MEDICAL SCIENCES

EXPRESSION OF p53 AND Ki67 IN TYPE 1 AND TYPE 2 ENDOMETRIAL CARCINOMAS AND IN LOW AND HIGH GRADE SEROUS OVARIAN CARCINOMAS

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Abstract

Ovarian cancer is the deadliest of gynecological malignancies, and despite the efforts to improve existing treatment methods and early diagnosis, no progress has been made. Endometrial carcinoma (EC) is the fourth most common malignancy in women.

The p53 gene has a leading role in the control of the cell cycle and the initiation of carcinogenesis. The p53 protein induces apoptosis, or cell cycle arrest, which allows the cell to repair genomic damage. Loss of p53 function plays a central role in the development of malignant tumors. P53 is a tumor suppressor gene whose expression in tumors is associated with progression and poor prognosis.

Ki67 protein is a cell proliferation marker. Immunohistochemical staining with Ki-67 provides reliable data on the growth fraction of tumors. The Ki67 marker reflects cell proliferation in the tissue being examined. By reading the immunohistochemical expression of Ki67, we can obtain information about the proliferative index and about the growth fraction of tumors. The number of Ki67 positive tumor cells often correlates with the clinical course.

Keywords: p53, Ki67, endometrial cancer, ovarian cancer.

Introduction

The mechanisms underlying the progression of noninvasive serous borderline ovarian tumors to low-grade invasive carcinomas are poorly understood. Cheng J-C et al. demonstrated that inhibition of p53 induces tumor cell invasion in serous borderline ovarian tumors through activation of the PI3K/Akt pathway and transcriptional repression of E-cadherin (1). The other histological subtypes of ovarian carcinoma, which more often present at an early stage (endometrioid, clear cell, mucinous), have a much lower frequency of TP53 mutations (2). The heterogeneous nature of adenocarcinomas is the reason for the different results of the study of this expression.

Ki67 is an indicator of cell activity and proliferation. Its gene is located on chromosome 10 and its protein product is located in the nucleus. The expression of ki67 is closely related to cell proliferation and differentiation and is often used as an indicator of carcinogenesis and cell activity. Data can be obtained from immunohistochemical results at the time of initial diagnosis.

10 - 20% of endometrial carcinomas and 90% of serous carcinomas may have a P53 mutation (3). P53 is mostly expressed in high-grade, poor-prognosis non-endometrioid endometrial carcinoma (4).

Ki67 expression in endometrial carcinoma is associated with prognosis (5). This study shows that when Ki67 is higher than 35%, it is necessary to be careful about the possibility of deep invasion of EC into the myometrium.

P53 plays an important role in the regulation of cell proliferation, DNA repair, apoptosis, genomic stability, and metabolic homeostasis.

The p53 gene has a leading role in cell cycle control and initiation of carcinogenesis. It is localized in the short arm of chromosome 17 (6). It is a tumor suppressor gene that encodes a nuclear phosphoprotein. The p53 gene is one of the most frequently mutated tumor suppressor genes and is damaged in 40-80% of ovarian carcinomas. The p53 protein accumulates in the cell nucleus and is activated as a transcription factor in response to DNA damage, hypoxia, oncogene activation, and other genotoxic injuries. It has a negative regulatory effect on the cell cycle. Loss of p53 function plays a central role in the development of malignant tumors (7).

Aim

The present study aims to compare the expression of p53 and ki67 in type 1 and type 2 endometrial carcinomas and in low and high grade serous ovarian carcinomas.

Materials and methods

We studied 58 cases of patients with type 1 and type 2 endometrial carcinomas (n=30), and low and high grade serous ovarian carcinomas (n=28) in the age range 43-82 years. 4 μm thick paraffin sections were dewaxed and rehydrated through descending alcohols. Haematoxylineosin staining was performed according to standard methods.

The immunohistochemical study was performed according to standard protocols. Antibodies used, manufactured by Leica Biosystems, Newcastle:

- Bond p53 Protein, clone DO-7, ready to use, 7 ml, cat. No. PA0057. Nuclear staining and diffuse patterns were reported.

- Bond Ki67, clone K2, ready to use, 7 ml, cat. No. PA0230. We reported nuclear expression.

DAB was used as the chromogen. An automated coloring platform was used.

Expression estimation system

Giurgea L et al. defined the following patterns for p53- and Ki-67-positivity of tumor cells (6): focal pattern, which shows a small number of expressing tumor cells; heterogeneous pattern, with islands of strong positive expression alternating with regions of low positive expression; diffuse model representing diffuse positivity. Also, they use a quantitative assessment, depending on the number of stained cells: 0: 0; 1-10%: score 1; 10-50%: score 2; 50-100%: score 3.

Immunohistochemical expression is the sum of the percentage of positive cells and the intensity. At least 10 x400 magnification fields are examined from each preparation.

Statistical analysis

Statistical hypotheses with accurate p-value calculation are applied. To accept the null hypothesis (H0) the criterion "p-value" ≥ 0.05 was used (the probability of making a first-order error is below 5%), and to accept the alternative hypothesis (H1) the criterion "p-value" was applied < 0.05 (the probability of a correct decision is over 95%).

To detect a statistical difference between the values of a parameter representing a continuous random variable, the non-parametric Mann-Whitney U tests (for the comparison of two defined groups of patients) and Kruskal-Wallis with Dunn's post hoc analysis for more than two groups were applied. For comparisons regarding variables taking discrete values, Fisher's Exact Test was applied.

In all cases, a null hypothesis (H0) is defined - the values of the studied parameter for the compared groups originate from the same general population and an alternative hypothesis (H1) - the values of the studied parameter for the studied groups differ statistically.

Presence of statistical significance is determined at a value of p< 0.05; at a value of p ≥ 0.05, there is no statistically significant difference.

Results

In our studies, we reported interesting results and dependencies of these two markers in ovarian and endometrial carcinomas.

Outcomes in endometrial carcinomas

The analysis of our results regarding the difference in p53 expression in relation to the degree of differentiation of endometrial carcinomas found that such a difference existed and it was significant (p=0.017).

Highly differentiated endometrial carcinomas (G1) were negative for p53 expression.

The more poorly differentiated the carcinoma, the stronger the expression of p53 in it. This dependence is shown in Fig. 1.

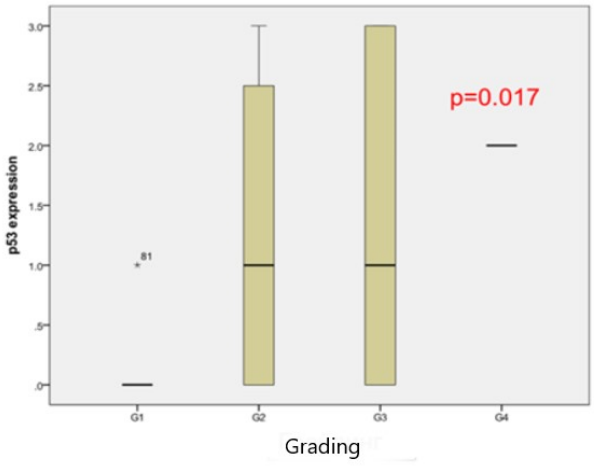
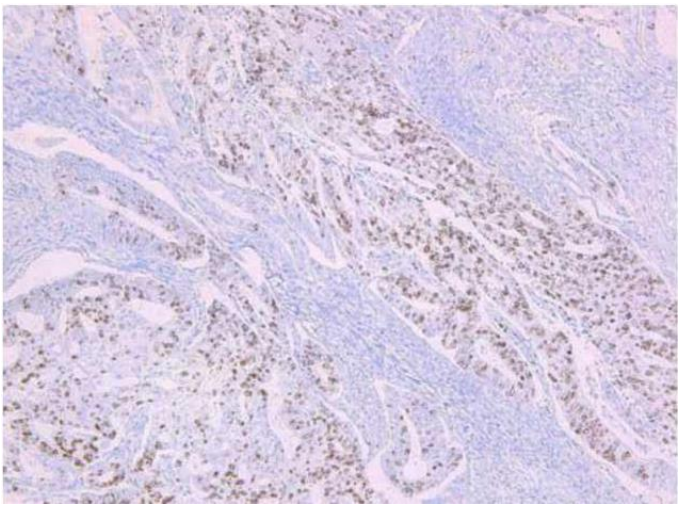


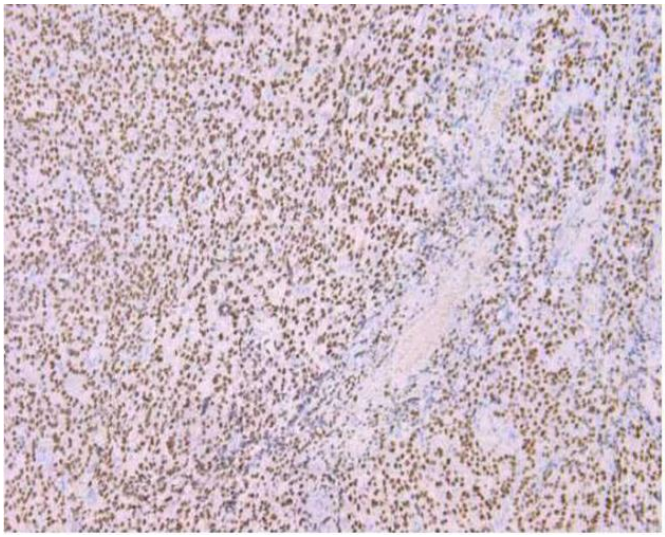
Fig.1 p53 expression

According to the histological type of the carcinomas, we observed a higher expression of p53 in the group of carcinomas type 2, the difference between the two types being significant (p=0.025).

In cases with endometrial carcinomas, we found a statistically significant difference in Ki67 expression relative to the degree of carcinoma differentiation (p=0.018). The expression of the marker becomes stronger as the degree of differentiation of the carcinoma decreases.



Picture 1. Moderate nuclear expression of Ki67 in highly differentiated endometrioid carcinoma of the endometrium, x100



Picture 2. Strong and diffuse nuclear expression of Ki67 in poorly differentiated endometrioid carcinoma of the endometrium, x100

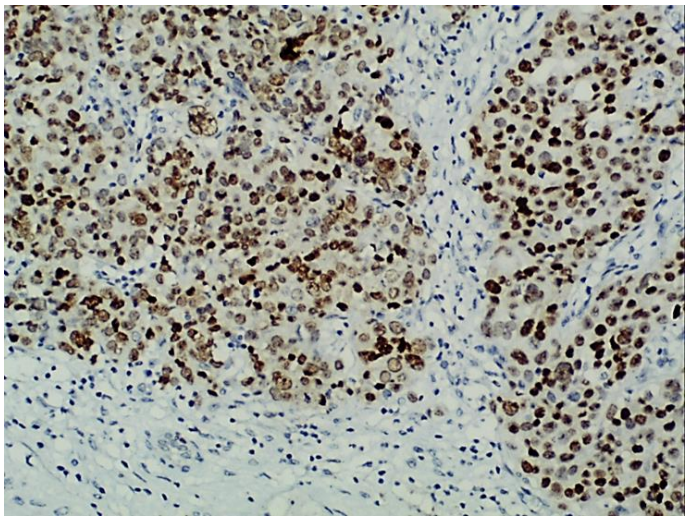
Outcomes in ovarian carcinomas

When comparing the expression of p53 with the proliferative index reported with Ki67 we found an association between the two markers. It is most strongly expressed in the negative expression of p53. 88% of Ki-67 negative cases were p53 negative. P53 negative cases were associated with weak or negative expression for Ki67.

The expression of Ki-67

Data from our study show a direct correlation between p53 and Ki-67 expression in serous ovarian carcinomas, i.e. in the presence of IXX reported mutant p53 molecules, a higher proliferative index of the corresponding tumor was also observed.

The majority of immunohistochemically examined ovarian carcinomas were of a high degree of malignancy. In the p53 positive cases with the highest frequency, we observed tumors with 50 - 100% p53 positive tumor cells.



Picture 3. High-grade serous carcinoma. IHC study: p53: nuclear expression in 50-100% of tumor cells, x100.

Although we did not find a statistical relationship between EMT status and proliferative index in each of the studied groups of working material, we also performed a comparative analysis of the same tumor areas in cases with EMT positive status with a heterogeneous pattern and Ki-67 expression in 50-100% from the tumor cells also with a heterogeneous pattern.

Discussion

Since EMP represents a transition from epithelial to mesenchymal morphology of tumor cells, reduced immunohistochemical expression (negative, weak and moderate expression) of epithelial markers such as E-cadherin is counted as a positive EMP status of the studied tumor (8). The decrease in E-cadherin expression can only occur in groups of cells that represent specific branches in the tumor with different adhesive properties. In order to refine the results for EMF positive status, in our study the E-cadherin positive cases with the presence of regions with negative expression, i.e. with a heterogeneous staining pattern, were assigned to the group of tumors with EMF positive status.

Some authors do not find a correlation of nuclear expression of the p53-marker with clinicopathological factors, incl. age, histological subtype, grading and staging (9). Other authors have reported that p53 overexpression is more common in serous ovarian carcinomas than in mucinous carcinomas and correlates with the malignant potential of serous tumors. In most studies, there was a positive association between p53-expression and higher grade (lower differentiation) of ovarian serous carcinomas (6).

The role of mutant p53 molecules in the initiation of EMP in the progression of carcinomas is unclear. Loss of p53-protein function suppresses epithelial markers such as E-cadherin and activates mesenchymal markers such as Vimentin and N-cadherin (10). As a result, epithelial-mesenchymal transition is induced in borderline serous ovarian tumors (1). Suppression of mutant p53 molecules in cancer cells leads to reversal of epithelial-mesenchymal transition into mesenchymal-epithelial transition and inhibition of cell invasive ability (11).

Ki67-positive tumor cells often correlate with the clinical course (6). The Ki-67 index is higher in advanced stage tumors and a higher Ki-67 index indicates a more aggressive tumor behavior and a worse clinical course.

A significant difference in Ki67 immunohistochemistry was found between ovarian carcinomas and benign tumors, and between borderline tumors and carcinomas, but not between benign and borderline tumors (12). The frequency of Ki-67-positive carcinomas increases with increasing grade of malignancy (13). These results indicate that a morphologically problematic serous carcinoma with a significantly elevated Ki-67-index is unlikely to be of low grade. Other authors found no correlation between the degree of differentiation and the Ki-67-index (12).

The p53 gene is an EMF inhibitor. When mutations occur in the p53 gene, the mutant molecules can be visualized immunohistochemically. They suppress epithelial markers such as E-cadherin and induce a positive EMP status in the corresponding tumors (11).

We analyzed our results regarding the difference in p53 expression in relation to the degree of differentiation of endometrial carcinomas. We found that such a difference existed and it was significant (p=0.038). The lower the differentiation of the carcinoma, the stronger the expression of p53 in it. Our results agree with those of other authors (14).

Unlike other authors, however, we did not find a statistically significant difference in p53 expression in the group of carcinomas according to the depth of invasion and FIGO stage (p=0.442). There was no such difference between the expression of p53 in the tumor parenchyma and in the invasive front of the carcinoma (p=0.787). The marker Ki67 is used to assess cell proliferation and is increasingly used in the preoperative phase in patients with endometrial carcinoma as a prognostic marker (15).

In our study, Ki67 expression was nuclear in all cases with positive expression. In endometrial carcinomas, we scored Ki67 expression according to the degree of differentiation. We found that in highly differentiated carcinomas (G1), 2(40%) had negative expression, 2(40%) had expression in 1-10% of tumor cells and only 1(20%) case had expression in 50 -100% of tumor cells. In moderately differentiated carcinomas (G2), those with negative expression were 4(26.7%), those with expression in 1-10% of tumor cells were

6(40%) cases, and those with expression in 10-50% of tumor cells, were 5 (33.3%). We had no cases of highly or moderately differentiated carcinomas with expression in 50-100% of tumor cells.

In poorly differentiated carcinomas, the number of Ki67-negative cases was only 1 (8.3%). Those with expression in 1-10% of tumor cells were 3(25%), 7(58.3%) had expression in 10-50% and one (8.3%) case had Ki67 expression in 50-100% of tumor cells. cells. Our results are similar to those of other authors (5).

In the group of endometrial carcinomas, we found a statistically significant difference in Ki67 expression relative to the degree of carcinoma differentiation (p=0.046). The expression of the marker becomes stronger as the degree of differentiation of the carcinoma decreases. These data correspond to the observations of other authors (5).

Regarding the expression pattern of Ki67 in the group of endometrial carcinomas, 7(21.2%) of the cases had a negative expression, those in a focal expression pattern were 13(39.4%), with heterogeneous - 11 (33.3%), and with diffuse - 2 (6.1%) cases. These results are similar to other studies (6), where the focal pattern predominates in the more highly differentiated carcinomas, and the heterogeneous and diffuse pattern predominates in higher grade.

Conclusion

On the difference in p53 expression in relation to the degree of differentiation of endometrial carcinomas. We found that such a difference existed and it was significant (p=0.038). The lower the differentiation of the carcinoma, the stronger the expression of p53 in it.

In the group of endometrial carcinomas, we found a statistically significant difference in Ki67 expression relative to the degree of carcinoma differentiation (p=0.046). The expression of the marker becomes stronger as the degree of differentiation of the carcinoma decreases.

There is a strong correlation between p53 expression and proliferative index (Ki67) in endometrial carcinoma cases, with more aggressive forms of carcinomas with a higher FIGO stage having stronger expression of these markers.

The expression of p53 and Ki67 depended on the degree of differentiation of endometrial carcinomas, with less differentiated carcinomas showing stronger expression of p53 and Ki67 in tumor cells.

In p53-positive high-grade serous ovarian carcinomas, a higher proliferative index is reported, which is a sign of more aggressive tumor growth.

Acknowledgments

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