

## ORIGINAL PAPER

# P53 and PCNA immunoexpression in endometrial carcinomas

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### Abstract

This paper analyzes 18 endometrial carcinomas with different degrees of differentiation, the objective being to estimate involvement of p53 oncoprotein in the mechanism of endometrial carcinogenesis and the possible correlations with the tumoral proliferative activity evaluated by PCNA. The p53 immunoexpression was positive in 44.4% of the studied endometrial carcinomas, divided in three groups, the intensity of the immunostaining for p53 being increased for the low differentiated and undifferentiated endometrial carcinomas, whereas the differentiated endometrial carcinomas were moderate or low p53 positive. All the investigated tumors were PCNA positive, the PCNA index being of 40% in well-differentiated carcinomas, while in low differentiated and undifferentiated endometrial carcinomas the medium values of PCNA were of 60%, respectively 85%. Correlating the p53 and PCNA findings, we noted that PCNA was expressed especially in the cases with increased proliferative activity, without a significant statistic correlation between p53 and PCNA expression.

**Keywords:** endometrial carcinomas, p53, PCNA.

### Introduction

Endometrial carcinomas represent a common group of cancers in the oncologic pathology of women, additionally representing the most frequent invasive neoplasm of the feminine genital tract.

The most frequent subtype of endometrial carcinomas is endometrioid carcinoma (type I), representing approximately 80% of them, often preceded by histopathological identifiable precursors lesions [1].

Molecular events that contribute to occurrence and progression of the endometrial precancerous and cancerous lesions are yet little understood.

If the normal endometrium is characterized by a well-controlled hormone dependent menstrual cycle, the system is disturbed in endometrial hyperplasia and carcinomas were initiated a series of changes able to induce the progression of the precursor lesions to a malign phenotype.

It is considered that apparition and progression of the endometrial carcinomas is a multi stadium process, without defining the entire mutations specific to tumoral progression.

The key of the endometrial carcinogenesis is represented by two suppressor tumoral genes, phosphatase gene and the homologous of tensine on the 10 chromosome (PTEN), for the type I endometrial

tumors, and p53 for type II endometrial tumors, both regulating apoptosis in a different manner.

In carcinogenesis correlated with PTEN anomalies at the debut, estrogen impregnation possesses the promoter role, than, as the tumor accumulates cellular clones, the tumoral proliferation becomes estrogen-independent [1, 2].

It is considered that simultaneous PTEN and p53 anomalies expression are implied in the tardy stages of the endometrial carcinogenesis [3, 4].

### Material and methods

This study included 18 endometrial carcinomas diagnosed in the Pathology Lab of the Emergency County Hospital of Craiova. The classification of the lesions was made in conformity with the last WHO classification [1].

Investigated cases were immunohistochemical processed by LSAB/HRP technique in the Lab of the Center of Researches for Microscopic Morphology and Immunology of the University of Medicine and Pharmacy of Craiova.

We used antibodies for appreciation of the proliferative activity – PCNA and p53 oncogene implicated in endometrial carcinogenesis, pursuing them immunoexpression in the neoplastic epithelial cells.

To appreciate the intensity of p53 gene immunoexpression we used a system of appreciation in four degrees: absence of immunostaining 0, low intensity immunostaining +, moderate intensity immunostaining ++, and high intensity immunostaining +++.

For PCNA immunostaining was calculated a rate index of the immunostaining referring the number of positive cells (nuclei) to the total of the numbered cells (positive and negative), the result being multiplied by 100, numbering minimum 1000 nuclei for each case.

## Results

The 18 cases of endometrial carcinomas corresponded histopathological to 10 cases of endometrioid well differentiated cases, five cases of low differentiated endometrioid carcinomas and three cases of undifferentiated carcinomas.

For the selected cases, we followed the degree of cellular proliferation by PCNA antibodies and the detection of the genomic anomalies or DNA alterations by immunostaining with anti-p53 antibody.

P53 immunostaining revealed that 44.4% of the endometrial carcinomas were p53 positive, respectively 8 of the 18 investigated endometrial carcinomas.

The higher frequency of the p53 positive cases was in undifferentiated endometrial carcinomas (66.6%) and in the low differentiated endometrioid carcinomas (60%), comparative with well-differentiated forms of the endometrioid carcinomas (30%).

The majority of the carcinomas had a diffuse distribution of immunostaining in the tumoral cells, the pattern being nuclear in all the p53 positive cases.

The intensity of the p53 immunostaining was low (+) in one case of well-differentiated endometrioid carcinoma and moderate (++) in the other two cases (Figure 1).

All the low differentiated and undifferentiated carcinomas were p53 intense positive (+++) (Figures 2 and 3). Thus, the intensity of the p53 immunostaining was higher as the degree of differentiation of the carcinoma was lower (Table 1).

**Table 1 – P53 immunostaining intensity distribution**

Immunostaining intensity	-	+	++	+++
Endometrial carcinomas	10	1	2	5

Analyzing the incidence of p53 positivity, we noted that 30% of the type I endometrial carcinomas, respectively 62.5% of the type II, expressed p53 protein.

Correlating the p53 immunostaining results with the pathogeny of the two types of endometrial carcinomas, estrogenic and non-estrogenic, we can note the net differences between type I and II of endometrial carcinomas.

Therefore, type I estrogen dependent was much less p53 positive comparative with type II, estrogen-independent.

These observations are in concordance with the dual pattern of the endometrial carcinogenesis, according to whom type I carcinoma is correlated with

non-antagonized estrogenic stimulation, whereas in type II carcinoma development are associated mutations of the p53 gene.

Analyze of PCNA immune marking in the studied cases reveals the presence of the cellular proliferation both in the carcinomatous structures and in the tumoral stroma, demonstrated by positive nuclear PCNA staining. For this reason we calculated the PCNA index of positivity for each case (IP-PCNA), noting the medium values if IP for each lesional type analyzed (Table 2).

**Table 2 – PCNA index appreciated in the carcinomatous cells**

Lesional type	Well differentiated endometrioid carcinoma	Low differentiated endometrioid carcinoma	Undifferentiated carcinoma
IP-PCNA	40%	65%	85%

In the analyzed lesional groups, PCNA positivity was different. The medium IP to PCNA was of 40% in the well-differentiated endometrioid carcinomas, 65% in the low differentiated carcinomas and 85% in the undifferentiated lesions (Figures 4–6).

The PCNA immune marking was diffuse distributed in the glandular structures and in the compact neoplastic islands. These values indicate a proliferative cellular activity progressive increased with the decrease of the degree of differentiation of the endometrial carcinomas.

Grouping the analyzed endometrial carcinomas in type I and II, we noted that the higher degree of PCNA expression was present in II group of carcinomas, therefore in the category with the most reserved prognostic.

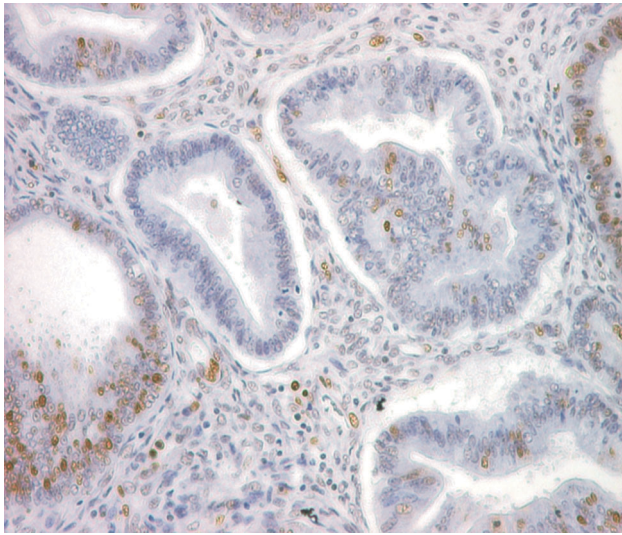
## Discussions

The comparative study of the 18 cases of endometrial carcinomas with different degrees of differentiation aim both to distinguish the degree of implication of the p53 oncoprotein in endometrial carcinogenesis and the intensity of the proliferative activity appreciated by PCNA rate index and the possible correlations between the two markers.

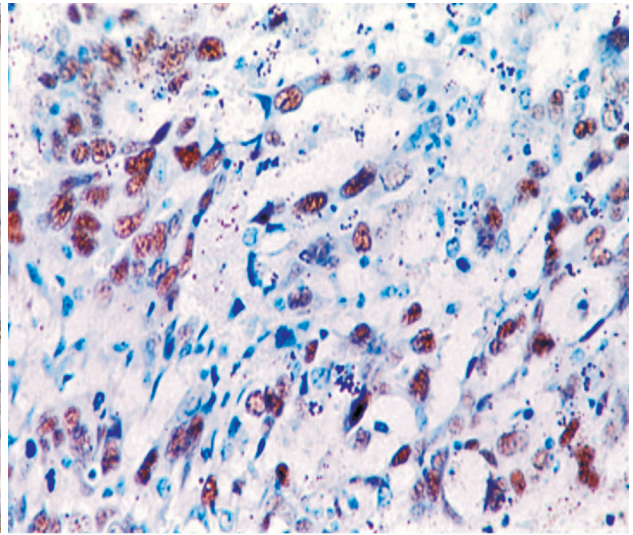
The fact that only 44.4% of the analyzed endometrial carcinomas were p53 positive suggests the p53 implication only in certain types. The higher frequency of the p53 positive cases was present in the type II carcinomas (63.3%) comparative with the type I where only 30% were positive. The obtained results are similar with the one from other studies that indicated the presence of p53 mutation in 34% of the type I carcinomas and in 66% of the type II carcinomas [5].

The analyze of the intensity of immunostaining for p53 for the three groups of endometrial carcinomas revealed an increased intensity of the immunostaining for the low differentiated endometrioid carcinomas and undifferentiated endometrial carcinomas, whereas the differentiated endometrioid carcinomas were moderate or low positive. In the specialty papers, the intensity of the p53 glandular expression is correlated with the histologic subtype, being higher at the lesions with the worse prognostic [6].

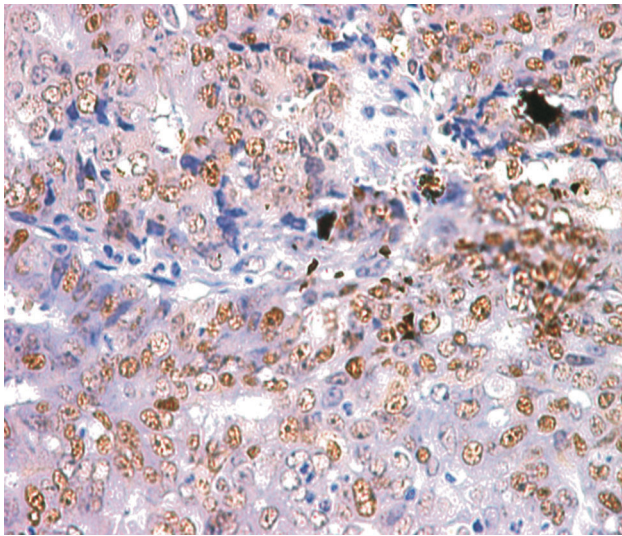




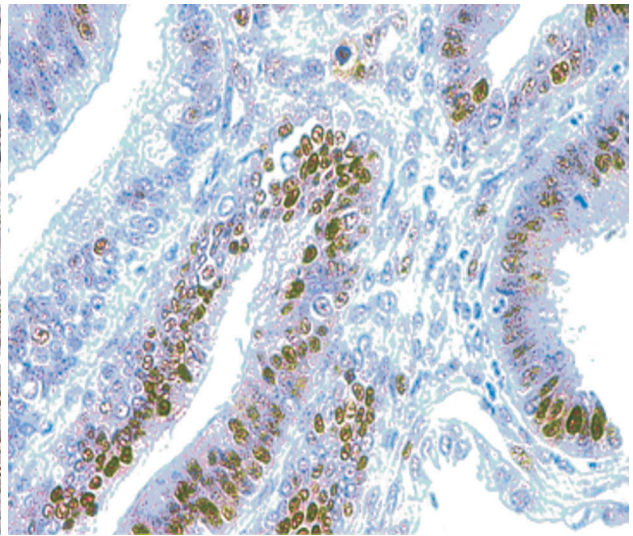
**Figure 1 – Well differentiated endometrial carcinomas (p53, ob. ×10)**



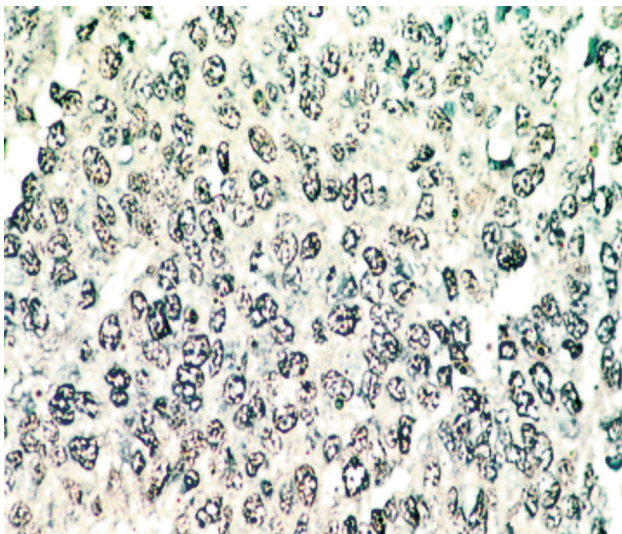
**Figure 2 – Low differentiated endometrioid carcinomas (p53, ob. ×20)**



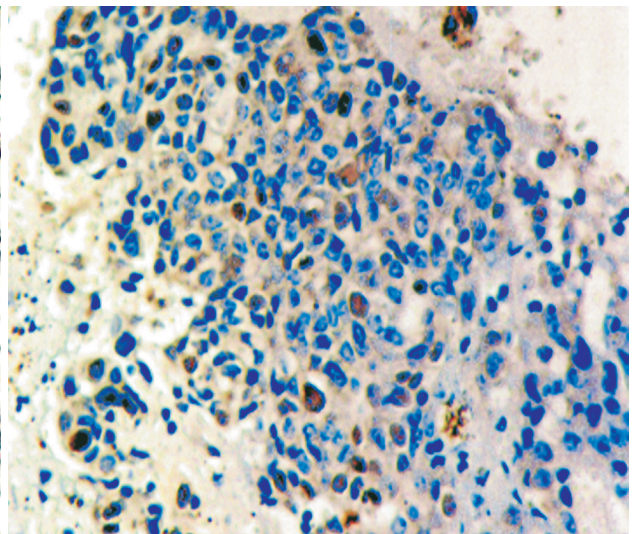
**Figure 3 – Undifferentiated endometrial carcinomas (p53, ob. ×20)**



**Figure 4 – Well differentiated endometrial carcinomas (PCNA, ob. ×20)**



**Figure 5 – Low differentiated endometrioid carcinomas (PCNA, ob. ×20)**



**Figure 6 – Undifferentiated endometrial carcinomas (PCNA, ob. ×20)**



The analyze of the p53 expression with the same type of antibody, or with antibodies that detect only mutant p53, indicated the fact that both endometrial hyperplasias with atypia and the well differentiated carcinomas had a focal distribution of the immunoreactions, while the low differentiated carcinomas have a diffuse immunoreactivity [3, 6–8].

The immunohistochemical marker with low and focal intensity from the well-differentiated endometrial carcinomas represents the consequence of the increasing of the intracellular level of the wild type of the p53 protein, in order to stop the cellular division and correct the DNA errors.

Therefore, the true p53 mutations were present only in the tardy phases of evolution of an endometrial carcinoma, namely in the cases of undifferentiated and low differentiated lesions when the p53 immunostaining was intense and diffuse.

In this sense, the literature mention the fact that immunohistochemical evaluation of the overexpression of the p53 protein using antibodies that do not make distinction between wild and mutant types of p53 must take in consideration the pattern of staining, because only a intense and diffuse marking can predict mutations of the p53 gene [3].

It is considered that p53 anomalies appear associated with the tardy stages of the endometrial carcinogenesis. In the precocious stages of the endometrial carcinogenesis were noted reciprocal anomalies of PTEN and then of p53, whereas in the tardy stages are described simultaneous anomalies of p53 and PTEN [3, 8].

PCNA positivity was present in all the investigated tumors, but was different in the analyzed groups. For the well differentiated forms (type I) of the endometrioid carcinomas the PCNA index had reduced medium values, 40%, comparative with low differentiated and undifferentiated endometrial carcinomas (type II) where the medium values of PCNA index were 60%, respectively 85%.

It can be said that these values indicates a proliferative cellular activity progressively increased with the decrease of the degree of differentiation of the endometrial carcinomas, the higher degree of the PCNA expression being present in the type II carcinomas, therefore in the category with the worst prognostic.

In the literature are communicated similar results, but with statistical significance, by PCNA morphometric analyze which indicates a significant correlation with the degree of differentiation of the endometrioid carcinomas and with the subtype of endometrial carcinomas [6].

Correlating the results obtained at p53 immunomarking with the one for PCNA we noted that p53 was expressed especially in the cases with increase proliferative activity, without a statistically significant correlation between p53 and PCNA expression.

The data from the literature for different types of tumors revealed different relationships between p53 and PCNA. If for mammary and central nervous system neoplasia the researches revealed no significant association with the expression of the levels of these genes, for esophageal carcinoma and some colon-rectal adenoma with dysplasia are communicated some correlations between p53 and PCNA immunorexpression [9–12].

## Conclusions

This study, which included 18 cases of endometrial carcinomas followed, both implication of the p53 suppressor tumoral gene expression in endometrial carcinogenesis, by the p53 oncoprotein immunorexpression, and the intensity of the proliferative activity for endometrial carcinomas with different degrees of differentiation, appreciated by PCNA index.

P53 expressions were correlated with the histologic degree of the studied lesions, the p53 immunostaining being intense and diffuse in the lesions with a badly prognostic.

P53 was expressed especially in the cases with increased cellular proliferative activity, detected with PCNA, without a statistically significant significance between p53 and PCNA expression.

The development of the low differentiated and undifferentiated carcinoma is associated with mutations of the p53 protein, which represent a tardy event in endometrial carcinogenesis.

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