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ARTICLE *in* CANCER · DECEMBER 2001

Impact Factor: 4.89 · DOI: 10.1002/1097-0142(20011215)92:12<3005::AID-CNCR10153>3.0.CO;2-6

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Expression of the Cell Cycle Regulator $p27^{Kip1}$ in Normal Squamous Epithelium, Cervical Intraepithelial Neoplasia, and Invasive Squamous Cell Carcinoma of the Uterine Cervix

Immunohistochemistry and Functional Aspects of $p27^{Kip1}$

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Supported in part by Grants-in-Aid for Scientific Research (06454468 and 07807154) from the Ministry of Education and Culture of Japan.

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Received February 1, 2001; revision received August 17, 2001; accepted August 31, 2001.

DOI 10.1002/cncr.10153

BACKGROUND. Abnormality of cell cycle regulators and tumor suppressors, such as cyclin dependent kinase inhibitors (cdkIs), has been reported in malignant tumors. The current study was undertaken to examine the involvement of a cdkI, $p27^{Kip1}$ (p27), in the neoplastic process of the uterine cervical epithelium.

METHODS. Immunohistochemical staining of p27 was performed in samples of normal cervical tissue (30 samples), cervical intraepithelial neoplasias (CINs; 17 samples), and invasive squamous cell carcinoma (SCC; 25 samples). The results were compared with the expression levels of Ki-67, cdk2, and cyclin E. The functional aspects of the p27 protein, such as its ability to bind to cdk2 and the phosphorylation activity of p27-bound cdk2, also were evaluated with an immunoprecipitation and histone H1 kinase assay.

RESULTS. In normal cervical epithelia, the expression of p27 was strong in the intermediate and superficial cells but very weak in the parabasal cells. In CIN samples, the expression of p27 was negligible. The expression of p27 in these tissues showed an inverse topologic correlation to that of Ki-67, cdk2, and cyclin E. However, it is noteworthy that the number of p27 positive cells increased in SCC samples that also showed increased expression of Ki-67, cdk2, and cyclin E. The p27 protein in SCC samples was bound to cdk2 and cyclin E. However, cdk2 that was bound to p27 still possessed histone H1 kinase activity.

CONCLUSIONS. The expression of p27 may be involved in the growth regulation of the normal squamous epithelium in the uterine cervix. However, aberrant function of p27 expression may occur in invasive SCC of the cervix. ***Cancer* 2001;92: 3005–11.** *© 2001 American Cancer Society.*

KEYWORDS: cervical carcinoma, $p27^{Kip1}$, Ki-67, cyclin dependent kinase, histone H1.

It has been shown recently that cell growth is controlled tightly by interactions of cyclins, cyclin dependent kinases (cdks), and cdk inhibitors (cdkIs).[1,2] The cyclin/cdk complexes stimulate cell cycle progression, and the cdkIs induce cell cycle arrest by counteracting cdks.[1,2] Deranged expression of these cell cycle-related molecules, such as overexpression of cyclins and cdks, is linked closely to the malignant transformation of the cell.[3] Loss of function of tumor suppressor gene products, such as pRB[4] and p53,[5] due to gene mutations or deletions is the most common genetic abnormality observed in human malignancies. We previously reported the expression of cyclins/cdks and p53 in normal and neoplastic tissues of the

uterine cervix,[6] although little is known concerning the negative regulators of the cell cycle. In the current study, to clarify further the growth-regulatory mechanism of uterine cervical tissues, we focused on the expression of $p27^{Kip1}$ (p27), which is a negative regulator of the G1-phase of the cell cycle and is regarded as a tumor suppressor: 1) p27 associates mainly with the cyclin E/cdk2 complex and inhibits pRB phosphorylation through the cyclin E/cdk2 complex,[7] 2) overexpression of p27 blocks the cell from entering the S-phase,[7,8] and 3) mice lacking p27 exhibit multiorgan hyperplasia.[9–11] In this study, to clarify the role of p27 in the malignant transformation of the cervical epithelium, we examined the expression of p27 in normal and neoplastic squamous epithelia of the uterine cervix by immunohistochemistry and Western blot analysis, and the immunolocalization of p27 was compared with that of Ki-67, cyclin E, and cdk2. In addition, to analyze further the functional aspects of p27 in normal and neoplastic cervical tissues, the complex formation between p27 and cyclin E/cdk2 was examined by immunoprecipitation. The phosphorylation activity of the cdk2/cyclin E complex that bound to p27 also was examined with a histone H1 kinase assay.

MATERIALS AND METHODS

Immunohistochemistry

Histologic materials

Formalin fixed, paraffin embedded sections of normal and neoplastic cervical tissues were obtained from 72 women who underwent biopsy or hysterectomy at Shinshu University Hospital. Serial sections of 3 μm thickness were prepared and used for hematoxylin and eosin staining and immunostaining. Among the 72 cervical tissue samples, there were 30 samples of normal tissue (15 samples were obtained in the proliferative phase, and 15 samples were obtained in the secretory phase), 17 samples of cervical intraepithelial neoplasia (CIN; Grade 1, 3 samples; Grade 2, 7 samples; Grade 3, 7 samples), and 25 samples of squamous cell carcinoma (SCC; 18 samples were of the nonkeratinizing type, and 7 samples were of the keratinizing type). Sections obtained from breast carcinoma tissue were used for positive tissue control.[12] Each tissue was used with the approval of the Ethical Committee of Shinshu University after obtaining written consent from the patients.

Staining procedures

Indirect immunostaining was performed using specific antibodies against p27, cyclin E, cdk2, and Ki-67. Antibodies for p27 (C-19), cyclin E (HE11), and cdk2 (M2) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-Ki-67 antibody (MIB-1) was obtained from Immunotech (Marseille, France). Each immunohistochemical staining was performed by the avidin-biotin-peroxidase complex method using a Histofine SAB-PO detector kit (Nichirei, Tokyo, Japan). Briefly, after routine deparaffinization and rehydration, sections were treated with microwaves in a 0.01 M citrate buffer, pH 6.0, for 15 minutes. After blocking of endogenous peroxidase activity, the sections were then incubated with specific primary antibodies (diluted 1:100 with phosphate-buffered saline [PBS] and bovine serum albumin) or control nonimmunized mouse or rabbit serum at 4 °C overnight. After washing with PBS, biotinylated antimouse or antirabbit immunoglobulin G (IgG) was applied for 30 minutes at room temperature. For negative control of the secondary antibody, biotinylated antirabbit or antimouse IgG was applied for mouse or rabbit primary antibodies, respectively. After washing with PBS, peroxidase-conjugated streptoavidin solution was applied for 30 minutes and visualized by 0.05% 3′,3′-diaminobenzidine. Counterstaining was performed lightly with hematoxylin.

Interpretation of immunohistochemical staining

The specific staining of each antibody was identified in the nucleus and/or cytoplasm. All control slides yielded negative staining. Each stain was evaluated by the percentage of positive cells and was described as follows: negative, no cells were stained; sporadically positive, $< 1\%$ of cells were positive; focally positive, 1–5% of cells were positive; regionally positive, 5–50% of cells were positive; and diffusely positive, $> 50\%$ of cells were positive. For the statistical analysis, the positivity of each staining also was described as a positivity index (PI), which indicated the percentage of positive cells in 1) 200 arbitrarily selected cells from 3 high-power fields in sections of normal epithelium and CINs and 2) 1000 arbitrarily selected cells from 10 high-power fields in sections of invasive carcinoma. The PI of the normal epithelia was obtained from parabasal cells, because they are the most active replicating cells in the normal epithelia. Statistical analysis for the PI comparison between normal and neoplastic tissues was done with the Mann–Whitney test. A tied P value of < 0.05 was considered significant.

Western Blot Analysis

To confirm the specificity of the antibodies used for immunostaining, Western blot analysis was performed as described previously.[13] In brief, six tissue specimens (two specimens of normal ectocervical epithelia from which intervening stroma and inflammatory cells were scraped with a scalpel under stereomi-

croscopy, one specimen of Grade 3 CIN, and three specimens of invasive SCC) were homogenized and lysed in 0.5 mL of a cell lysis buffer consisting of 50 mM Tris-HCl, pH 8.0; 0.25 M NaCl; 0.5% NP-40; 1 mM phenyl methyl sulfonyl fluoride (PMSF; Sigma Chemical Co., St. Louis, MO); 1 μg/mL aprotinin (Boehringer-Mannheim, Indianapolis, IN); 1 μg/mL leupeptin (Boehringer-Mannheim); and 20 μg/mL *N*-tosyl-L-phenylalanie chloromethyl ketone (TPCK) (Boehringer-Mannheim). The lysates were centrifuged at ×13,000 *g* for 20 minutes at 4 °C, and the supernatants were stored at −70 °C. Extracts equivalent to 50 μg of total protein were separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels (10% acrylamide). The proteins were then transferred to supported nitrocellulose membranes (Amersham, Buckinghamshire, England) by applying 20 volts for 180 minutes with a plate electrode apparatus (Semi Dry Blotter II; Ken En Tec, Copenhagen, Denmark). The filters were blocked for 1 hour in Tris-buffered saline/Tween-20 (TBST: 0.2 M NaCl, 0.2% Tween-20, and 10 mM Tris, pH 7.4), containing 5% nonfat dry milk and 0.02% NaN_3. Subsequently, the filters were incubated with antibodies against p27 (Santa Cruz Biotechnology) and β-actin (for the internal standard; AC-15; Biomakor, Rehovot, Israel; each diluted 1:500) in TBST containing 5% milk, and then incubated in antirabbit IgG (1:1000 dilution; Amersham) in TBST containing 2% milk. The filters were washed several times with TBST after each step. The bound antibodies were detected with an enhanced chemiluminescence system (Amersham).

Immunoprecipitation

Complex formation between p27 and cyclin E/cdk2 was examined using immunoprecipitation. The lysates obtained from the six cervical tissue sections used for Western blot analysis and from a cervical carcinoma cell line, HeLa cells (purchased from American Type Culture Collection, Rockville, MD), in which the binding of p27 with cdk2 has been reported[14] for the positive control, were subjected to immunoprecipitation. Briefly, 50 μg of the lysates were immunoprecipitated with 2 μL of the anti-p27 antibody (Santa Cruz Biotechnology) for 60 minutes at 4 °C. The p27 precipitates were collected for 1 hour on protein G-Sepharose beads (Boehringer-Mannheim). After washing with a lysis buffer, precipitates were resuspended in a Laemmli SDS sample buffer and resolved with SDS-polyacrylamide gel electrophoresis (PAGE). The immunoprecipitated protein complexes were resolved and probed for immunoblots to detect associated proteins using antibodies against cdk2 and cyclin E (Santa Cruz Biotechnology).

Histone H1 Kinase Assay

Because the nuclear protein histone H1 is a substrate of phosphorylation by the cyclin E/cdk2 complex, the kinase activity of the complex can be evaluated by the detection of radiolabeled histone H1 protein (histone H1 kinase assay). In the current study, to measure the phosphorylation activity of p27-associated cdk2, histone H1 kinase assay was performed. The 50 μg of lysates obtained from the six tissues used for Western blot analysis were immunoprecipitated with the anti-p27 antibody (Santa Cruz Biotechnology). After washing the precipitate with a kinase lysis buffer (150 mM NaCl; 50 mM Tris, pH 7.6; 0.1% NP-40; 1 mM PMSF; 10 μg/mL leupeptin; and 2 μg/mL aprotinin) and a reaction buffer (22 μM ATP; 7.5 μM $MgCl_2$; and 20 mM Tris, pH 7.5), 0.25 μg of histone H1 (Boehringer-Mannheim), and 10 μCi of ^{32}P-rATP were added to the reaction buffer for 30 minutes at 37 °C. Reaction products were resolved with SDS-PAGE, and the autoradiographic bands in the gels were analyzed with a Mac-BAS system (Fuji Film, Tokyo, Japan).

RESULTS

Immunohistochemistry

Normal cervical squamous epithelium

The expression of p27 in normal cervical epithelium was observed in the cytoplasm and the nucleus. Both cytoplasmic expression and nuclear expression of p27 were weak in basal and parabasal cells (PI: cytoplasm, 4.5 ± 2.1; nucleus, 3.9 ± 3.2; mean ± standard deviation) but increased in cells of the intermediate and superficial layers (Fig. 1a, Tables 1 and 2). There was no apparent change in p27 expression during the menstrual cycle. The expression of Ki-67 was observed mainly in the parabasal cells. The intermediate and superficial cells were almost negative for Ki-67 (Fig. 1b, Table 1). Expression of cdk2 and cyclin E was also observed in parabasal cells (Fig. 1c,d, Table 1). The distribution pattern of p27 positive cells in normal squamous epithelium showed an inverse correlation with the distribution patterns of Ki-67, cyclin E, and cdk2.

CIN

In CINs, the cytoplasmic and/or nuclear expression of p27 was slightly weaker than in normal parabasal cells (PI: cytoplasm, 3.8 ± 1.6; nucleus, 6.0 ± 5.8) (Fig. 2a, Table 2). In contrast, the PI of Ki-67, cyclin E, and cdk2 increased in CINs compared with normal squamous epithelium (Fig. 2b–d, Table 2). The low labeling of p27 also was correlated inversely with that of Ki-67, cyclin E, and cdk2 in CINs. In addition, an inverse topologic correlation between the expression of these

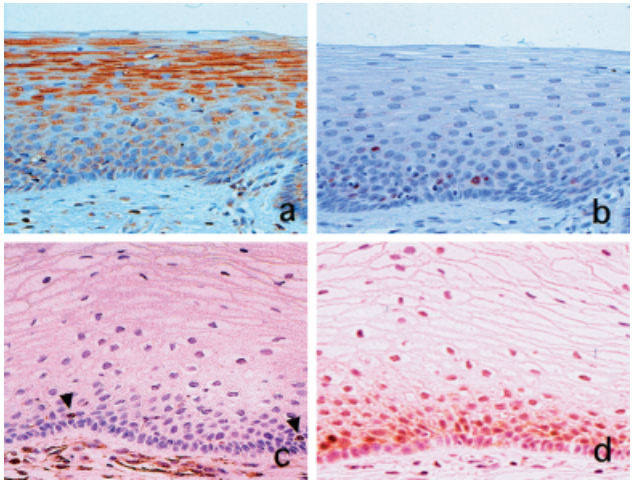

FIGURE 1. The expression of $p27^{Kip1}$ (p27) (a), Ki-67 (b), cyclin E (c), and cyclin dependent kinase 2 (cdk2) (d) in normal squamous epithelium (a,b; serial sections). Cells that were positive for p27 were seen in the intermediate and superficial layer. Ki-67 positive, cyclin E positive (arrowhead), and cdk2 positive cells were noted mainly in the parabasal layer (original magnification, ×175).

molecules also was observed; p27 was localized only in superficial cells of the CIN that were negative for Ki-67, cyclin E, and cdc2. Neoplastic cells in the basal and intermediate layers were negative for p27 but positive for Ki-67, cyclin E, and cdc2.

Invasive SCC

In SCC, all seven specimens showed positive staining for p27, and the p27 positive cells were intermingled with p27 negative cells (Fig. 3a). The expression of p27 increased (PI: cytoplasm, 28.1 ± 12.7; nucleus, 37.2 ± 11.8; Table 2), with a significant difference compared with the normal epithelium (cytoplasm, $P = 0.004$; nucleus, $P = 0.001$). There was no apparent correlation between p27 expression and histologic subtypes. The PI of Ki-67, cyclin E, and cdk2 also increased compared with the PI in normal epithelium (Fig. 3b–d, Table 2).

Western Blot Analysis

All tissue samples showed a single band of with a molecular weight of 27 kDa. Two samples of normal ectocervical epithelia showed a weak band (Fig. 4, lanes 1 and 2). A sample obtained from a patient with Grade 3 CIN showed a very weak band (Fig. 4, lane 3). All three SCC samples (Fig. 4, lanes 4–6) showed strong bands, with the strongest band observed in lane 4 of Figure 4. These results were consistent with those obtained by immunostaining.

Immunoprecipitation

When the lysates were immunoprecipitated with the anti-p27 antibody and the precipitates were examined with an anti-cdk2 antibody, the bands were observed weakly in tissues from the normal epithelia and Grade 3 CIN samples but appeared strongly in the three SCC samples (Fig. 4). When the lysates were immunoprecipitated with the anti-p27 antibody and examined with an anticyclin E antibody, the bands were observed weakly in the SCC samples (Fig. 4).

Histone H1 Kinase Assay

The lysates were immunoprecipitated with the anti-p27 antibody, and the p27-bound protein obtained from the three SCC tissues showed histone H1 kinase activity (Fig. 4). In contrast, no kinase activity was observed in tissue samples from the normal epithelium and Grade 3 CIN. These results seemed parallel the level of p27-bound cdk2 noted with immunoprecipitation.

DISCUSSION

The current study revealed that the expression of p27 in normal squamous epithelium of the uterine cervix is observed mainly in the cells of the intermediate and superficial layers. In contrast, the expression of p27 was weak in the parabasal cells, which replicate most actively.[15] This is consistent with a previous study by Troncone et al.,[16] and a similar staining pattern was also reported in the squamous epithelia of normal tonsils.[17] P27 has been identified in large amounts in quiescent cells, like those in the middle and apical regions of the intestinal villi, whereas p27 positive cells were rare in the proliferating basal region of the villi.[11] In the human endometrium, we previously reported that the expression of p27 was negligible in the glandular cells of the proliferative phase but increased markedly in cells during the secretory phase.[13] Thus, the expression and localization of p27 in the normal cervical squamous epithelium seems to represent the fundamental characteristics of p27 as a tumor suppressor gene product. Concerning the subcellular localization of p27, the immunoreactivity of p27 was observed both in the cytoplasm and in the nucleus. Although one study reported that the immunoreactivity of p27 was exclusively nuclear,[11] another study reported both nuclear and cytoplasmic staining.[18]

In the normal squamous epithelia, p27 positive cells were observed in the superficial layer, which is known as a terminal differentiation (maturation) site.[15] Recent studies have suggested that cell differentiation is achieved during cell growth arrest, which is mediated by cdk inhibitors, such as p27 and

TABLE 1
Results of Immunostaining of the Normal Squamous Epithelium of the Uterine Cervix

Immunostain	Site stained	Cell layer							
		Basal		Parabasal		Intermediate		Superficial	
		Pro.	Sec.	Pro.	Sec.	Pro.	Sec.	Pro.	Sec.
p27	C	+	+	+	+	+++	+++	+++	+++
p27	N	+–++	+–++	+	+	++	++	++	++
Ki-67	N	−/+	−/+	+–++	+–++	−/+	−/+	−	−
cyclin E	N	−	−	+	+	−	−	−	−
cdk2	N	−/+	−/+	+–++	+–++	−	−	−	−

Pro.: proliferative phase; Sec.: secretory phase; C: cytoplasm; N: nucleus; p27: p27[Kip1] cyclin dependent kinase 1; cdk2: cyclin dependent kinase 2; −: negative; −/+: sporadically (< 1%) positive; +: focally (1–5%) positive; ++: regionally (5–50%) positive; +++: diffusely (> 50%) positive.

TABLE 2
Positive Index for Each Staining in Normal and Neoplastic Squamous Epithelia

Immunostain	Site stained	Normal epithelium	CIN 1–3	Invasive carcinoma
p27	C	4.5 ± 2.1 (pc)	3.8 ± 1.6	28.1 ± 12.7[a]
p27	N	3.9 ± 3.2 (pc)	6.0 ± 5.8	37.2 ± 11.8[a]
Ki-67	N	15.3 ± 4.3 (pc)	36.1 ± 8.8[a]	51.9 ± 14.1[a]
cyclin E	N	0.5 ± 0.3 (pc)	3.6 ± 5.9	12.5 ± 11.8[a]
cdk2	N	0.9 ± 0.2 (pc)	60.3 ± 24.2[a]	62.1 ± 17.3[a]

CIN 1–3: Grade 1–3 cervical intraepithelial neoplasia; p27: p27[Kip1] cyclin dependent kinase 1; C: cytoplasm; N: nucleus; pc: parabasal cells; cdk2: cyclin dependent kinase 2.
[a] Significantly different from normal epithelia.

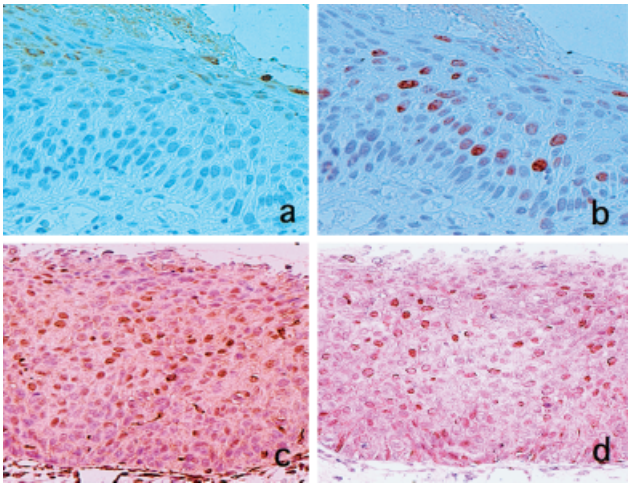

FIGURE 2. The expression of p27[Kip1] (p27) (a), Ki-67 (b) cyclin E (c), and cyclin dependent kinase 2 (cdk2) (d) in Grade 3 cervical intraepithelial neoplasia (a,b; serial sections). The expression of p27 in atypical cells was negligible, and only several cells located at the surface showed positive staining. Conversely, positive staining for Ki-67, cyclin E, and cdk2 were noted in atypical cells (original magnification, ×175).

p21[WAF-1/CIP-1] (p21).[19,20] Growth inhibition of tumor cells by differentiation-inducing agents, such as vitamin D, is associated with the increased expression of p21 and p27.[21,22] We previously reported that the expression of p27 was enhanced in endometrial hyperplasia by the administration of medroxyprogesterone acetate, which is a potent growth inhibitor and induces differentiation in the endometrial glands.[13] Growth arrest and differentiation of epidermal keratinocytes was associated with increased expression of p27.[23] Thus, it is likely that p27 is capable of changing the cell function from proliferation to differentiation, and the current findings in the cervical epithelia may be an additional example of such a switching function for p27.

In CINs, which are preinvasive lesions of the uterine cervix, the expression of p27 was negligible in atypical cells, in which cell growth was accelerated, as shown by the increased expression of Ki-67, cyclin E, and cdk2. Only cells in the superficial layer were positive for p27 expression and negative for Ki-67, cyclin E, and cdk2 expression. Therefore, the topologic correlations observed between p27 and Ki-67, cyclin E, and cdk2 observed in the normal epithelia also were noted in the CINs. These findings suggest that the expected function of p27 as a tumor suppressor seems to be preserved in CINs and that a decrease in p27 expression may contribute to the early stage of the malignant transformation of the cervical epithelia.

It is noteworthy, however, that the number of p27 positive cells increased significantly in SCC cells, which have more aggressive growth potential than normal and dysplastic cells. Such a paradoxic finding also was reported in a study by Dellas et al.,[24] although other two studies reported that the number of p27 positive cells decreased in SCC compared with normal epithelium.[16,25] The latter two studies measured the number of positive cells of all cell layers together, and

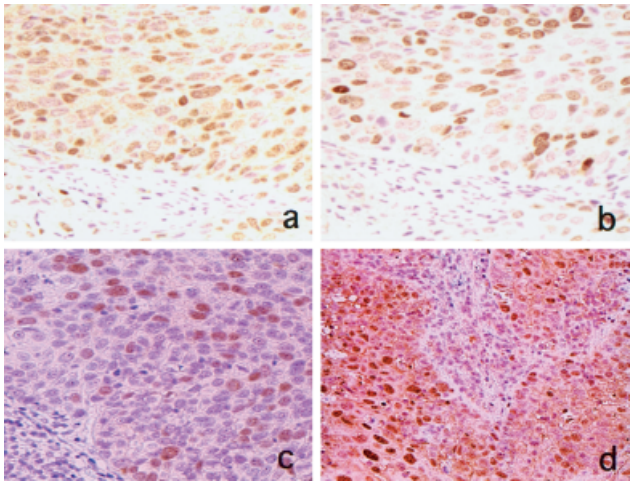

FIGURE 3. The expression of $p27^{Kip1}$ (p27) (a), Ki-67 (b) cyclin E (c), and cyclin dependent kinase 2 (cdk2) (d) in invasive squamous cell carcinoma (a,b; serial sections). The number of p27 positive cells as well as Ki-67 positive, cyclin E positive, and cdk2 positive cells increased (original magnification, ×220).

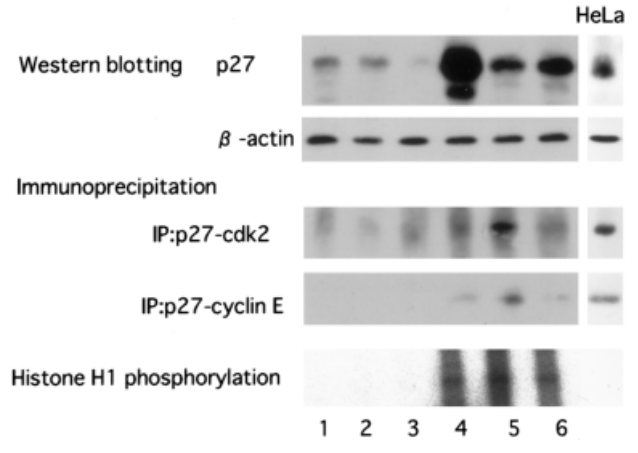

FIGURE 4. Results of Western blot analysis, immunoprecipitation (IP), and a histone H1 kinase assay. Lanes 1 and 2, samples obtained from normal squamous epithelium; lane 3, samples of Grade 2 cervical intraepithelial neoplasia; lanes 4–6, invasive squamous cell carcinoma. HeLa cells were used for the positive control of immunoprecipitation and Western blotting. β-actin was used as an internal control (p27, $p27^{Kip1}$; cdk2, cyclin dependent kinase 2).

the discrepancy may have been due to the different methods of positive cell counting. In other tissues, such as the pituitary and the thyroid, p27 expression reportedly decreased in neoplastic lesions compared with its expression in normal tissues.[17,26] In hepatocellular carcinoma[17] and prostatic carcinoma,[27], however, neoplastic cells frequently overexpressed the p27 protein compared with their normal counterparts. Highly proliferative breast carcinoma cells also reportedly expressed high levels of the p27 protein.[28] The reason for p27 accumulation in malignant tumor cells is largely unknown. In this study, we tried to address this issue by examining the functional aspects of the p27 protein. The results of immunoprecipitation studies using SCC tissues indicated that the p27 molecules were capable of binding to cdk2. The detection of cyclin E when the lysate was immunoprecipitated with p27 suggested that cyclin E also bound to the p27 complex. Increased Ki-67 labeling in SCC may have been due to the effect of cyclin E/cdk2 complexes, which are not bound to p27. In the current study, however, a histone H1 kinase assay revealed that the p27-bound protein, which contained at least the cyclin E/cdk2 complex, still had phosphorylation activity. Therefore, a speculative explanation for this result may be insufficient p27 function. It has been shown that the phosphorylation of threonine-187 of the p27 protein by the cyclin E/cdk2 complex induces p27 protein degradation.[29,30] In this regard, even if the antigenic epitope of the immunoreactive p27 is preserved in SCC, the growth-suppressive function of p27 already may be impaired by the threonine phosphorylation. In addition, it is possible that the ubiquitin/proteosomal pathway[18,31] is up-regulated in SCC to degrade the higher levels of p27. Another alternative possibility is that other cdk families, such as cdk4, which can bind to p27,[2] sustain the kinase activity, because Fredersdorf et al.[28] suggested that the overexpression of growth-stimulative factors, such as cyclin D/cdk4 complexes, were capable of overcoming the growth-suppressive effect of p27.

In summary, p27 seemed to be involved in the regulation of growth and differentiation in the normal squamous epithelia of the uterine cervix. The apparently normal function of p27 may be preserved in preinvasive CIN lesions. However, invasive SCC samples showed paradoxic overexpression of p27, which may represent aberrant regulation and/or function of the p27 protein.

REFERENCES

1. Nurse P. Ordering S phase and M phase in the cell cycle. *Cell* 1994;79:547–50.
2. Sherr CJ, Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev* 1995;9:1149–63.
3. Hartwell LH, Kasten MB. Cell cycle control and cancer. *Science* 1994;266:1821–8.
4. Goodrich DW, Lee WH. Molecular characterization of the retinoblastoma susceptibility gene. *Biochem Biophys Acta* 1993;1155:43–61.
5. Vogelstein B, Kinzler KW. p53 function and dysfunction. *Cell* 1992;70:523–6.

6. Kanai M, Shiozawa T, Xin L, Nikaido T, Fujii S. Immunohistochemical detection of sex steroid receptors, cyclins and cyclin-dependent kinases in the normal and neoplastic squamous epithelia of the human uterine cervix. *Cancer* 1998;82:1709–19.
7. Polyak K, Lee MH, Erdjument-Bromage H, Koff A, Roberts JM, Tempst P, et al. Cloning of p27^{Kip1}, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell* 1994;78:59–66.
8. Toyoshima H, Hunter T. p27, a novel inhibitor of G1 cyclin-cdk protein kinase activity, is related to p21. *Cell* 1994;78: 67–74.
9. Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, et al. Mice lacking p27^{Kip1} display increased body size, multiorgan hyperplasia, retical dysplasia, and pituitary tumors. *Cell* 1996;85:707–20.
10. Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, et al. Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27^{Kip1}. *Cell* 1996;85:721–32.
11. Fero ML, Rivkin M, Tasch M, Porter P, Carow CE, Firpo E, et al. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27^{Kip1}-deficient mice. *Cell* 1996;85:733–44.
12. Porter PL, Malone KE, Heagerty PJ, Alexander GM, Gatti LA, Firpo ED, et al. Expression of cell-cycle regulators p27^{Kip1} and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. *Nat Med* 1997;3: 222–5.
13. Shiozawa T, Nikaido T, Nakayama K, Lu X, Fujii S. Involvement of the cyclin-dependent kinase inhibitor p27^{Kip1} in the growth inhibition of endometrium in the secretory phase and in hyperplastic endometrium treated with progesterone. *Mol Hum Reprod* 1998;4:899–905.
14. Kwon TK, Nordin AA. Overexpression of cyclin E and cyclin-dependent kinase inhibitor (p27^{Kip1}): effect on cell cycle regulation in HeLa cells. *Biochem Biophys Res Commun* 1997;238:534–8.
15. Ferenczy A, Wright TC. Anatomy and histology of the cervix. In: Kurman RJ, editor. Blaustein's pathology of the female genital tract. 4th ed. New York: Springer-Verlag, 1994:185–90.
16. Troncone G, Vetrani A, de Rosa G, Gerbasio D, Palombini L. Cyclin dependent kinase inhibitor p27^{Kip1} expression in normal and neoplastic cervical epithelium. *J Clin Pathol* 1999; 52:880–7.
17. Lloyd RV, Jin L, Qian X, Kulig E. Aberrant p27^{Kip1} expression in endocrine and other tumors. *Am J Pathol* 1997;150:401–7.
18. Loda M, Cukor B, Tam SW, Lavin P, Fiorentino M, Draetta GF, et al. Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. *Nat Med* 1997;3:231–4.
19. Halevy O, Novitch BG, Spicer DB, Skapek SX, Rhee J, Hannon GJ, et al. Correlation of terminal cell cycle arrest of skeletal muscle with induction of p21 by Myo D. *Science* 1995;267:1018–21.
20. Parker SB, Eichele G, Zhang P, Rawls A, Sands AT, Bradley A, et al. p53-independent expression of p21^{Cip1} in muscle and other terminally differentiating cells. *Science* 1995;267: 1024–7.
21. Wang QM, Jones JB, Studzinski GP. Cyclin-dependent kinase inhibitor p27 as a mediator of the G1-S phase block induced by 1,25-dihydroxyvitamin D3 in HL60 cells. *Cancer Res* 1996;56:264–7.
22. Kawa S, Nikaido T, Aoki Y, Zhai Y, Kumagai T, Furihata K, et al. Vitamin D analogues up-regulate p21 and p27 during growth inhibition of pancreatic cancer cell lines. *Br J Cancer* 1997;76:884–9.
23. Harvat BL, Wang A, Seth P, Jetten AM. Up-regulation of p27^{Kip1}, p21$^{WAF/Cip1}$ and p16^{ink4a} is associated with, but not sufficient for, induction of squamous differentiation. *J Cell Sci* 1998;111:1185–96.
24. Dellas A, Schultheiss E, Leivas MR, Moch H, Torhorst J. Association of p27^{Kip1}, cyclin E and c-myc expression with progression and prognosis in HPV-positive cervical neoplasms. *Anticancer Res* 1998;18:3991–8.
25. Skomedal H, Kristensen GB, Lie AK, Holm R. Aberrant expression of the cell cycle associated proteins TP53, MDM2, p21, p27, cdk4, cyclin D1, RB, and EGFR in cervical carcinomas. *Gynecol Oncol* 1999;73:223–8.
26. Resnick MB, Schacter P, Finkelstein Y, Kellner Y, Cohen O. Immunohistochemical analysis of p27/kip1 expression in thyroid carcinoma. *Mod Pathol* 1998;11:735–9.
27. Cordon-Cardo C, Koff A, Drobnjak M, Capodieci P, Osman I, Millard SS, et al. Distinct altered pattern of p27^{KIP1} gene expression in benign prostatic hyperplasia and prostatic carcinoma. *J Natl Cancer Inst* 1998;90:1284–91.
28. Fredersdorf S, Burns J, Milne AM, Packham G, Fallis L, Gillet CE, et al. High level expression of p27(kip1) and cyclin D1 in some human breast cancer cells: inverse correlation between the expression of p27(kip1) and degree of malignancy in human breast and colorectal cancers. *Proc Natl Acad Sci USA* 1997;94:6380–5.
29. Vlach J, Hennecke S, Amati B. Phosphorylation-dependent degradation of the cyclin-dependent kinase inhibitor p27. *EMBO J* 1997;16:5334–44.
30. Morisaki H, Fujimoto A, Ando A, Nagata Y, Ikeda K, Nakanishi M. Cell cycle-dependent phosphorylation of p27 cyclin-dependent kinase (cdk) inhibitor by cyclin E/cdk2. *Biochem Biophys Res Commun* 1997;240:386–90.
31. Pagano M, Tam SW, Theodoras AM, Romero-Beer P, Del Sal G, Chau V, et al. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. *Science* 1996;269:682–5.