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.

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. The mechanism of carcinogenesis in the endometrium is strongly associated in most cases with unopposed estrogen influence on the mucosal turnover. Nevertheless, the molecular mechanisms which negatively regulate the growth of endometrial cells are not fully understood. 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. 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. 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...
growth factor- (TGF-β), contact inhibition and serum deprivation of epithelial cells. Normal levels of p27 might be important in controlling cellular proliferation and opposing tumor progression. 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. P27 in endometrial carcinomas has frequently been found to be downregulated with not much controversy. There are, however, much less studies and much more controversy concerning the clinicopathologic significance of this phenomenon.

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. 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. 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. However, it has also been reported that endometrioid carcinomas can overexpress p53 without gene alteration and, such overexpression is related to mdm2 overexpression.

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.

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**

Thirty-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 histopathologically 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, angiolympathic 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 suffered 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 μm 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 retrieval and standard streptavidin-