Cyclin dependent kinase inhibitor p27^{Kip1} expression in normal and neoplastic cervical epithelium

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Abstract

Aim—To investigate whether there is loss of the p27^{Kip1} protein in developing cervical cancer and whether p27^{Kip1} immunoreactivity has any relation to the proliferative indicator Ki-67.

Methods—The expression of p27^{Kip1} and Ki-67 was assessed by immunohistochemistry in serial sections from normal epithelium (13), low grade (27) and high grade (19) squamous intraepithelial lesions (LSIL, HSIL), and invasive cervical cancer (23). In the SIL cases the presence of human papillomavirus (HPV) genomic sequences was assessed by in situ hybridisation. The results were evaluated by image analysis, and reported as mean score of the percentage of p27^{Kip1} and of Ki-67 positive cells in each histological group.

Results—In general, p27^{Kip1} immunostaining was related to squamous differentation, and was intense in normal epithelium (47%), while it was reduced in SIL lesions as an effect of the decreased number of differentiating cells. However, decrease in the p27^{Kip1} expression was more evident in LSIL (36%) than in HSIL (39%); in the latter, p27^{Kip1} had a different intraepithelial distribution in that the staining extended to the basal cells. The average levels of p27^{Kip1} were similar in SIL lesions associated to low, intermediate, and high risk HPV types. Compared with normal epithelium and dysplasia, invasive cancer showed significantly lower p27^{Kip1} levels (23%). There was no relation between p27^{Kip1} and Ki-67 labelling indices in any of the histological groups examined.

Conclusions—A reduction in p27^{Kip1} protein occurs in cervical cancer independently of the proliferative status. The changes in p27^{Kip1} expression may be related to the unregulated kinetics of developing cervical cancer.

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It is widely held that the interactions between high risk human papillomavirus (HPV) oncoproteins and the cellular proteins involved in the cell cycle regulation play a central role in the development of cervical cancer,[1] the second most common malignancy affecting women worldwide.[2] Recently, investigation on the kinetics of the cervical epithelium has extended to those proteins where activity has an inhibitory effect on the cell cycle progression, such as the cyclin dependent kinase (CDK) inhibitors, among which the p21^{WAF1} protein has been the most widely studied.[3–6] In a previous report, we showed that p21^{WAF1} expression is normally restricted to the suprabasal layers, while in invasive cervical cancer this protein is overexpressed.[3] Therefore, it may be expected that other CDK inhibitors are analogously affected and that the topographic analysis of their expression can yield information on the pathogenesis of cervical cancer.

A CDK inhibitor closely related to p21^{WAF1} is the p27^{Kip1} protein,[7] which has an inhibitory effect on the G1/S progression in response to extracellular signals.[8–10] In experimental models, the p27^{Kip1} protein is conspicuously present in quiescent cells and in cells undergoing terminal differentiation, whereas it is less expressed when cell division occurs.[10] There is increasing evidence that the loss of p27^{Kip1} protein expression is a common feature of the more advanced and poorly differentiated tumours of different linkages,[11] and low or absent p27^{Kip1} protein in tumour cells is an important clinical marker of disease progression in many tumour types.[12–18]

In this study we investigate whether the alteration of p27^{Kip1} expression also occurred in the neoplasms arising from cervical squamous epithelium. This is also suggested by the finding that, in vitro, the HPV E7 oncoprotein inactivates p27^{Kip1}, disassociating it from the cyclin–Cdk complexes,[19] thus underlining the possibility that there is loss of p27^{Kip1} expression in developing cervical cancer as well. This issue has been explored in a previous study, in which, surprisingly, it was reported that invasive cancer may express high levels of p27^{Kip1}.[20] This is in contrast with other reports, where loss of p27^{Kip1} protein was found in squamous carcinomas arising from other anatomical sites, such as the bronchial and oral epithelia.[21][22] In the present study we addressed this conflicting issue from a histopathological point of view by using immunohistochemistry in paraffin embedded material. We investigated whether the expression of p27^{Kip1} in squamous intraepithelial lesions (SIL) and in invasive cancers differs from that in normal tissue by assessing first, the immunocytochemical staining pattern of p27^{Kip1} in the normal, dysplastic, and neoplastic cervix, and second, whether there is any relation between the Ki-67 protein and the p27^{Kip1} immunoreactivity in cervical lesions.
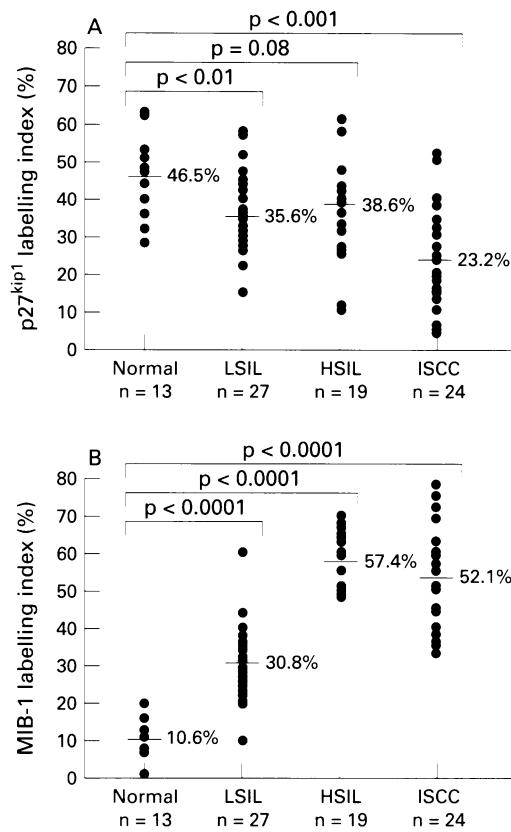
Figure 1 Comparison of the mean values of p27^{Kip1} and MIB-1 expression in normal cervix and in cases with low grade squamous intraepithelial lesions (LSIL), high grade lesions (HSIL), and invasive squamous cell carcinoma (ISCC).

Methods

TISSUE SAMPLES

Eighty two formalin fixed, paraffin embedded cervical biopsy specimens were obtained from the files of the department of pathology at the University Federico II of Naples, Italy. These included 13 examples of non-neoplastic squamous epithelium (including seven cases with regions of chronic inflammation and/or squamous metaplasia), 27 cases of low grade SIL (12 HPV infections and 15 cervical intraepithelial neoplasia (CIN) I), 19 cases of high grade SIL (four CIN II, six CIN III, and nine carcinomas in situ), and 23 cases of invasive squamous cell cancer of the cervix. These latter were graded by a modification of the Broder system[23] into grade 1, well differentiated neoplasms (2), grade 2, moderately differentiated (15), and grade 3, poorly differentiated (6). Consecutive 5 µm sections were cut from each block and placed on charged slides.

ANTIBODIES

p27^{Kip1} Protein was detected with the monoclonal antibody from Transduction Laboratories, Lexington, Kentucky, USA (K 25020; 1:1000 dilution), generated against the full length mouse KIP1. The specificity of this antibody has been demonstrated previously.[14]

MIB-1 monoclonal antibody (Novocastra; 1:50 dilution) recognises Ki-67, a nuclear protein expressed in G1, S, and G2/M phases; this antibody, known to yield staining in the proliferating cervical epithelium, was used as control of antigenic preservation and of successful antigenic retrieval.

STAINING TECHNIQUES

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.[3] After heating, slides were thoroughly rinsed in cool running water for five
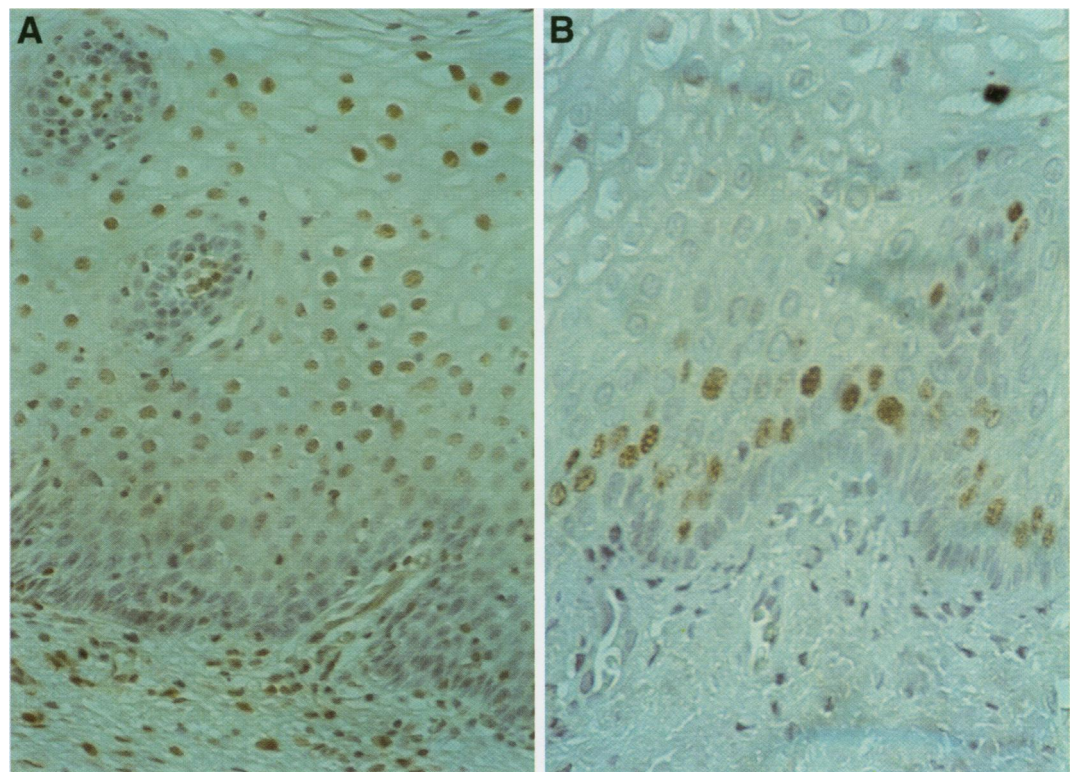

Figure 2 p27^{Kip1} (A) and Ki-67 (B) immunostaining in non-neoplastic epithelium. Staining for p27^{Kip1} was absent in the basal cell layer and in parabasal cells immediately above, the latter expressing Ki-67. The more superficial layers of the parabasal cells and the intermediate cells showed intense p27^{Kip1}.
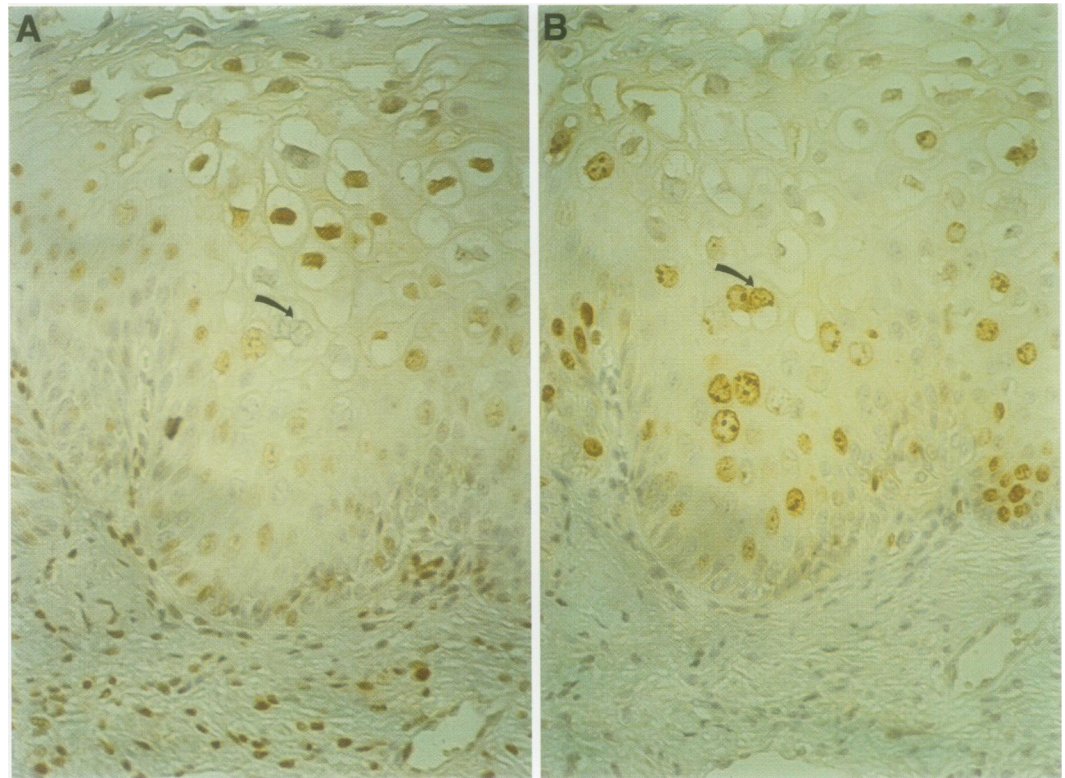
Figure 3   p27^{Kip1} (A) and Ki-67 (B) immunostaining of serial sections from a case of low grade SIL. Note the different distribution of the p27^{Kip1} positive cells in respect to the Ki-67 immunoreactive nuclei. The lower part of the epithelium was Ki-67 positive, while p27^{Kip1} stained the more superficial cells. The arrows indicate the same binucleate cell stained for Ki-67 and negative for p27^{Kip1}.

minutes. They were then washed in Tris buffered saline (TBS), pH 7.4, before incubating with the specific antibody.[3]

The incubation with the primary antibody was followed by incubation with biotinylated antimouse immunoglobulins, and by peroxidase labelled streptavidine (LSAB–Dako); the signal was developed by using diaminobenzidine (DAB) chromogen as substrate. Incubations omitting the specific antibody, as well as with unrelated antibodies, were used as controls of the technique.

For double immunostaining the sections were incubated first with the MIB-1 monoclonal antibody and the signal developed with DAB, as mentioned above. After the complete performance of the first staining sequence an antibody elution step was performed (Dako). The sections were then incubated with the p27^{Kip1} antibody, followed by alkaline phosphatase labelled antimouse immunoglobulin (Dako). The alkaline phosphatase staining was performed in red (Fast Red).

In cases of SIL, the presence of HPV DNA was detected by in situ hybridisation as previously reported,[24] using a cocktail of 6, 11, 16, 18, 31, 33, and 35 biotinylated HPV–DNA probes (Enzo). Each positive sample was then singly probed with combined 6/11, 16/18, and 31/33/35, respectively. The hybridisation was detected by using a catalysed signal amplification system (Dako) and developed by using DAB chromogen as substrate.
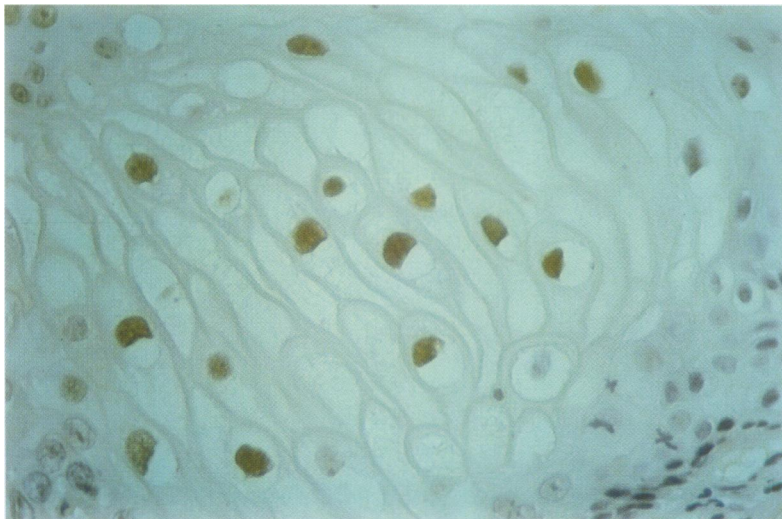
QUANTITATIVE STUDY AND STATISTICAL ANALYSIS
Labelling indices for p27^{Kip1} and MIB-1 antibodies were determined in the same manner. Adjacent sections were used, and counting was performed in similar areas; quantitative analysis performed with a computerised analyser system (CAS 200, Becton Dickinson) was used to score the nuclei of individual cells for expression of p27^{Kip1} and Ki-67 proteins. As already described, nuclear boundary optical density and antibody threshold were adjusted for each case examined.[3] In tissue sections from normal cases and from SIL cases, the entire squamous epithelial surface observed in any single slide was evaluated. In specimens from invasive cancer a minimum threshold was established by counting at least 1000 cells per sample. In each case the distribution of p27^{Kip1} and Ki-67 proteins in both normal and abnormal tissues was evaluated and expressed as a

Figure 4   p27^{Kip1} immunostaining in a case of low grade SIL showing morphological features of wart virus infection. p27^{Kip1} staining is intense, corresponding with the HPV cytopathic effect.
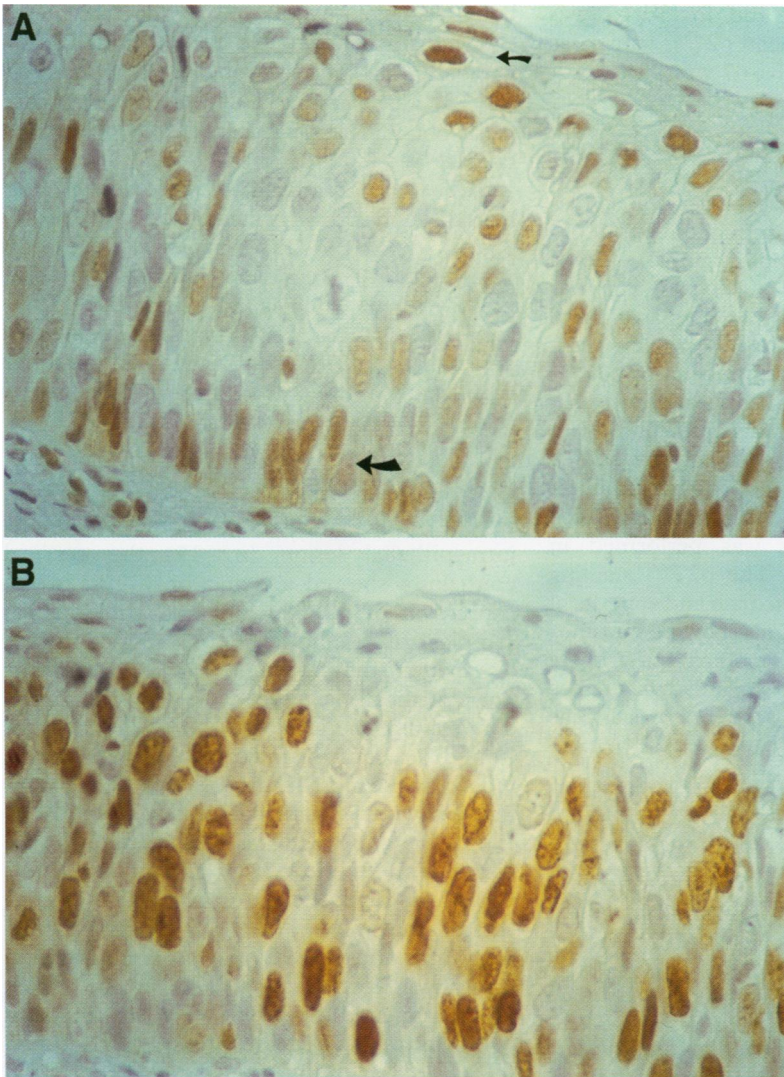
Figure 5   p27^{Kip1} (A) and Ki-67 (B) immunostaining of serial sections from a case with high grade squamous intraepithelial lesions (SIL). Note the characteristic pattern of staining of p27^{Kip1}, with intense staining of the basal epithelium (arrow) and of the more superficial cells displaying squamous differentation (arrow). The intermediate layers showed scattered p27^{Kip1} positive nuclei, being more intensely stained by the Ki-67 antibody.

percentage of the total cell population. Data were evaluated using the SPSS software for Microsoft Windows 6.1, 1994. In each histological group, the mean score, standard deviation, and 95% confidence intervals (CI) were calculated for both p27^{Kip1} and Ki-67 proteins and are reported in fig 1. The differences in the means were assessed by direct comparison of 95% CI, using the non-parametric Mann–Whitney U test. The association between p27^{Kip1} and Ki-67 for each histological group was verified by Spearman's correlation coefficient for continuous variables.

Results

p27^{Kip1} AND Ki-67 EXPRESSION IN NON-NEOPLASTIC CERVICAL EPITHELIUM

In the absence of neoplasia (n=13) the epithelium of the exocervix showed a topographically sequential expression of p27^{Kip1} and Ki-67 as there was complete segregation of the p27^{Kip1} and Ki-67 immunoreactive nuclei in all the cases studied. Indeed, although staining for p27^{Kip1} was absent in the basal cell layer and in parabasal cells immediately above (fig 2A), the latter expressed Ki-67 (fig 2B). On the other hand, nuclei of the midzone layer (stratum spongiousum), including the most superficial layers of the parabasal cells and the intermediate cells, showed intense p27^{Kip1} nuclear labelling, which was also evident in areas of squamous metaplasia. The staining for p27^{Kip1} in small pyknotic nuclei of the superficial layer was sporadic. Infiltrating mature lymphocytes showing positive p27^{Kip1} staining were used as an internal positive control. In each case of normal epithelium examined, the percentage of
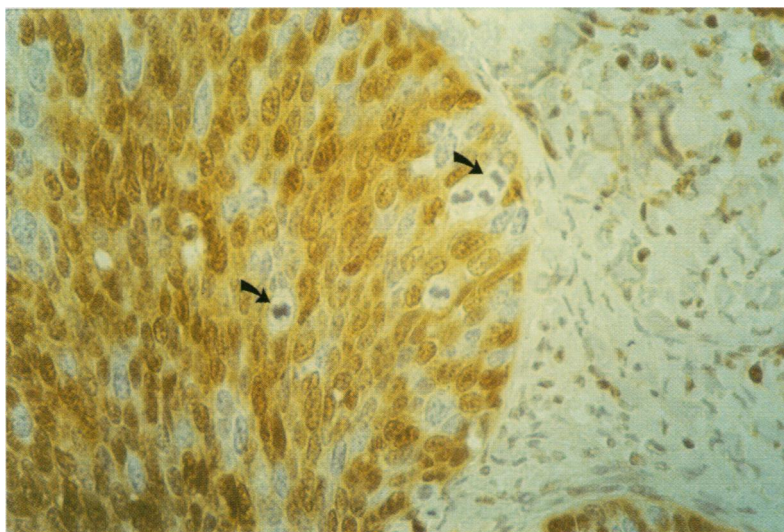

Figure 6   p27^{Kip1} immunostaining in a case with high grade high grade squamous intraepithelial lesions (SIL) showing intensely positive endocervical dysplastic glands. Note the lack of p27^{Kip1} staining in cells with mitotic figures, as the staining is abruptly interrupted by the presence of cell division.
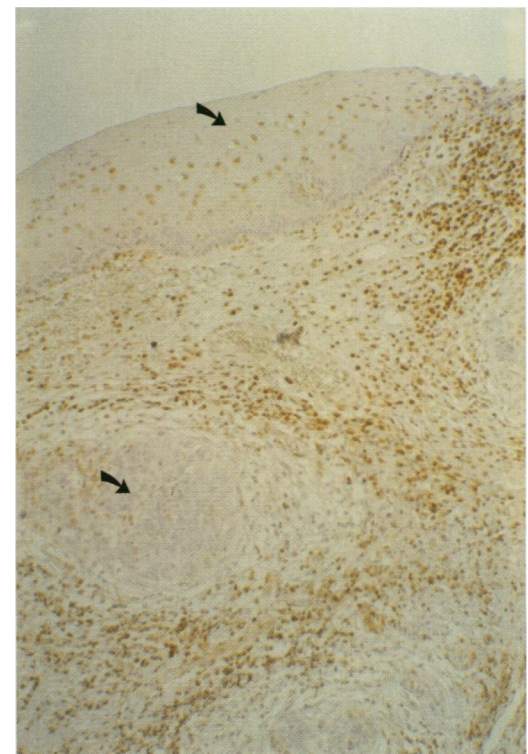

Figure 7   p27^{Kip1} Immunostaining in a tissue area showing normal epithelium adjacent to a nest of invasive neoplastic cells (arrows). The loss of p27^{Kip1} expression in the invasive area is evident when compared to the above normal epithelium. Infiltrating mature lymphocytes were used as an internal positive control.

cells positive for p27^{Kip1} and for Ki-67 was determined (fig 1); the mean p27^{Kip1} value was 47%; the mean value for Ki-67 was 11%.

p27^{Kip1} AND Ki-67 EXPRESSION IN LOW GRADE SIL

In the presence of low grade squamous intraepithelial lesion (LSIL, n = 27) the cervical epithelium showed a significant reduction (p < 0.01) in the mean expression of p27^{Kip1} (36%) compared with normal (47%) (fig 1). This was owing to the extension of the dysplastic basal and parabasal cells, which were in most instances negative for the expression of p27^{Kip1}. The staining pattern in LSIL was similar to that observed in normal squamous epithelium, with a nearly complete segregation of the p27^{Kip1} positive cells in respect to the Ki-67 immunoreactive nuclei, which was evident at the single cell level when the staining was performed in adjacent sections (fig 3). The p27^{Kip1} staining was confined to the intermediate and superficial squamous cells (fig 3A). Cells showing morphological feature of HPV infection were strongly reactive for p27^{Kip1} (fig 4). In only a few cases (n = 5) did we observe scattered basal cells showing immunoreactivity to the p27^{Kip1} anti-

body, the majority of cells in the basal layer showing no reaction. On the other hand, the parabasal cells showed strong labelling for Ki-67 (fig 3B), correlating well with the degree of dysplasia obtaining a mean score (31%) higher than normal (p < 0.0001; fig 1).

Twenty of 27 cases of LSIL were labelled after hybridisation with a cocktail of 6, 11, 16, 18, 31, 33, and 35 HPV–DNA probes. Of these 20 cases, eight (40%) contained low risk (6/11), five (25%) intermediate risk (30s), and seven (35%) high risk (16/18) HPV types. The average percentage of p27^{Kip1} positive cells did not differ significantly between the different HPV groups (6/11, 38%; 16/18, 36%; 30s, 36%).

p27^{Kip1} AND Ki-67 EXPRESSION IN HIGH GRADE SIL

In the presence of high grade squamous intraepithelial lesion (n = 19) the cervical epithelium showed a reduction (39%) in the expression of p27^{Kip1} compared with normal samples (47%); however, this reduction was less evident than that noted in LSIL and was not statistically significant (p = 0.08) (fig 1). The staining pattern was somewhat different from that seen in the majority of low grade SIL; indeed, while in LSIL the lower part of the epithelium was in most instances devoid of p27^{Kip1} expression and there was a segregation in the distribution of the p27^{Kip1} and of the Ki-67 nuclei, in high grade lesions (HSIL), conversely, the immunoreactivity to p27^{Kip1} antibody also extended to the proliferative area, positivity being demonstrated in the most undifferentiated dysplastic cells in the basal epithelium. In some cases (usually CIN II and CIN III), the immunocytochemical expression of p27^{Kip1} yielded a characteristic pattern of staining with strong labelling of the basal cells, the overlying intermediate layers showing only scattered p27^{Kip1} positive nuclei and the presence of abundant and intensely stained nuclei in the cells in the more superficial layers displaying squamous differentation (fig 5A). In other instances (usually in cases of carcinoma in situ), the staining for p27^{Kip1} was intense throughout the entire thickness of the dysplastic epithelium. As expected, Ki-67 immunoreactivity was higher (57%) than normal (p < 0.0001) and proportional to the severity of the dysplasia (fig 5B), with carcinoma in situ showing the strongest staining; in these cases, serial sections revealed overlapping between the p27^{Kip1} and the Ki-67 positive areas. Noteworthy was the absolute lack of p27^{Kip1} staining in cells containing mitotic figures, and even when all the cells in a given area of the dysplastic epithelium were heavily labelled the staining was abruptly interrupted by the presence of cell division (fig 6).

Thirteen of 19 cases of HSIL were positive after hybridisation with the cocktail of HPV–DNA probes. Of these 13 positive cases, four (31%) were positive for 30s HPV types and nine (69%) for 16–18 types (fig 9). The average percentage of p27^{Kip1} positive cells did not differ significantly between the two different HPV groups (16/18, 41%; 30s, 35%).
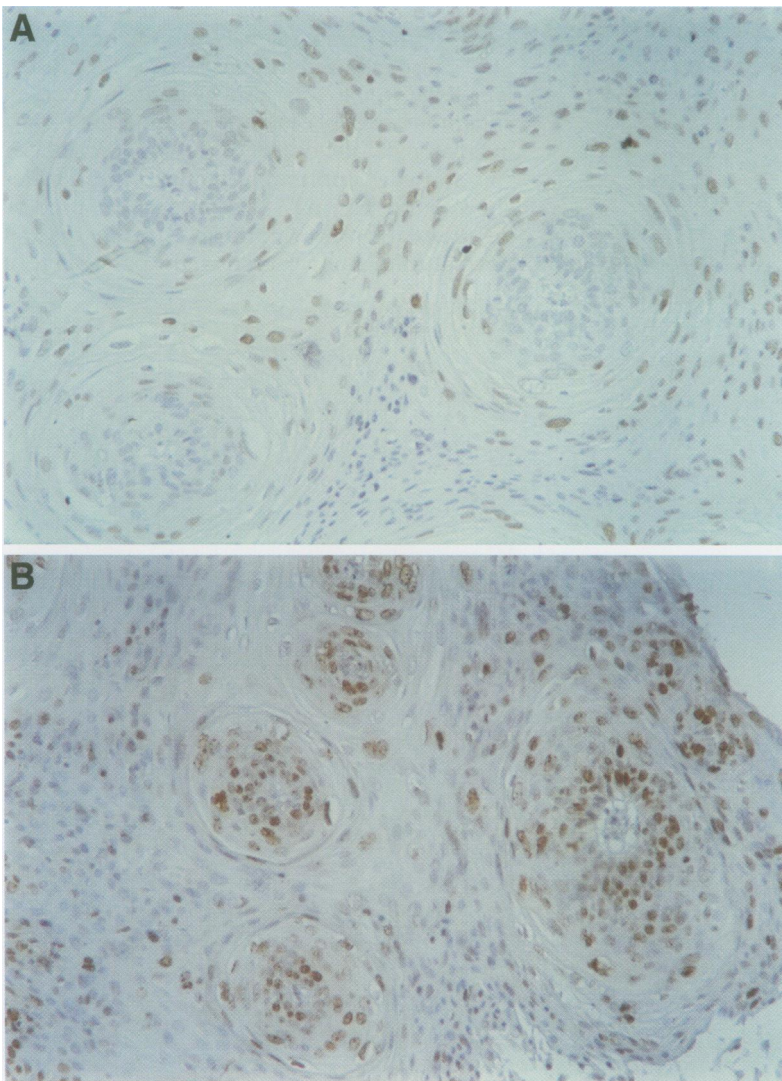

Figure 8 p27^{Kip1} (A) and Ki-67 (B) immunostaining in a case of invasive cancer showing features of squamous differentation. Note the abundant expression of p27^{Kip1} in the intermediate areas of keratinised squamous whorls, while Ki-67 is expressed by the cells of the peripheral layer.

p27^{Kip1} AND Ki-67 EXPRESSION IN INVASIVE SQUAMOUS CELL CARCINOMA

The immunocytochemical staining pattern of p27^{Kip1} in invasive cervical cancer differed from that observed in the normal epithelium and in the squamous intraepithelial lesions, in which we have generally observed a more marked expression of this protein. In invasive carcinomas there was variability in the number of positive cells, but as a group the p27^{Kip1} expression was significantly lower (23%) than that observed in normal cases (p < 0.001) and in both grades of dysplasia (p < 0.01). High nuclear p27^{Kip1} expression (> 50% of positive cells) was seen in only two cases (9%) (fig 1). In 15 of 23 cases (65%), invasive cancer showed less than 50% positive cells, while in the remaining 26% only focal staining (< 15% positive cells) was observed, the squamous neoplastic cells being nearly completely negative, in sharp contrast to the strong positivity of the infiltrating mature lymphocytes. The differ-
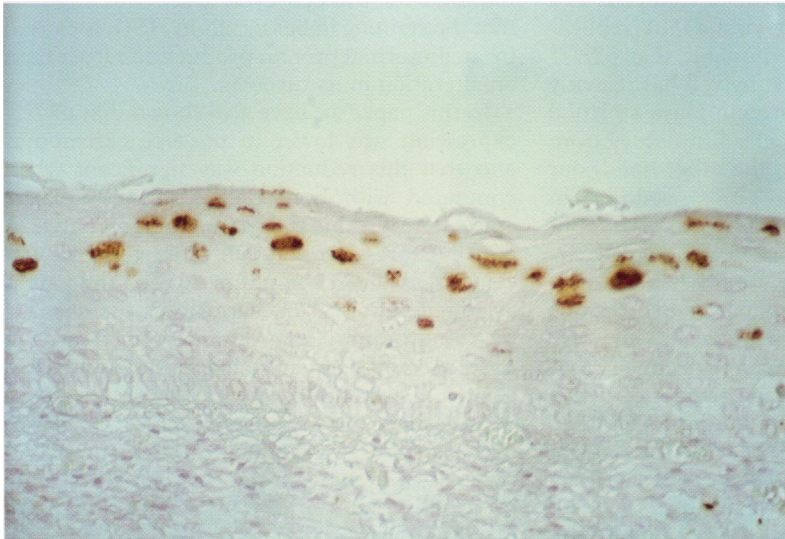

Figure 9   16/18 types HPV-DNA detection by non-isotopic in situ hybridisation in a case with high grade squamous intraepithelial lesions. Note the presence of a specific nuclear staining generated after hybridisation with 16/18 biotinylated HPV-DNA probes. The signal was developed by the DAB chromogen with a catalysed system for signal amplification.
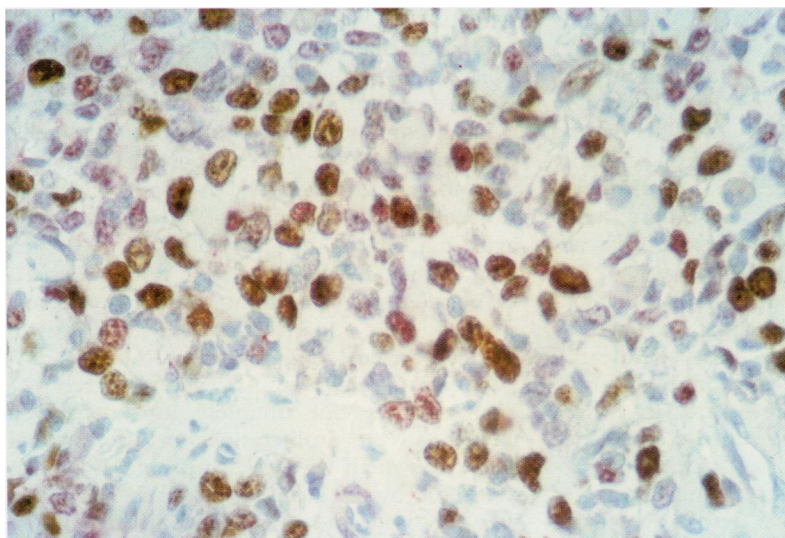

Figure 10   p27^{Kip1} and Ki-67 double immunoenzymatic staining in a case of invasive cancer. Ki-67 positive brown stained nuclei are mixed with cells showing a red nuclear signal specific for p27Kip1. The two antigens were localised in different cells and we did not observe coexpression of these two proteins in the same cells of invasive cervical carcinoma.

ent pattern of staining between intraepithelial and invasive lesions, with the loss of p27^{Kip1} expression in the latter, was evident when, in the same tissue blocks, areas of normal epithelium or SIL were located adjacent to the invasive cancer (fig 7). In the group of invasive carcinomas the mean Ki-67 score (52%) was significantly higher than normal (p < 0.0001).

Although there was a variable number of the cells expressing p27^{Kip1}, this was intensely expressed (46%) by the well differentiated neoplasms (G1) showing abundant keratin and squamous pearl formations and less intense mitotic activity (fig 8A). In these neoplasms, p27^{Kip1} was expressed in the intermediate areas of the tumour nests, and, in contrast to HSIL, the peripheral cell layer of these nests remained negative for p27^{Kip1} while showing reactivity for the Ki-67 antibody (fig 8B). Conversely, the poorly differentiated neoplasms (G3), composed of nests of small undifferentiated cells with a high Ki-67 index and minimal keratinisation, showed the lowest level (8%) of average p27^{Kip1} expression. An intermediate level (27%) of average p27^{Kip1} expression was observed in tumours showing features of moderate differentiation (G2).

To evaluate whether there was coexpression of p27^{Kip1} and Ki-67 in individual cells we performed double immunoenzymatic staining in selected cases of invasive cervical carcinoma. The double staining revealed two distinct cell populations: those that were positive for Ki-67 and those that were positive for p27^{Kip1} (fig 10). We did not observe coexpression of these two proteins in the same cells of invasive cervical carcinoma.

RELATION BETWEEN p27^{Kip1} AND Ki-67 EXPRESSION

We analysed the correlation among the p27^{Kip1} and Ki-67 scores for all diagnostic categories reported in this paper. The Spearman correlation coefficient for continuous variables did not reveal any correlation between p27^{Kip1} and Ki-67 in any of the histological groups examined.

Discussion

Alteration in the p27^{Kip1} expression has been described in a number of human cancers[11]; in this study we have investigated whether altered expression also occurred in neoplasms arising from cervical squamous epithelium. This issue was addressed by using immunohistochemistry analysis of paraffin embedded material. We investigated whether the immunocytochemical staining pattern of p27^{Kip1} in SILs and in invasive carcinomas differed from normal patterns, and we are able to report that reduced amounts of p27^{Kip1} protein are associated with developing cervical cancer.

Our results show a significant decrease in the expression of p27^{Kip1} protein in low grade SIL. As the p27^{Kip1} protein is expressed during the terminal differentiation of keratinocytes, it is conceivable that p27^{Kip1} is less abundant in abnormal cell differentiation. The relation between p27^{Kip1} and cell differentiation is evident when considering the p27^{Kip1} staining pattern in normal and in LSIL samples. In

normal specimens the p27^{Kip1} expression appeared in the intermediate cells at the onset of squamous differentiation; similarly, in LSIL only, the differentiated upper half of the squamous epithelium showed p27^{Kip1} labelling, with only sporadic positive basal cells. Therefore in low grade cervical dysplastic lesions, the expansion of the undifferentiated basal and parabasal dysplastic cells and the smaller number of differentiated squamous cells could well explain our finding of reduced p27^{Kip1} expression.

The presence of the p27^{Kip1} protein in the upper part of the mucosa was also observed in the LSIL cases showing prominent HPV cytopathic effect (koilocytosis). In these cases, in which the presence of HPV–DNA was demonstrated by in situ hybridisation, the koilocytes were strongly reactive for p27^{Kip1}. Because the G1 block is released in these cells in order to ensure viral replication, the presence of p27^{Kip1} is intriguing. It was previously shown that p21^{WAF1} is expressed in HPV infected cells as a host response to prevent viral DNA replication[5]; therefore it may be possible that p27^{Kip1} has similar activity. Interestingly, it has recently been proposed that the protein plays a pivotal role in the protection of cells and tissues from inflammatory injury.[25] On the other hand, our demonstration of strong p27^{Kip1} staining in HPV infected cells may suggest that viral replication occurs despite the presence of high levels of p27^{Kip1}. In our study, we observed a similar level of p27^{Kip1} expression in SIL lesions associated with low, intermediate, and high risk types. These observations concur with a recent study by Zehbe et al,[26] which provides evidence for the overriding of CDK inhibitors by high and low risk human papillomavirus types.

The relation between p27^{Kip1} and cell differentiation was less clear when considering the p27^{Kip1} staining pattern in cases of high grade SIL. In this group the reduction of p27^{Kip1} expression also occurred but was less evident, as the staining pattern was somewhat different from that seen in normal samples and in the majority of cases of low grade SIL. In HSIL we observed immunoreactivity to p27^{Kip1} antibody in superficial cells showing squamous differentiation and also in the most undifferentiated dysplastic cells at the base of the epithelium, in which p27^{Kip1} normally is absent. This finding concurs with that observed in dysplastic colonic mucosa, in which p27^{Kip1} protein— normally expressed in superficial differentiated cells—appears at the bottom of crypts adjacent to areas of invasive cancer.[12] Moreover, p27^{Kip1} is strongly expressed through the entire thickness of the epithelium in areas of Barrett's associated esophageal in situ adenocarcinoma.[27] On the other hand, in high grade squamous dysplasia of the oral cavity, where loss of p27^{Kip1} protein also occurs, the presence of p27^{Kip1} basal staining was not reported.[22] Concordant with our results, the occurrence of p27^{Kip1} staining in the basal dysplastic cells in 11 of 17 cases of cervical HSIL was shown by Zehbe et al, whereas such localisation was rarely observed in five cases of LSIL examined.[26] In our study, in which we corre-

lated the presence of HPV–DNA to the p27^{Kip1} staining pattern, we did not consistently observe basal staining in LSIL lesions associated with 16/18 HPV. We therefore conclude that the p27^{Kip1} staining pattern observed in HSIL cannot be explained by the increased prevalence of high risk HPV types. Our results show that in severe epithelial dysplasia, p27^{Kip1} protein is not only reduced but also has a different intraepithelial distribution, and the appearance of staining in the basal cell layer may suggest that in most advanced cases of intraepithelial proliferation, p27^{Kip1} protein might be expressed in order to control further proliferation. The abnormal p27^{Kip1} staining pattern of the dysplastic epithelium may therefore reflect changes in the growth control of precancerous cells. It should be noted that the cells showing mitotic figures were completely devoid of p27^{Kip1} staining. This finding is consistent with in vitro results which have consistently shown that p27^{Kip1} degradation is required for the cell cycle exit,[9] and suggests that the staining in basal cells in HSIL is caused by an abnormal protein production rather than an alteration in its turnover.

In this paper we have also shown that p27^{Kip1} expression was lower in invasive carcinomas and that this reduction was significant when compared with both normal and dysplastic epithelium. In this group, the relation between p27^{Kip1} expression and cell differentiation was evident, as the G3 tumours showed the lowest average level of p27^{Kip1} expression. Conversely, p27^{Kip1} was more intensely expressed in well differentiated neoplasms in areas of keratinisation and squamous pearl formation. The decrease in p27^{Kip1} expression in cervical carcinomas concurs with similar findings in several types of epithelial and non-epithelial tumours, in which there is loss of p27^{Kip1}, a feature usually shown by more advanced as well as by less differentiated neoplasms.[28]

The lower levels of p27^{Kip1} in invasive cervical carcinomas compared with normal epithelium might be explained simply as a direct consequence of the increased cell proliferation, thus reflecting the fact that the time spent by the neoplastic population in the early part of the cell cycle is reduced. It is noteworthy that in invasive cervical carcinoma the p27^{Kip1} protein is also consistently absent in mitotic cells. Interestingly, Zehbe et al showed by double immunofluorescence that in invasive cervical cancer the coexpression of the positive cell cycle marker cyclin A and p27^{Kip1} is observed only in a small fraction (< 2%) of the neoplastic population.[26] We did not observe coexpression of p27^{Kip1} and Ki-67 in individual cells of invasive cervical carcinoma by double immunoenzymatic technique. However, altered p27 expression is not uniquely attributable to increased cell proliferation: in the present study, in which the cellular proliferation was measured by the Ki-67 indicator in each case, we did not find a significant correlation between the reduced p27^{Kip1} expression levels and the proliferative status. Therefore the decrease in p27^{Kip1} observed in this study has to be considered a primary event, which may also

explain the different staining pattern of p27^{Kip1} compared with the closely related p21^{WAF1} protein, overexpression of which has been shown previously.[3] An inconsistent relation between p27^{Kip1} expression and the percentage of cells in the S phase has also been noted in other studies in breast cancer,[13] in colorectal tumours,[12] and in the neoplastic lesions arising from the squamous oral epithelium.[22] These concurring data add further importance to studies aimed at unravelling the molecular mechanisms responsible for altered p27^{Kip1} in cervical cancer. As the p27^{Kip1} cellular levels are regulated by both translation and ubiquitin mediated degradation,[29 30] the decrease in p27^{Kip1} observed in this study may be the result of abnormal mRNA or protein regulation. In breast,[13] colon,[12] and lung cancer,[31] there is evidence to suggest that the loss of p27^{Kip1} protein expression is caused by the enhanced protein degradation by the ubiquitin–proteasome pathway. More recently still, it has been shown that methylation may be another mechanism regulating p27^{Kip1} expression.[32] Preliminary data derived from the analysis of p27^{Kip1} mRNA levels by in situ hybridisation in cervical epithelium seem to suggest that the loss of p27^{Kip1} expression occurs at a post-transcriptional level (Troncone G, personal communication).

CONCLUSIONS

In conclusion, in this study we have reported the following observations: (1) there is a reduction in p27^{Kip1} protein levels in SIL lesions and invasive cancer compared with normal epithelium; (2) in HSIL the p27^{Kip1} protein has a different intraepithelial distribution; and (3) the decreased p27^{Kip1} expression is not related significantly to the Ki-67 proliferative activity. More must be learned about the mechanisms and the significance of p27^{Kip1} reduction in invasive cervical cancer, as our observations suggest that changes in p27^{Kip1} protein contribute to the altered kinetics of the dysplastic and neoplastic cervical epithelium.

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