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ARTICLE

Significantly Decreased P27 Expression In Endometrial Carcinoma Compared to Complex Hyperplasia with Atypia (correlation with p53 expression)

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P27 expression was examined on paraffin-embedded specimens in proliferative, secretory, hyperplastic and neoplastic human endometrium by immunohistochemistry. The results of p27 immunoreactivity in endometrial carcinomas were compared with clinicopathological indicators as well as with p53 expression. Thirty-eight cases of endometrial carcinoma, 30 normal functional (15 proliferative, 15 secretory), 24 hyperplastic endometrium (12 without atypia, 12 with atypia) specimens were studied by using monoclonal p27 and p53 antibodies. The streptavidin-biotin-peroxidase detection system was used and the intensity and the distribution of immunoreactivity was evaluated semiquantitatively. p27 expression was present both in the proliferative and secretory phases; the expression being stronger in the secretory period. In complex hyperplasia with atypia, p27 expression was even higher and it was significantly reduced in the endometrial carcinoma group (p<0. 05). No significant correlation was found between p27 expression and any of the clinicopathologic prognostic parameters (p>0.05). Nuclear p53 expression was detected in 13 (34. 2%) patients with endometrial carcinoma and was higher in non-endometrioid carcinomas and in tumors with increasing FIGO grade (p<0.05). High expression of p53 was not found to be a significant prognostic indicator of survival (p> 0.05). No p53 expression was detected in the endometria with proliferation, secretion or hyperplasia either simple without atypia or complex with atypia. Surprisingly, tumors with absent/low p27 expression showed absent/low p53 expression. Our data suggest that p27 is necessary to control the proliferation of endometrium and its loss of expression seems to play a role in some aspects of endometrial carcinogenesis. (Pathology Oncology Research Vol 10, No 2, 89–97)

Keywords: Endometrium, carcinoma, hyperplasia, p27, p53

Introduction

Proliferation and differentiation of the human endometrium are controlled by ovarian steroids via their receptors. Estrogen stimulates the proliferation of glandular cells, whereas progesterone inhibits their growth and induces secretory changes.[17] The mechanism of carcinogenesis in the endometrium is strongly associated in most cases with unopposed estrogen influence on the mucosal turnover.[6] Nevertheless, the molecular mechanisms which negatively regulate the growth of endometrial cells are not fully understood.[17,22]

Advances in cell cycle research have revealed that cell proliferation is regulated by the interactions between cyclins/cyclin dependent kinases (cdks), cyclin-dependent kinase inhibitors and tumor suppressor gene products.[5,17] Cyclin E with its partner cdk 2 is thought to be the rate-limiting activator of the mitotic G1 to S phase transition, where the cyclin dependent kinase inhibitors prevent this cycle progression.[5,10,17,21,22]

P27 Kip1 (p27) is one member of a group of proteins identified as cdk inhibitors, which cause G1 arrest when overexpressed and functions as a tumor suppressor. It has been suggested that p27 mediates G1 arrest induced by transforming

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growth factor- (TGF), contact inhibition and serum deprivation of epithelial cells. Normal levels of p27 might be important in controlling cellular proliferation and opposing tumor progression[2]. Low levels of p27 are associated with poor prognosis in a variety of gynecological tumors, including breast, ovarian, and cervical carcinomas. On the other hand, the role of p27 in endometrial cancer has been investigated in limited number of studies.[13] P27 in endometrial carcinomas has frequently been found to be downregulated with not much controversy.[2,13,15,19] There are, however, much less studies and much more controversy concerning the clinicopathologic significance of this phenomenon.[13,21]

P53 protein, on the other hand, regulates cell-cycle inhibition and apoptosis in response to DNA damage, and mutation of the p53 gene has been found to be the most common genetic defect in human cancers.[3,6,8] Loss of wild-type p53 function predisposes cells to malignant transformation. In normal cells, p53 protein is rapidly degraded (and therefore rarely immunohistochemically detectable), whereas mutant p53 protein resists degradation and accumulates in the nucleus, where it can be demonstrated by immunohistochemistry.[1,3,8,12] In addition, immunoreactivity of p53 protein was detectable in over 90% of p53 mutations. Therefore, in general, the immunohistochemical staining of p53 could be considered to represent the overexpression of p53 consistent with p53 gene alteration.[12] However, it has also been reported that endometrioid carcinomas can overexpress p53 without gene alteration and, such overexpression is related to mdm2 overexpression.[20]

More recently, a putative tumor suppressor gene called PTEN has been shown to contribute specifically to the development of endometrioid carcinomas of endometrium, especially of microsatellite instability-positive endometrial carcinomas, as an early genetic change. It inhibits cell proliferation by regulating intracellular signaling pathways and its level of expression was found to be significantly correlated with cell cycle regulators including p27 and p53.[11]

The aim of this study was to examine p27 expression in neoplastic as well as in the proliferative, secretory and hyperplastic endometrium by using immunohistochemical methods. The intensity of p27 expression was compared to and correlated with clinicopathological features of the disease and with p53 accumulation in human endometrial carcinomas. Although these two proteins are not in the same pathway nor is there any direct relationship between them, as both are involved in cell cycle regulation, p27 expression is compared with p53 which has been intensively studied in many tumors as well as in endometrial carcinomas.

Materials and Methods

Patients and samples

Thirth-eight patients with endometrial adenocarcinoma, who were consecutively diagnosed in Kocaeli University Medical Faculty Hospital between 1996–2002 were included in the study. The control group were composed of 15 cases of proliferative, 15 cases of secretory functional endometrium and 12 simple and 12 atypical hyperplastic endometria.

Normal proliferative and secretory as well as simple hyperplastic endometria were obtained from consecutive archival hysterectomy specimens from women aged 35–65 years, who underwent hysterectomy for benign gynecological diseases unrelated to endometrial pathology, such as uterine leiomyoma. Atypical hyperplastic endometria specimens were curettage materials and no progesterone derivative had been given to these patients previously. These patients were also followed-up for at least 2 years with no symptomatology.

All sections prepared from the paraffin blocks of each specimen were histopatologically examined and evaluated according to the published criteria. Endometrial adenocarcinoma cases were analyzed for age, menopausal status, tumor size, histopathologic tumor subtype, FIGO grade, depth of myometrial invasion, lymph node involvement, peritoneal washings, stage, angiolymphatic invasion, interval to recurrence and death.

Hysterectomy with salpingo-oophorectomy and surgical staging procedures were performed in all tumor patient's. Selective pelvic and paraaortic lymph-node sampling was performed in patients with risk of recurrence. Postoperative radiotherapy was offered depending on the patients characteristics. There was follow-up information on all patients with endometrial carcinoma.

Three of 38 patients who were followed up were dead of disease. Additional three patients sufferred from recurrent disease 12, 30 and 36 months postoperatively. Disease specific deaths but not recurrences were used for cumulative survival analysis.

Hematoxylin-eosin stained microscopic slides and May-Grünwald-Giemsa stained peritoneal washings were reevaluated by the same pathologist (SKÖ), and grading and staging was assessed according to FIGO 1988 criteria. Histopathologic subtyping was assessed according to WHO classification schemes.

Adenocarcinomas other than endometrioid carcinomas were graded solely by their nuclear features. Endometrioid carcinomas with squamous differentiation were classified according to the nuclear grade of the glandular component. Depth of myometrial invasion was recorded as 50% or > 50 % of the myometrial thickness.

Immunohistochemical staining was performed on formalin-fixed, paraffin embedded specimens. A representative area of the tumor was selected and serial sections of 4–6 m thickness were cut from the paraffin blocks and mounted on positively charged slides, deparaffinized in xylene and rehydrated in graded alcohol. Immunohistochemistry procedure was performed using a combination of microwave-oven heating for antigen retriaval and standard streptavidin-

biotin-peroxidase complex methods. After microwave heat treatment with three 10-minute cycles in 10 mM citrate buffer (pH 6.0), sections were cooled at room temperature for 1 hour. Endogenous peroxidase activity was blocked by hydrogen peroxide (0.86%). Tissue sections were then stained immunohistochemically with prediluted p27 (clone: DCS-72.F6; 2 g/ml) and p53 (clone: DO-7+BP53-12; 1 g/ml) purified mouse monoclonal antibodies purchased from Neomarkers (distributed by Lab Vision Corporation, Fremont, CA, USA) for 1 hour incubation period at room temperature. Amino-ethyl-carbazole (AEC) was used as chromogen. Sections were counterstained with Mayer's hematoxylin, dehydrated and mounted.

Infiltrative ductal breast carcinoma was used for both markers as positive control. Negative controls were incubated with tris-buffered saline (TBS) instead of the primary antibody.

For p27, endothelial cells within each section, as the stromal components or infiltrating lymphocytes. were found to act as an internal positive control as well

Immunohistochemistry Evaluation

Every tissue sample was assessed by the same pathologist and given a score reflecting both the intensity of p27 and p53 staining and the percentage of stained cells.

Clear nuclear staining in tumor cells was regarded as positive in sections subjected to immunohistochemical staining. Cytoplasmic reactivity for the p27 antibody used was disregarded and only nuclear staining above a cytoplasmic background was considered as evidence of expression.

Nuclear staining was considered positive for p53 when >5% of the neoplastic cells showed nuclear staining. P27

Table 1. **p27 and p53 expressions with respect to patient characteristics in cases of endometrial adenocarcinoma**

Clinicopathologic Variables		*Total number of cases (n= 38) (%)*	*Low/absent p27 expression (%)*	*Presence of p53 expression (%)*
Age mean: 58.8+/–10.8	< 45	5 (13.2)	5 (100.0)	1 (20 0)
range: 32–75	45	33 (86.8)	23 (69.7)	12 (36.4)
Menopausal status	Premenopausal	12 (31.6)	9 (75.0)	5 (41.7)
	Postmenopausal	26 (68.4)	19 (73.1)	8 (30.8)
Tumor size	< 2 cm	23 (60.5)	18 (78.3)	8 (34.8)
	2 cm	15 (39.5)	10 (66.7)	5 (33.3)
Histopathologic subtype	Endometrioid (classical)	29 (76.3)	23 (79.3)	8 (27.6)
	Endometrioid (squamous dif.)	7 (18.4)	4 (57.1)	4 (57.1)
	Clear cell adenocarcinoma	1 (2.6)	0 (0.0)	0 (0.0)
	Papillary serous adenocarcinoma	1 (2.6)	1 (100.0)	1 (100.0)
Grade	I	18 (47.4)	14 (77.8)	4 (22.2)
	II	14 (36.8)	10 (71.4)	6 (42.9)
	III	6 (15.8)	4 (66.7)	3 (50.0)
Myometrial invasion	None	14 (36.8)	9 (64.3)	7 (50.0)
	< ½	12 (31.6)	9 (75.0)	4 (33.3)
	> ½	12 (31.6)	10 (83.3)	2 (16.7)
*Lymph nodes**	Negative	29 (93.6)	23 (79.3)	11 (37.9)
	Positive	2 (6.5)	2 (100.0)	0 (0.0)
Peritoneal cytology	Negative	34 (89.5)	25 (73.5)	12 (41.4)
	Positive	4 (10.5)	3 (75.0)	1 (25.0)
Stage	I	31 (81.6)	22 (71.0)	11 (35.5)
	II	2 (5.3)	2 (100.0)	1 (50.0)
	III	5 (13.2)	4 (80.0)	1 (20.0)
Hyperplasia	Present	16 (42.1)	11 (68.8)	4 (25.0)
	Absent	22 (57.9)	17 (77.3)	9 (40.9)

* Thirty-one of the thirty-eight cases had lymph node dissection.

and p53 staining intensity was graded from zero (no staining) to 3 (strong staining) and percentage of cells showing nuclear staining was graded as zero (no tumor cells positive), 1 (positive staining in <10% of the tumor cells), 2 (positive staining in 10–50 % of the tumor cells), 3 (positive staining in >50 % of the tumor cells). A staining score was calculated as the product of percentage of cells showing nuclear staining and staining intensity. Patients were then categorized according to their p27 and p53 staining scores as high in expression (staining score = 7–9), moderate expression (staining score = 4–6), low in expression (staining score = 1–3) and absent expression (staining score = 0).

Patients were followed up for 81–7 months (mean follow-up for 41. 7 months, SD = 19.9 mo).

Statistical Analysis

The statistical comparison of groups was performed by using Pearson's chi-square test. Survival analysis was performed by the Kaplan-Meier method. The log-rank test was used to compare the survival results. A value of $p<0.05$ was considered significant.

Results

The antibodies against both p27 and p53 reacted with nuclear sites, demonstrating a heterogenous distribution of immunopositive cells and various levels of immunointensity within the tumors and controls. Stromal staining for p27 was also observed. The results of p27 and p53 immunostaining scores with respect to patient characteristics in cases of endometrial carcinoma are given in *Table 1*.

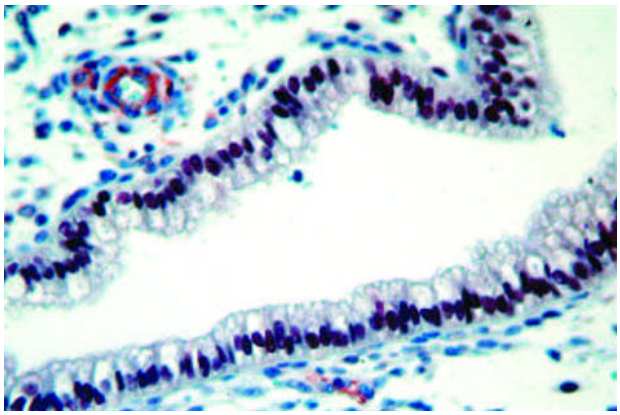

Figure 1. *Strong nuclear immunoreactivity with p27 in the glandular epithelium of secretory endometrium (Streptavidin-biotin, x300).*

Results with p27

In the normal endometrium, p27 expression was observed throughout the menstrual cycle, both in the proliferative and the secretory phase. Of the proliferative phase samples (n = 15), none showed strong (score = 7–9) p27 expression while 93.3% had a score of 0–4. Expression was stronger in the secretory phase compared to that in the

Table 2. **Significantly decreased p27 expression in endometrial carcinoma when compared with complex hyperplastic endometrium with atypia (p = 0.000)**

Groups	*P27 expression*				*P value*
	Absent (score = 0)	*Low (score = 1–3)*	*Moderate (score = 4–6)*	*High (score = 7–9)*	
Proliferative endometrium (n = 15) (%)	8 (53.3)	6 (40.0)	1 (6.7)	0 (0.0)	
Secretory endometrium (n = 15) (%)	6 (40.0)	2 (13.3)	1 (6.7)	6 (40.0)	
Simple hyperplasia without atypia (n = 12) (%)	9 (75.0)	2 (16.7)	1 (8.3)	0 (0.0)	0. 000
Complex hyperplasia with atypia (n = 12) (%)	0 (0.0)	2 (16.7)	4 (33.3)	6 (50.0)	
Endometrial carcinoma (n = 38) (%)	20 (52.6)	8 (21.1)	7 (18.4)	3 (7.9)	

Table 3. **Significantly different p27 expression between the cases of complex hyperplasia with atypia and endometrial carcinoma confined to the endometrium, with no myometrial invasion (p = 0.028)**

Groups	*P27 expression*				*P value*
	Absent (score = 0)	*Low (score = 1–3)*	*Moderate (score = 4–6)*	*High (score = 7–9)*	
Complex hyperplasia with atypia (n = 12) (%)	0 (0.0)	2 (16.7)	4 (33.3)	6 (50.0)	0.028
Endometrial carcinoma (stage Ia) (n = 14) (%)	7 (50.0)	2 (14.3)	3 (21.4)	2 (14.3)	

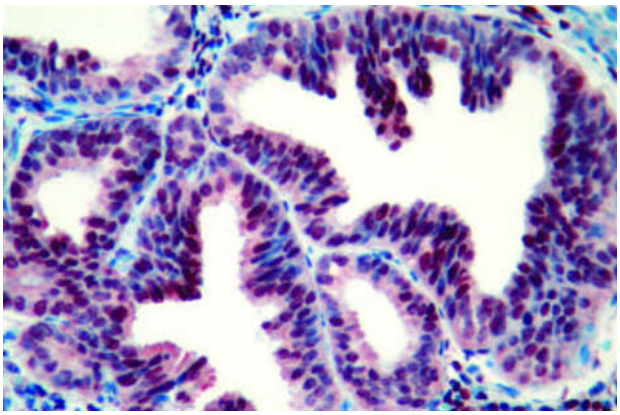

Figure 2. *Strong nuclear immunoreactivity with p27 in the glandular epithelium of complex hyperplastic endometrium with atypia (Streptavidin-biotin, x300).*

proliferative phase. Fourty percent of these samples showed high level of expression (score = 7–9) (*Figure 1*) (*Table 2*).

In the hyperplastic endometrium, no p27 expression was observed in the 75% of the cases of simple hyperplasia without atypia, while no cases showed strong expression of p27. In the group of complex hyperplasia with atypia, 50.0% showed strong p27 expression (*Figure 2*) while 16. 7% had low immunoreactivity scores (*Table 2*).

In thirty-eight cases of endometrial carcinoma that were analysed, 73.7% showed reduced or absent (score = 0-4) p27 staining (*Figures 3a, b.*). On the other hand, 7.9% of the cases showed strong p27 expression (*Table 2*). Although some trend toward absent/low p27 expression with myometrial invasion depth, lymph node involvement, positive peritoneal cytology, high stage and absence of hyperplasia was present (*Table 1*); the differences were not statistically significant.

Statistical analysis between the functional, hyperplastic and neoplastic endometrium in terms of p27 expression revealed that there is significant difference between the groups. The analysis was repeated when stage Ia cases here excluded and when only stage Ia cases here put into the endometrial carcinoma group (*Table 3*). The *p* value remained to be significant.

There was no statistically significant relationship between p27 expression and clinicopathologic prognostic variables (age, menopausal status, tumor size, histopathologic tumor subtype, FIGO grade, stage, myometrial invasion, lymph node involvement, peritoneal cytology, lymphovascular invasion) (p > 0.05). Cumulative survival was not different in tumors with absent/low (score = 0–3) or moderate/high (score: 4–9) p27 expression (92. 9 % vs 90 %; p = 0.9933). The disease-free survival rate was 82. 1% in patients with absent/low p27 expression while it was 90. 0% in patients with moderate/high p27 expression. The difference was again not statistically significant (p = 0.3553).

Results with p53

P53 was detected in none of the proliferative, secretory or hyperplastic endometrium (either with or without atypia) samples. In the endometrial carcinoma group, nuclear p53 expression was detected in 13 of 38 patients (34.2%) with variable staining intensities (*Table 4*).

P53 was expressed in higher percentages with increasing FIGO grade *(Figures 4a and 4b.*). (linear by linear association, p = 0.037). P53 expression was detected in 50% of non-endometrioid tumors and 33.3% of endometrioid tumors (p = 0. 024) (*Table 4*).

There was no statistically significant association between p53 expression and other clinicopathologic prognostic variables (age, menopausal status, tumor size, FIGO stage, myometrial invasion, lymph node involvement, peritoneal cytology, lymphovascular invasion) (p > 0.05). Surprisingly, both cumulative (100% vs 90.9%; p = 0.5259) and disease-free survivals (100% vs 81.8%; p = 0.3257) were better in tumors with moderate/high (score = 4–9) p53

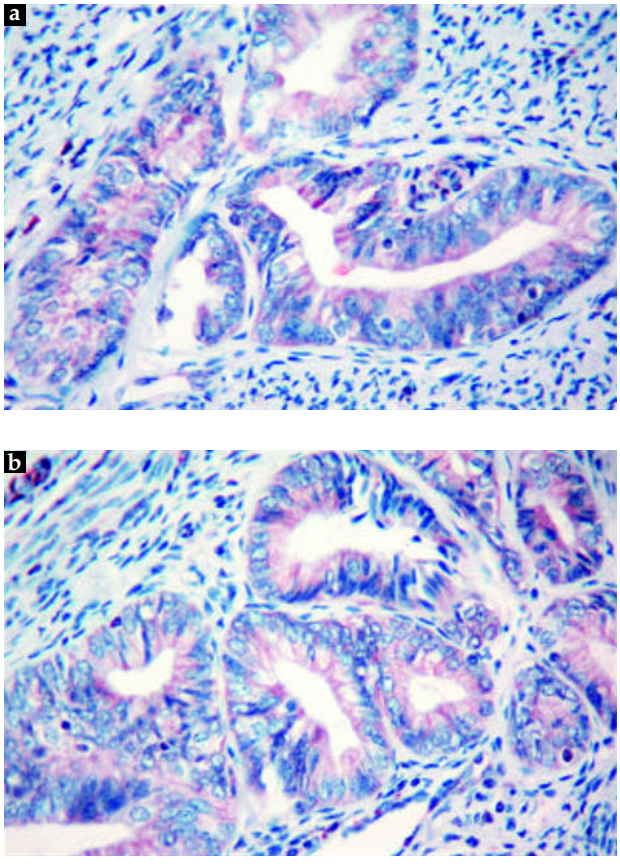

Figure 3. (a) *Weak cytoplasmic/no nuclear immunoreactivity with p27 in the malignant glandular epithelium of endometrium invading the myometrium (Streptavidin-biotin, x300).* ***(b)*** *Weak nuclear immunoreactivity with p27 in the malignant glandular epithelium of endometrium invading the myometrium (Streptavidin-biotin, x300).*

expression, but neither of these differences reached statistical significance.

Comparison of p27 expression with p53 expression revealed that tumors with no p53 expression had also absent/low p27 staining scores (p = 0. 003) (*Table 5*). Significant linear by linear association was present among the grade III endometrial carcinoma cases in terms of p27 and p53 expressions (p = 0.031).

Nevertheless, five of the six patients whose disease recurred or metastasized, or who were dead of disease, showed no p53 expression, while the remaining one showed low staining score. However, no significant association was present between p53 expression and disease-free or cumulative survivals (p> 0.05).

Discussion

There is increasing evidence that the molecules involved in cell cycle control are also involved in oncogenesis. CDK inhibitors are potential tumor suppressors, and loss of their expression is accepted as an important step in promoting tumor growth.[2]

Our data showed that p27 is expressed in the nuclei of normal endometrial cells throughout the menstrual cycle, and that expression is higher during the secretory phase. In simple hyperplasia without atypia the expression pattern is similar to that observed in the proliferative endometrium, whereas, in complex hyperplasia with atypia, the expression increased significantly. Of the 38 endometrial adenocarcinomas analysed, 73.7% revealed diminished or absent expression of p27, suggesting that loss of p27 expression plays a part in the pathogenesis of endometrial carcinoma. In the majority of grade I tumors, p27 expression was already low-absent (77.8%), which suggests that loss of p27 expression may be an early event in the development of endometrial carcinoma. Although some trend toward the association of absent/low p27 expression with myometrial invasion depth, lymph node involvement, positive peritoneal cytology, high stage and absence of hyperplasia was present *(Table 1.);* the differences were not statistically significant. We did not find significant association between p27 expression score and any of the prognostic parameters or recurrence-free or cumulative survival rates.

In the most recent study by Mascuillo et al, immunohistochemical analysis revealed a significant loss of p27 expression from normal (33%) through hyperplastic endometrium (50%) to endometrial adenocarcinomas (71%) in their large series of 217 endometrial adenocarcinoma cases. On the other hand, despite the suggested role of p27 protein in determining the prognosis of several human tumors, no significant correlation between the presence of p27 staining and clinicopathologic parameters or survival was found in this large group of patients with endometrial cancer.[13]

Considering the overlap between the various entities, the morphologic assessment of endometrial lesions is challenging particularly in the curettage specimens.[6] Our data also showed that p27 expression of the endometria with complex atypical hyperplasia was significantly higher when compared to stage Ia tumors which are limited to endometrium and have no myometrial invasion. This finding may be useful for the differential diagnosis of complex atypical hyperplasia and endometrial carcinoma, which is a common diagnostic problem, especially in the curettage specimens.

Mutations of the p53 tumorsuppresor gene are associated with alterations in cell proliferation, and mutant p53 protein has been found to be the most common genetic defect in human cancers.[3,6,8] In the endometrium, in the majority of the studies, p53 has been shown only in

Table 4. **Significantly increasing p53 expression with respect to decreasing degree of differentiation in endometrial carcinomas. Also, significant difference in p53 expression with respect to histopathological subtype**

Endometrial adenocarcinomas (n = 38)	*P53 expression*				*P value*
	Absent (score = 0)	*Low (score = 1–3)*	*Moderate (score = 4–6)*	*High (score = 7–9)*	
Grade I (n = 18) (%)	14 (77.8)	3 (16.7)	1 (5.6)	0 (0.0)	
Grade II (n = 14) (%)	8 (57.1)	4 (28.6)	1 (7.1)	1 (7.1)	0.037
Grade III (n = 6) (%)	3 (50.0)	1 (16.7)	0 (0.0)	2 (33.3)	
Endometrioid (classical) (n = 29) (%)	21 (72.4)	4 (13.8)	2 (6.9)	2 (6.9)	
Endometrioid with squamous differentiation (n = 7) (%)	3 (42.9)	4 (57.1)	0 (0.0)	0 (0.0)	0.024
Clear cell adenocarcinoma (n = 1) (%)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Papillary serous adenocarcinoma (n = 1) (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	
Total (n = 38) (%)	25 (65.8)	8 (21.1)	2 (5.3)	3 (7.9)	

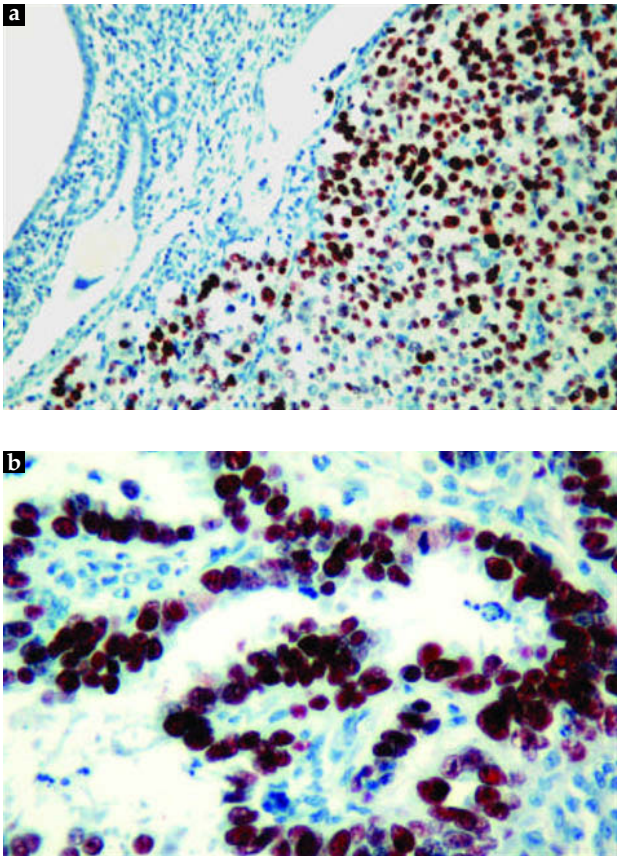

Figure 4. (a) *Strong nuclear immunoreactivity with p53 in grade III endometrioid adenocarcinoma with diffusely solid pattern; no hyperplasia in the adjacent tumor free endometrium (Streptavidin-biotin, x200).* ***(b)*** *Strong nuclear immunoreactivity with p53 in grade III endometrial carcinoma with papillary serous pattern (Streptavidin-biotin, x300).*

endometrial carcinoma with a staining rate of 10–50%.[1,3,8,12] On the other hand, some researchers found much higher (65%) p53 expression in endometrial carcinoma and as high as 30% p53 expression in endometrial hyperplasia with atypia.[6,14] In addition, others observed p53 expression in disordered proliferative endometrium as well as in hyperplastic endometrium[4]. We observed p53 expression only in endometrial carcinoma cases. As Elhafey et al stated, this observation may be due to the difference in the sensitivities of the immunohistochemical methods to evaluate the staining results, the antibodies, used as well as the higher number of their cases. Elhafey et al also added to their comments that the immunoreactivity pattern seen in the hyperplasia cases was focal and weak, which might be detectable only with computerized image analysis used in their study.[6]

Mutant p53 has also been found to be associated with advanced FIGO stage, non-endometrioid histopathologic subtypes – especially papillary serous –, and with higher grade of the tumor in endometrial carcinoma.[1,3,4,6,8,12] P53 overexpression is seen in up to 85% of papillary serous carcinomas.[1] There are also differences in the literature regarding p53 expression in relation to FIGO stage, histopathologic subtype and grade of the tumor. In our study, although a higher expression of p53 was found in non-endometrioid tumors and high p53 expression was significantly associated with increasing FIGO grade, it was not related to advanced FIGO stage.

P53 overexpression has been reported to be associated with a poor outcome for patients with low-risk (grade I or II, stage Ia or Ib) endometrial carcinoma.[1] We did not find such significant association, either. All of the patients having progression of their disease were those with high risk (grade III or stage Ib). However we observed that patients with low risk had higher incidence (40.9%) of p53 expression (scores = 1–9) compared to those with high risk (25%).

Interestingly, cumulative survival rate was interestingly better in patients with p53 expression in their tumors, but statistical analysis failed to demonstrate a significant association with prognosis in our study group. However, it has previously been shown that p53 expression was an independent prognostic indicator and also could be used to predict recurrence in endometrial carcinoma.[8] Presence or absence of p53 expression or low or high expression of p53 did not predict the recurrence or death in our study group ($p > 0.05$). The p53 results of this study might be quite perplexing as there is a significant body of literature suggesting that p53 overexpression is correlated with tumor grade and clinical behavior. Although we found significant correlation with tumor grade, we did not find significant association with prognosis.

Since p53 expression was not detected in cases with endometrial hyperplasia including those of complex hyperplasia with atypia, this may support the idea that, mutation of the p53 gene is a relatively late event in endometrial carcinogenesis.[3,7]Alternatively, because p53 overexpression is much more frequent in cases of papillary serous adenocarcinoma, it is possible that acquisition of a p53 mutation, reflected by immunohistochemically, overexpression leads to the development of an aggressive type of endometrial carcinoma that does not pass through a phase of hyperplasia.

Kaku et al found a statistically significant difference of p53 expression between endometrial carcinoma with hyperplasia (30.8% p53 expression) and those without hyperplasia (59.1% p53 expression)[9]. In our study, we observed p53 expression in carcinomatous areas of 25% of endometrial carcinomas with hyperplasia, while 40.9% in those without hyperplasia although the difference was not statistically significant ($p = 0.5$).

Comparison with p53 expression levels revealed that tumors with no p53 expression had also low/absent p27

Table 5. **Correlation between p27 & p53 expressions of endometrial carcinomas (n = 38)**

p53 expression	*P27 expression*				*P value*
	Absent (score = 0)	*Low (score = 1–3)*	*Moderate (score = 4–6)*	*High (score = 7–9)*	
Absent (score = 0) (n = 25) (%)	15 (60.0)	7 (28.0)	2 (8.0)	1 (4.0)	0.003
Low (score = 1–3) (n = 8) (%)	4 (50.0)	1 (12.5)	3 (37.5)	0 (0.0)	
Moderate (score = 4–6) (n = 2) (%)	1 (50.0)	0 (0 0)	0 (0.0)	1 (50.0)	
High (score = 7–9) (n = 3) (%)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)	
Total (n = 38) (%)	20 (52.6)	8 (21.1)	7 (18.4)	3 (7.9)	

Pearson correlation coefficient = 0.49. Significance (2-tailed) = 0.002

staining (*Table 5*). Of the grade III tumors (both endometrioid and non-endometrioid) 66.7 % showed low/absent p27 expression in addition to absent/low p53 expression (p = 0. 031). This finding is in contrast to the results of Bamberger et al in which comparison with p53 expression levels revealed that tumors with strong p53 expression had low/absent p27 staining. Additionally, the inverse association between p27 and p53 expression were concluded to indicate that both loss of p27 expression and p53 overexpression may be implicated in progression to grade III or the development of a more aggressive non-endometrioid (papillary-serous) histopathology.[2] Our results did not support this finding.

In some studies, the pattern of p27 expression clearly showed age dependence, demonstrating down-regulation among tumors of pre-, peri-, and post-menopausal patients, while p53 accumulation was frequent in tumors of older patients.[16] We also observed high p53 expression in patients over 45 years of age. Under this age group, we observed p53 expression weakly in only one case. We did not detect p27 expression at all under this age group.

There also exist other studies reporting paradoxically increased p27 expression in higher histological grades and cell proliferation[21]. We observed similar results as 77.8% of the grade I tumors showed low/absent p27 expression, while 71.4% of grade II and 66.7% of grade III tumors showed diminished p27 expression.

Western blot analysis confirmed elevated p27 protein levels in samples with positive p27 immunostaining in a study by Oshita et al. In the same study, considerable levels of p27 mRNA were detected in all normal and cancerous samples examined by semiquantitative PCR and it has been suggested that the decreased expression of p27, caused by posttranslational mechanism, might play an important role in endometrial cancer development.[15] Shiozawa et al also supported the posttranslational mechanisms in the up-regulation of p27 protein by progestins.[17] Despite an extensive search for molecular aberrations, no significant alterations of p27 gene have been reported[2]. As we were unable to perform these molecular analyses at our institution yet, we could not comment on the molecular mechanisms of p27 expression.

The importance of p27 in regulating normal proliferative processes and its reduction in endometrial carcinoma make it an interesting target for therapy of such states. In the studies of Shiozawa, medroxyprogesterone acetate (MPA) treatment induced p27 expession in endometrial hyperplasia.[18] Therefore, p27 has been suggested to be involved in growth suppression by progestins in endometrial glandular cells. On the other hand, the expression of p27 mRNA did not show any marked difference between the proliferative and secretory phase endometria in contrast to the expression of the p27 protein. The growth suppression of endometrial glandular cells by progesterone treatment is thought to be associated with elevated expression of p27 protein, as well as with reduced expression of cyclin D1, cyclin E and cdk 4.[17,18]

In addition, MPA is a potent differentiation inducer and has antitumor activities; therefore, it has been used to treat advanced and recurrent endometrial carcinoma with a response rate of 30–35%. Endometrial carcinomas with progesterone receptors showed even a higher response rate of 80%.[17] Prolonged progesterone administration can suppress cell proliferation in endometrial carcinomas through tumor cell differentiation without altering apoptosis, resulting in a shift in tissue cell kinetics toward a relative predominance of cell deletion.[16]

In conclusion, our data suggest that p27 seems to be necessary to control the proliferation of endometrium and its loss of expression might be correlated with some aspects of endometrial carcinogenesis. Future research is necessary studying on much higher numbers of cases and controls to highlight the molecular markers that might be the targets for the future therapy models to prevent as well as to treat endometrial carcinoma. Also, molecular changes occurring in the premalignant and malignant conditions have been proposed as an adjunct to differential diagnosis.

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