results are consistent with several other reports[7-10] indicating that p53 immunostaining occurs in the vast majority of cervical cancers and that nearly all cases show at least some degree of nuclear signal (table 2). We also confirmed that higher p53 protein levels are not dependent on the absence of high risk viral infection,[8] as shown by the similar immunoreactivity score of the HPV positive and HPV negative groups. Thus what needs to be investigated is not simply the demonstration of p53 reactivity in cervical epithelium, but also whether the protein retains its activity, or alternatively whether there are differences in the staining pattern of the p53 downstream proteins between high risk HPV positive and negative groups. To this end, we investigated the immunohistochemical expression of p53, mdm2, and p21^{WAF1} in normal and neoplastic cervical epithelium.

Antigen retrieval by hot pressure cooker heating enhances p53 immunoreactivity, thus enabling this protein to be found in non-neoplastic conditions, where a specific nuclear signal is shown by the basal layer cells.[9] In our series, the average value of p53 in absence of neoplasia was 5%, and this level of expression probably reflects the normal cellular p53 homeostasis in actively replicating cells. This pattern of staining is consistent with a recent immunohistochemical analysis of organotypic cultures of normal human foreskin which verified detectable p53 only in basal keratinocytes,[24] while in suprabasal cells the p53 mediated transcriptional activity was enhanced, leading to increased mRNA levels of the endogenous p53 inducible proteins mdm2 and p21^{WAF1}.[24] Consistent with these findings derived from in vitro studies, we observed in vivo, in non-neoplastic cervical epithelium, that mdm2 and p21^{WAF1} have a suprabasal pattern of staining (fig 1, B and C). These findings suggest that in a non-neoplastic setting a functional p53 protein is associated with the expression of its target proteins mdm2 and p21^{WAF1}; conversely, the expression of p21^{WAF1} is not always dependent on p53, as shown by recent evidence in cell lines[25 26] and tissues.[16 27 28]

As well as eliminating the p53 cellular content, the HPV E6 oncoprotein may also lead to non-mutational inactivation of the protein and to the lack of the expression of its downstream proteins, mdm2 and p21^{WAF1}. We tested this hypothesis in our series of 31 invasive cervical cancers. Our results showed that immunostaining for mdm2 and p21^{WAF1} was not abrogated in invasive cervical cancer by high risk HPV genomic sequences. Indeed, there were no significant differences in the expression of p53, mdm2, and p21^{WAF1} between the HPV-DNA positive and negative groups, as similar levels expression were observed (table 3). These data concur with the recent demonstration of the immunocytochemical overexpression of p21^{WAF1} in cutaneous squamous cell carcinoma arising from anogenital sites, where high risk HPV is often involved.[29]

Thus, in this study we have shown: (1) that p53 accumulates in almost all cases of cervical neoplasia; (2) that this finding is associated with the expression of its downstream proteins mdm2 and p21^{WAF1}; and (3) that the pattern of staining of these proteins is not abrogated by high risk HPV infection. These observations suggest that the p53 transcriptional function is not lost as a result of the viral infection and that the p53 protein accumulated in cervical cancer cells is still capable of inducing the expression of its downstream proteins in presence of high risk HPV genomic sequences. This is also indicated by the heterogeneous pattern of staining of p53, mdm2, and p21^{WAF1} observed in invasive neoplasms, similar to that recently reported in invasive colon cancer.[28] On the other hand, the expression of p21 in invasive cervical cancer might also be partly independent of p53—as indicated in this study by the more significant correlation between p53 and mdm2 ($R = 0.49$; $p < 0.01$) than between p53 and p21^{WAF1} ($R = 0.31$; $p < 0.05$)—and may not therefore be completely accurate in assessing the transcriptional activity of p53. A recent study in cervical dysplastic lesions and in a few cases of invasive cervical cancer showed that p21^{WAF1} is highly expressed in cervical neoplastic epithelium and that this finding is not correlated with the p53 staining pattern.[16] More must be learned concerning the relation between p53 protein accumulation and mdm2 and p21^{WAF1} expression in invasive cervical cancer. Other studies correlating the immunocytochemical expression of these proteins with p53 gene analysis are required to investigate the functional status of p53 protein in HPV related cervical carcinoma.

GT holds a postdoctoral fellowship from the University of Naples. This study was supported by a grant from the Fundo de Investigation Sanitaria (FIS) 92/0721, from the P F ACRO-CNR, and from the Associazione Italiana Ricerca contro il Cancro (AIRC). We thank Mr Pasquale Signoriello for his valuable technical support.

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