Immunoexpression of CAS protein is augmented in high grade serous ovarian tumors

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Ovarian cancer is one of the most important causes of death from gynecological malignancies in Poland. Recent investigations took a note of possible relationship between tumor histological grading and immunoexpression of apoptosis and proliferation related proteins in serous ovarian cancers. The aim of the study was to assess the immunoexpression of CAS protein in serous ovarian tumors of different histological grade, as well as to find possible relationships between this immunoexpression and tumor proliferation activity expressed by immuneexpression of Ki-67 protein. The analysis comprised of 66 women diagnosed and treated for malignant epithelial ovarian tumors. The immunoexpression of CAS protein was assessed semiquantitatively whereas immunoexpression of Ki-67 was performed using computer image analysis system. On immunohistochemical examinations it was found a significantly higher immunoexpression of both examined proteins in invasive serous ovarian cancers than in cystadenomas. Also, the significant positive correlation has been shown between immunoexpression of Ki-67 and CAS protein in particular group of tumors. In conclusion, our data suggest that increased immunoexpression of CAS protein in serous ovarian tumors may be useful in identifying the patients with more aggressive disease.

Key words: serous ovarian tumors, apoptosis, proliferation, CAS, Ki-67.

Introduction

Ovarian cancer is one of most dangerous gynecological malignancies in Poland [1] and in the World. It takes 6th place in Poland and 5th place in United States as a cause of death from all malignancies. Serous epithelial tumors are the most frequent neoplasms of the ovary – they state 60% of all ovarian tumors and 90 % of all ovarian malignancies [2, 3]. Therefore it is important to find a useful marker of malignant potential of serous ovarian tumors. Last investigations indicates th role of apoptosis – related proteins in carcinogenesis of ovarian cancers. The CAS (cellular apoptosis susceptibility) protein, product of the CAS gene is a homologue of the chromosome segregation gene (CSE) [5, 6]. It is connected with the microtubules of karyokinetic spindle during mitosis. The CAS is a protein which probably works as a switch between apoptosis and proliferation signalling, but it's mechanism is not fully clear yet. A scant literature shows possible relationships between CAS expression and histological grade of ovarian tumors {13]. The Ki-67 is well – known protein which immunoexpression is strictly connected with cell cycle: it's expressed in G1, S and G2 phases of cell cycle and remains unexpressed in G0 phase {4]. This protein is useful proliferation marker for malignant tumors.

The aim of our study was to assess the immunoexpression of CAS protein in serous ovarian tumors of different histological grade, as well as to find the possible relationships between this immunoexpression and tumor proliferation activity expressed by immuneexpression of Ki-67 protein.

Materials and methods

Patients

The analysis comprised of 66 women diagnosed and treated for epithelial ovarian tumors at Gynecology and Obstetrics Institute of Medical University of Lodz between 1997 and 2002. Patients were divided into five groups according to histological grade of tumor. Fifteen women were treated from benign tumors – cystadenomas of the ovary. Eight women had borderline serous ovarian tumors. Forty three women suffered from ovarian serous cancer: twenty one women of this group were treated from G3 serous cancer. Fourteen women had G2 serous cancer. Eight women suffered from G1 serous cancer.

Light microscopy

Paraffin embedded tissue sections taken from postoperative material were diagnosed using a standard haematoxylin and eosin staining. Tumors were assesed using WHO 2002 classification of ovarian tumors [7].

Immunohistochemistry

Paraffin sections were mounted on Superfrost slides, deparaffinised, then treated in a microwave oven in a solution of citrate buffer, pH 6.0 for 2×5 min and transferred to distilled water. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide in distilled water for 5 min, and then sections were rinsed with Tris-buffered saline (TBS, DakoCytomation, Denmark) and incubated overnight at 4°C with the primary antibody for CAS (mouse monoclonal anti-human CAS, Novocastra; dilution 1 : 80) and for Ki-67 (mouse monoclonal anti-human Ki-67; Novocastra; dilution 1 : 100). Afterwards LSAB+/HRP Universal kit (DakoCytomation, Denmark) prepared according to the instructions of the manufacturer was used. Visualisation was performed by incubating the sections in a solution of 0.5 mg 3.3'-diaminobenzidine (DakoCytomation, Denmark), per ml Tris-HCl buffer, pH 7.6, containing 0.02% hydrogen peroxide, for 10 min. After washing, the sections were counterstained with haematoxylin and coverslipped. For each antibody and for each sample a negative control was processed. Negative controls were carried out by incubation in the absence of the primary antibody and always yielded negative results.

Morphometry

The immunoexpression of CAS protein was assesed semiquantitatively, using the scale shown below:
0 – negative immunoexpression of CAS protein in neoplastic cells;
1 – weak immunoepression of CAS protein in small ($< 25\%$) count of neoplastic cells;
2 – moderate immunoepression of CAS protein in 26 -75% of neoplastic cells;
3 – strong immunoepression of CAS protein in the most ($> 76\%$) of neoplastic cells;

The Ki-67 immunoexpression was assessed by means of image analysis system consisting of a PC computer equipped with a Pentagram graphical tablet, Indeo Fast card (frame grabber, true-color, real-time), produced by Indeo (Taiwan), and color TV camera Panasonic (Japan) coupled to a Carl Zeiss microscope (Germany). This system was controlled by MultiScan 8.08 software, produced by Computer Scanning Systems, Poland, working under macroinstructions written specially for this analysis. A percentage of immunopositve cells in a 1000 tumor cells for each slide was measured.

Statistical analysis

Differences between groups were tested using one-way ANOVA with post-hoc RIR Tukey test, preceeded by evaluation of normality. The Kruskall-Wallis test was used where appropriate. Correlation coefficient was calculated using Spearman's method. Results were considered statistically significant if $p < 0.05$.

Results

On immunohistochemical examinations the immunoexpression of CAS protein was the lowest in cystadenomas and it grows up with histological grade of tumors (Table I). We found significantly higher immunoexpression of analysed protein in G3 and G2 groups of cancers as compared to cystadenomas and borderline tumors (Fig. 1, 2). No significant differences were found as between G3 and G2 groups of cancers as well as between cystadenomas and borderline tumors. The immunoexpression of CAS was significantly increased in G1 group of cancers in comparison with cystadenomas.

The immunoexpression of Ki-67 protein was also growing up with histological grade of tumors (Table II). We found statisticaly higher immunoexpression of Ki-67 protein in G3 and G2 groups of cancers than in all groups of lower histological grade (Fig. 3, 4). We did not observe any significant differences between cystadenomas and borderline tumors and between G1 group of cancers and borderline tumors.

Strong positive correlation existed between the immunoexpression of CAS and Ki-67 protein (Fig. 5).

Discussion

The apoptosis and proliferation processes plays an important role in tumorgenesis of most neoplasms,

Table I. The immunoexpression of CAS protein in serous ovarian tumors

Group	Cystadenomas (N = 15)	Borderline tumors (N = 8)	G1 Cancers (N = 8)	G2 Cancers (N = 14)	G3 Cancers (N = 21)
Mean ± SD	0.6 ±0.37	0.8 ±0.59	1.6 ±0.91	1.9 ±0.8	2.1 ±0.6
p-value	0.987 (NS)[2]	0.987 (NS)[1]	< 0.05[1]	< 0.0005[1]	< 0.0005[1]
	< 0.05[3]	< 0.05[3]	< 0.09 (NS)[2]	< 0.05[2]	< 0.005[2]
	< 0.0005[4]	< 0.0005[4]	< 0.93 (NS)[4]	< 0.93 (NS)[3]	< 0.60 (NS)[3]
	< 0.0005[5]	< 0.0005[5]	< 0.60 (NS)[5]	< 0.90 (NS)[5]	< 0.90 (NS)[4]

[1] *vs. cytadenomas*
[2] *vs. borderline tumors*
[3] *vs. G1 cancers*
[4] *vs. G2 cancers*
[5] *vs. G3 cancers*

including serous ovaian tumors [8, 9]. The classic apoptosis markers are bcl2 – family proteins, but recent investigations took a note for CAS protein as a possible marker of apoptosis. The mechanisms of CAS protein are not fully understood yet, but it is assumed that plays a role in both cell apoptosis and in the cell proliferation. The CAS protein is a homologue of protein responsible for the chromosome segregation during G1 phase of cell cycle. Therefore the CAS protein is highly expressed in proliferating cells whereas it remains unexpressed in nonproliferating cells. On the other hand, the CAS protein is strictly connected with apoptosis, induced by tumor necrosis factor α (TNF-α) [10]. This mechanism also remain unclear. It is supposed, that CAS plays it's role as a nuclear membrane transport protein for other apoptosis – related proteins. According to this dual role, CAS protin is considered as a switch between cell death and proliferation. In rather scant yet literature, the authors take a note of high corelation between CAS immnoexpression and high grading of breast, liver and ovarian malignancies [11, 12]. Brustmann [13] docummented the positive corelation between immunoexpression of CAS protein and histological grading of serous ovarian tumors and mitotic index of these tumors. Our study confirmed these obervations. We noted strong positive corelation between tumor proliferation activity expressed by immunoexpression of Ki-67 protein and immunoexpresion of CAS protein in serous ovarian tumors. Moreover, we found statisticaly significant differences between immunoexpression of examined proteins in benign and malignant tumors. These findings are probably related to disturbances of CAS – dependent cell cycle regulation and with deregulation of apoptosis. These disturbances could be derived from mutations of CSE1 gene, which were described by Wantabe *et al.*, Tanner *et al.* [14, 15]. Peiro et. al. considered mutations of genes on chromosome 20q13.2 and mutations of CYCD1 gene as a main pathways leading to ovarian cancer tumorge-

nesis [16]. Interpretation of our findings should be considered carefuly, because of small amount of available data in literature, but our results suggest that innunoexpression of CAS protein may be used as agressiveness marker of serous ovarian tumors.
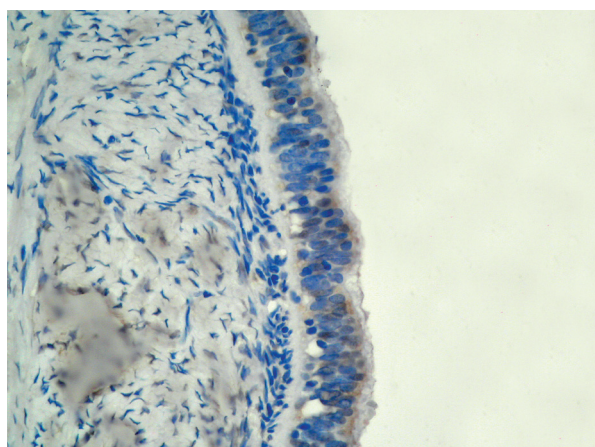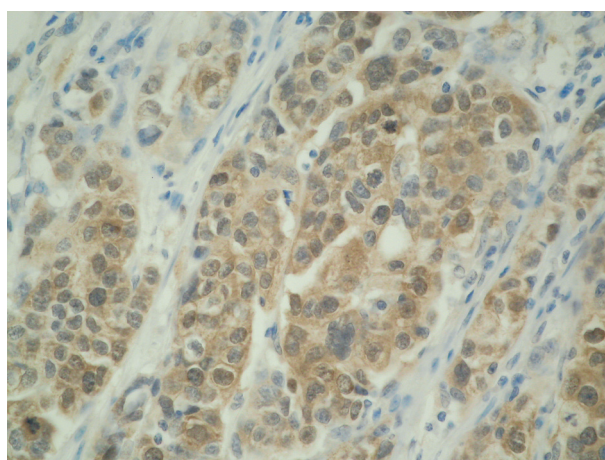

Fig.1. Immunoexpression of CAS protein in ovarian cystadenoma (magnification 100×)

Fig. 2. Immunoexpression of CAS protein in G3 ovarian serous cancer (magnification 200×)

Table II. The immunoexpression of Ki-67 protein in serous ovarian tumors

Group	Cystadenomas (N = 15)	Borderline tumors (N = 8)	G1 Cancers (N = 8)	G2 Cancers (N = 14)	G3 Cancers (N = 21)
Mean ± SD	0.6 ±0.37	0.8 ±0.59	1.6 ±0.91	1.9 ±0.8	2.1 ±0.6
Mean ± SD	1.50 ±1.53	4.27 ±4.59	14.08 ±7.33	31.59 ±8.94	39.86 ±8.35
P value	0.653 (NS)[2]	0.653 (NS)[1]	< 0.01[1]	< 0.0005[1]	< 0.0005[1]
	< 0.01[3]	0.183 (NS)[3]	0.183 (NS)[2]	< 0.0005[2]	< 0.0005[2]
	< 0.0005[4]	< 0.0005[4]	< 0.0005[4]	< 0.0005[3]	< 0.0005[3]
	< 0.0005[5]	< 0.0005[5]	< 0.0005[5]	< 0.05[5]	< 0.05[4]

[1]vs. cytadenomas
[2]vs. borderline tumors
[3]vs. G1 cancers
[4]vs. G2 cancers
[5]vs. G3 cancers

On the other hand, the Ki-67 protein is at present widely used as a marker of proliferating cells. Thus proliferation index, defined as percentage of tumor cells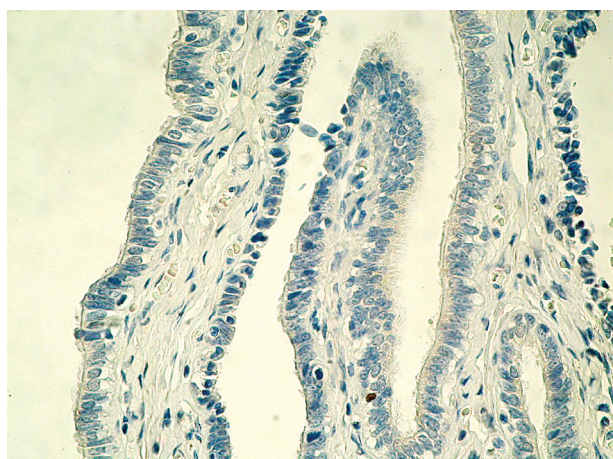 with positive nuclear immunoexpression of Ki-67, should grow up with an increase of tumor histological grade. That relationship was shown by many authors, not only in ovarian tumors [17, 18]. Numerous authors regard the proliferation index as prognosis factor in malignant neoplasms, including serous ovarian cancer [17-19]. Our results seem to confirm these observations.

In conclusion our results suggest that increase of immunoexpression of both examined proteins is a constitutive finding in malignant serous ovarian tumors and may be helpful in identyfing patients with more aggresive disease for better diagnosis and therapy.

Acknowledgement

Fig. 3. Immunoexpression of Ki-67 protein in ovarian cystadenoma (magnification 100×)

Fig. 4. Immunoexpression of Ki-67 protein in G3 ovarian serous cancer (magnification 200×)
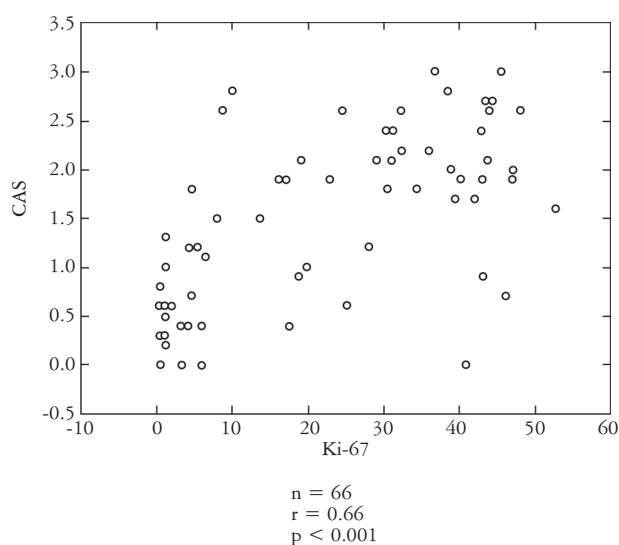

n = 66
r = 0.66
p < 0.001

Fig. 5. The correlation between CAS and Ki-67 protein

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