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Survivin in ovary tumors

Surwiwina a rak jajnika

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Abstract

Introduction: *Survivin is a member of the inhibitor of apoptosis protein (IAP) family, which are selectively overexpressed in human neoplasms, and its expression has been shown to be connected with cell proliferation. We analyzed survivin expression in ovarian epithelial neoplasms to evaluate its role in the development of ovarian tumors.*

Material and methods: *Immunohistochemistry assays were conducted in 137 cases (48 ovarian carcinoma, 43 borderline ovarian carcinoma, 46 benign ovarian tumor, and 20 samples of normal ovarian tissue of ovarian epithelial neoplasms. Histological types included serous (n=68) and mucinous (n=69) tumors. All tumors were reviewed histopathologically and classified according to the WHO criteria.*

Results: *Survivin expression in the group of serous neoplasms was detected in 24.0% (6 of 25) of benign cases, in 60.0% (12 of 20) of borderline tumors, and 91.0% (24 of 47) of ovarian carcinomas. In the group of mucinous tumors, survivin expression was found in 33.5% (7 of 21) of benign cases, 43.5% (10 of 23) of borderline tumors, and 80.0% (20 of 25) of malignant tumors.*

Conclusions: *Our results demonstrate that survivin overexpression may play a crucial role in the development of epithelial ovarian neoplasms and be an important prognostic factor for the influence of survivin expression on epithelial ovarian cancers.*

Key words: **ovarian cancer subtypes** / **IHC** / **survivin** /

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Streszczenie

Wstęp: *Surwiwina należy do rodziny inhibitorów białek apoptozy (IAP – inhibitor of apoptosis protein), cechujących się selektywną nadekspresją w procesie rozwoju nowotworów. Wykazało, że ekspresja surwiwiny jest związana z proliferacją komórek. Celem pracy było określenie roli surwiwiny w guzach jajnika poprzez badanie ekspresji tego białka w grupie nabłonkowych nowotworów jajnika.*

Materiał i metody: *Immunohistochemiczne badania przeprowadzono na 137 przypadkach nowotworów nabłonkowych jajnika (48 przypadków raka jajnika, 43 przypadki granicznych raków jajnika, 46 przypadków łagodnych guzów jajnika, oraz 20 próbek prawidłowej tkanki jajnikowej). Histologicznie badane nowotwory zakwalifikowano jako surowicze (n=68) oraz śluzowe (n=69) guzy. Wszystkie nowotwory były badane histopatologicznie I klasyfikowane zgodnie z kryteriami WHO.*

Wyniki: *W grupie nowotworów o charakterze surowiczym ekspresję surwiwiny wykryto w 24,0% przypadków z guzami łagodnymi (6 z 25), w 60,0% (12 z 20) w nowotworach granicznych oraz w 91,0% (24 z 47) z rakach jajnika. W puli nowotworów o charakterze śluzowym wykazano ekspresję surwiwiny w 33,5% przypadków (7 z 21) nowotworów łagodnych. Wśród nowotworów granicznych ekspresję surwiwiny stwierdzono w 43,5% przypadków (10 z 23), a wśród nowotworów złośliwych w 80% (20 z 25) badanych prób.*

Wnioski: *Nadekspresja surwiwiny może odgrywać kluczową rolę w rozwoju nowotworów nabłonkowych jajnika u kobiet i dodatkowo może stanowić istotną wartość prognostyczną wpływu tej ekspresji na nabłonkowe raki jajnika.*

Słowa kluczowe: **rak jajnika** / **IHC** / **surwiwina** /

Introduction

Survivin belongs to the inhibitor of apoptosis protein (IAP) family and is coded by the gene localized on the long arm of chromosome 17 (17q11) [1]. Survivin contains only one amino-terminal BIR domain and does not have zinc finger-like motif located near its carboxyl terminus [2]. The presence of at least one such domain is necessary to maintain anti-apoptotic protein activity [3]. This protein counteracts apoptosis through inhibition of initiator and effector caspases, and binds to mitotic spindle microtubules, thus inhibiting the mitochondrial pathway of apoptosis. Apart from cell death regulation, survivin plays a crucial role in the cell cycle, and its quantity is regulated during transcription.

Experimental proof confirmed the pivotal role of survivin in apoptosis modulation. Firstly, increased survivin expression in cell line cultures is associated with inhibition of apoptosis, induced through various factors stimulating apoptotic pathways. Secondly, the anti-apoptotic function of survivin has been demonstrated in experimental animals. Moreover, the use of survivin antisense induces apoptosis, increases caspase activity, and inhibits cells proliferation in multiple groups of animal cell cultures [4].

Survivin controls caspase-dependent and caspase-independent apoptosis [5]. Although there is no survivin in normal cell mitochondria, it is present in neoplastic cells, what shows its unique role in cancer etiology [6]. In response to cell death-inducing agents, survivin mitochondrial level decreases and is released into the cytoplasm, where it prevents caspase activation and inhibits apoptosis. Survivin inhibits the end effector, or caspase 3, 7 and 9 in cells receiving the signal to apoptosis, which implies tumor resistance to apoptosis promoters, including chemotherapy [7].

Ovarian cancer has the highest mortality rate among malignant tumors of the female reproductive system. Although the 5-year survival rate has improved recently, it remains low and does not exceed 30% of the affected individuals, largely due to lack of effective diagnostic methods and treatment [8]. Radical surgery, combined with chemotherapy, has failed to provide satisfactory therapeutic results for many years. Therefore, it is important to identify and verify a biomarker that might detect high-risk patients. Numerous studies [9-12] demonstrated survivin to be associated with tumor aggressiveness and adverse clinical effects.

According to some reports, expression of survivin mRNA correlates with other prognostic factors, such as clinical stage of a tumor, histological differentiation or lymph node involvement, and this correlation is associated with progression of ovarian cancer [13]. This correlation is as yet uncertain for survivin protein, although studies are ongoing. As survivin is found in tumor cells, it has become a well-established target in ovarian cancer therapies. The tertiary structure of survivin creates many problems, including the possibility of using specific antibodies [14, 15].

Survivin is expressed in human neoplasms but its level varies in different tissues and is directly associated with poor patient outcome. Numerous studies demonstrated the rate of expression and subcellular localization of survivin to be correlated with progression and prognosis of ovarian cancer [14, 16,18]. The aim of our research was to reveal these correlations by analyzing expression levels in epithelial ovarian neoplasms and different stages of ovarian cancer using immunocytochemical staining. We wished to investigate whether survivin protein expression is associated with progression of ovarian cancer and can be used as a prognostic marker for ovarian cancer patients.

Material and methods

Patients

The study was conducted in 48 cases of ovarian carcinoma, 43 cases of borderline ovarian carcinoma, 46 cases of benign ovarian tumor, and 20 samples of normal ovarian tissues from women who underwent total abdominal hysterectomy for non-gynecologic diseases. Histological types included serous (n=68, mean age 48.1±8.4, range 32-56 years) and mucinous (n=69, mean age 46.7±7.3 years, range 34-58 years) tumors. All tumors were reviewed histopathologically and classified according to the criteria of WHO.

The exclusion criteria were as follows: chemotherapy before staging laparotomy, diagnosed tumors of the uterus and other organs, hormonal substitute therapy or oral contraceptives, autoimmune diseases, pregnancy and breastfeeding.

All patients underwent laparotomy at Bielsko-Biała Center of Oncology in Poland. The study was accepted by the Bioethical Commission of the Silesian Medical University in Katowice, Poland (KNW/0022/KB/54/10).

Immunohistochemical studies

Tissue samples were fixed in 10% (v/v) solution of buffered formalin for 24h at 4°C, and then dehydrated, cleared in xylenes and embedded in paraffin. Paraffin sections (5 μm) were mounted on silane-coated slides, de-waxed, and rehydrated. The sections were treated with Tris-EDTA buffer, pH 9.0 in water bath (30 min. at 95°C) for antigen retrieval, then treated with 3% (v/v) H_2O_2 for 10 min. for quenching of endogenous peroxidase activity, and washed in 10 mM PBS-0.05% v/v Tween 20, pH 7.5. Non-specific binding was reduced by incubation with normal horse serum for 60 min. Next, the sections were incubated with mouse anti-survivin (Santa Cruz Biotech) monoclonal antibody in a humidified chamber overnight at 4° C. After washing in PBS-Tween 20, the sections were incubated with biotinylated horse anti-mouse immunoglobulins (Vector Laboratories, Burlingame, CA, USA) for 30 min., and next with avidin-biotinylated peroxidase complex (Vector) for 30 min. The bound antibodies were visualized with diaminobenzidine (DAB, Vector) and H_2O_2 in PBS, pH 7.5 according to supplier's instructions. Finally, the tissues were stained with Gill's hematoxylin, dehydrated, and cover-slipped. Negative controls were performed by substituting the primary antibody with mouse IgG at the same concentration.

Survivin expression was scored for nuclear staining. Light microscope (Nikon Eclipse E200 with DS-Fi1 camera) at 400x magnification was used to count 300 cells in each section. The nuclear expression of survivin was described as percentage of stained nuclei ± SD.

Statistical analysis

One-way ANOVA was used for statistical analysis. Due to heteroscedasticity, Games-Howell test was used for post-hoc multiple comparisons. Statistically significant differences were found in all stages of tumor differentiation, at a significance level of $p<0.01$. Ranges in the figures represent the 95% confidence interval.

Results

No survivin expression was observed in control group samples. The study group results showed nuclear expression of survivin in all examined cases which revealed the presence of neoplastic lesions (Fig. 1). These changes are associated with the degree of tumor differentiation. Survivin expression in the nucleus was observed in only 6/25 patients with benign serous tumors, 12/20 with borderline cancer, but 21/23 with malignant carcinomas.

A similar trend was observed in the group of mucinous ovarian cancers. Survivin expression in cell nuclei was detected in only 7/21 patients with benign mucinous cancer, 10/23 with borderline cancer, but 20/25 with malignant tumors.

The analysis of the number of nuclei expressing survivin in serous and mucinous benign tumors revealed it to be notably lower in the latter, constituting approximately 60% of the pool of nuclei expressing that protein in serous tumors (Table I). Similar findings were observed in the group of borderline tumors, where the number of nuclei with this protein expression represented 75% of the studied serous tumors. In primary malignant ovarian cancers, the number of nuclei with survivin expression varied slightly between serous and mucinous tumors (Table I). The number of these nuclei in mucinous cancers was 90% as compared to malignant serous cancers.

Evaluation of survivin expression in ovarian cancers showed that in the group of serous tumors the number of survivin-expressing nuclei was notably higher (by 210%) in borderline than benign tumors (Table I). This growth in malignant tumors, in comparison to benign tumors, was even higher (by 330%). A differential analysis of the number of survivin-expressing nuclei revealed it to be higher in the group of malignant tumors (by 160% as compared to borderline tumors).

A similar analysis relating to mucinous ovarian tumors showed that, in comparison to benign tumors, the number of nuclei with survivin expression was higher, reaching 245% of the pool of stained nuclei in benign tumors (Table I). In malignant tumors, there was an even greater increase in the number of nuclei with the expression of survivin (by 440%) as compared to benign tumors. A comparison between borderline and malignant tumors demonstrated that the number of nuclei with survivin expression increased by 180% in favor of malignant tumors.

Discussion

Ovarian cancer is one of the most common female cancers and the most frequent cause of gynecologic cancer-related deaths in the world. Although clinical and histological prognostic factors

Table I. Survivin expression in ovarian tumors.

Probe	Serous			Mucinous		
	Number of patient	Patients with positive reaction	Percent of stained nucleus	Number of patient	Patients with positive reaction	Percent of stained nucleus
Benign ovarian tumor	25	6 (24,0%)	23,8 ± 2,1	21	7 (33,5%)	15,4 ± 3,6
Borderline ovarian carcinoma	20	12 (60,0%)	49,7 ± 2,9	23	10 (43,5%)	38,4 ± 4,4
Ovarian carcinoma	23	21 (91%)	78,5 ± 5,1	25	20 (80,0%)	69,4 ± 6,5

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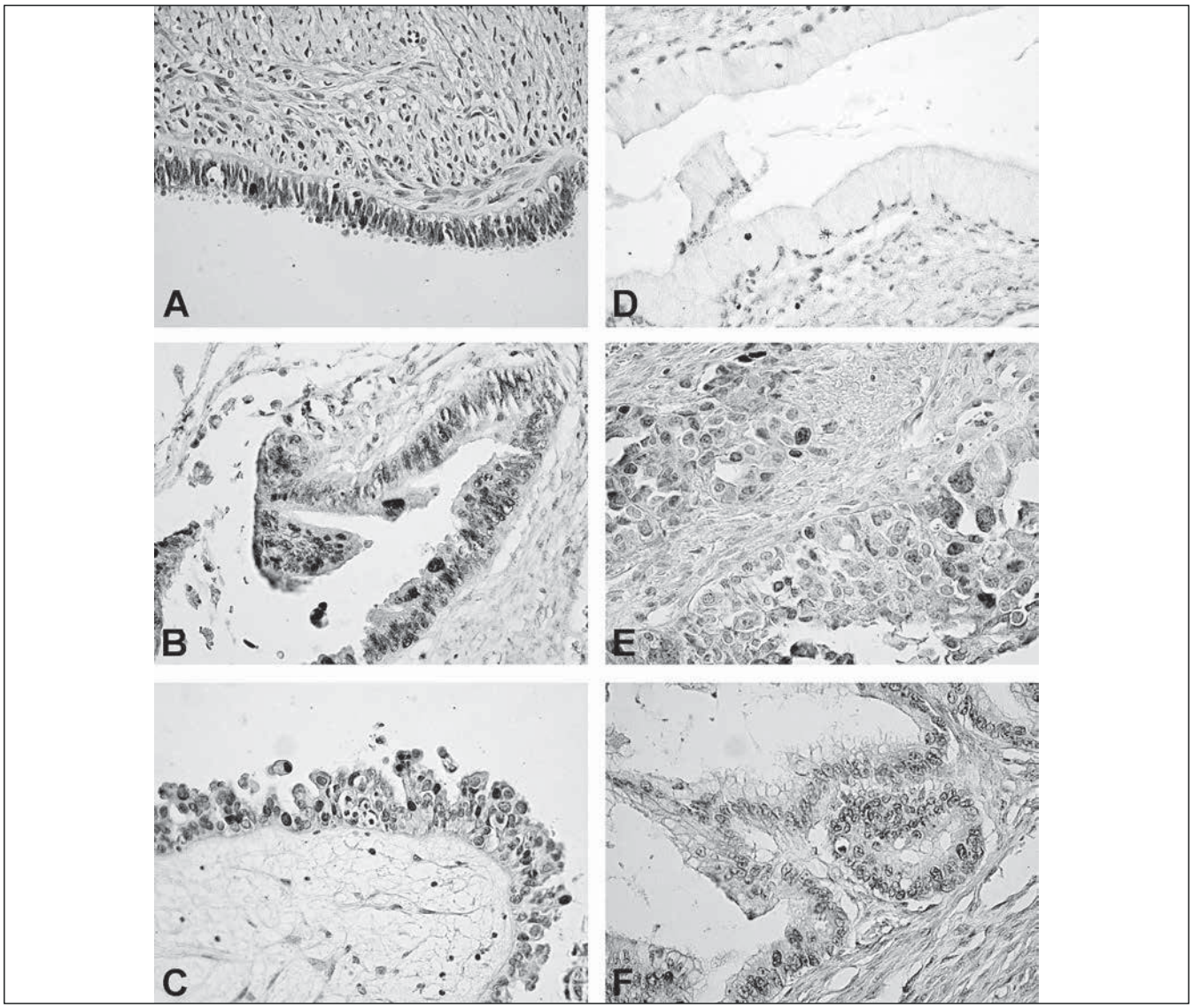

Image 1. Immunoreactivity of survivin in benign (A, D), borderline (B, E) and malignant (C, F), serous (A, B, C) and mucous (D, E, F) ovarian tumors. Magnification ×400.

such as tumor type and clinical stage have been proposed [19], it cannot be ruled out that biochemical assessment of cancer cells and its aggressiveness can help to identify high-risk patients [20].

Survivin is treated as a potential target in molecular therapy because of its strong anti-apoptotic activity. Nevertheless, the results of numerous studies on clinical significance of survivin in cancer are inconclusive.

Significant amount of research demonstrated the mRNA expression level of survivin to correlate with prognosis in cancer, for example in the osteosarcoma syndrome [21, 22]. Survivin expression level is proposed as a marker of progression and prognosis in ovarian cancer. Alas, reports about survivin expression and its influence on patient outcomes are scant [16-18].

Despite a growing number of analytical data on survivin expression in primary ovarian cancer, the conclusions on its clinical role in the development of this neoplasm are incomplete [9, 18, 22]. Obviously, differences between the studies may be explained by a varying number of groups and clinical characteristics of the examined cases [9, 15, 23, 24], as well as methodological differences. For example, different antibodies and different immune systems were used for the evaluation of survivin expression. Moreover, survivin staining in the cytoplasm and the nucleus is widely used, however some authors did not clarify whether the overall level of positive response came from one subcellular compartment or both [9, 15, 18, 24].

We demonstrated that the survivin expression rate is associated with the progression of ovarian cancer and other parameters, such as the degree of differentiation or histological type. Survivin expression levels were highest in ovarian cancers and lowest in the tissue of benign ovarian neoplasms. Moreover, survivin expression levels are associated with histological type of ovarian cancer, i.e., serous vs. mucinous tumors.

Many authors reported the expression of survivin in the nuclei of cancer cells to be associated with poor prognosis and high risk of death [25, 26], but several studies showed an inverse correlation [2, 27]. Two research centers investigated survivin expression and tumor response to chemotherapy in ovarian cancer [14, 28]. A correlation between cytoplasmic or nucleic expression

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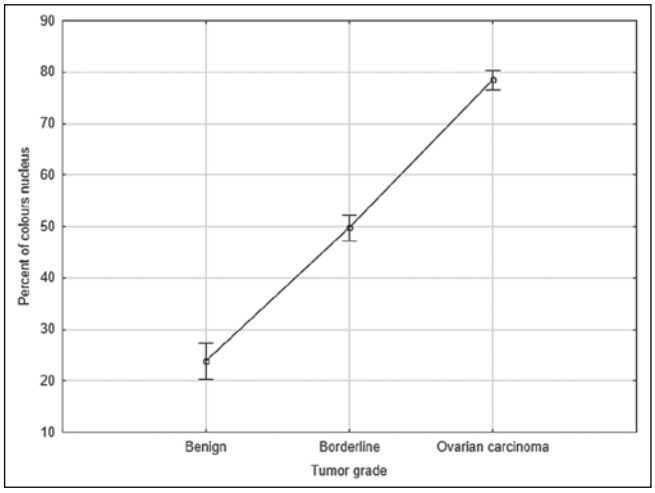

Figure 1. The percentage of stained nuclei depending on the malignancy stage of serous tumors.

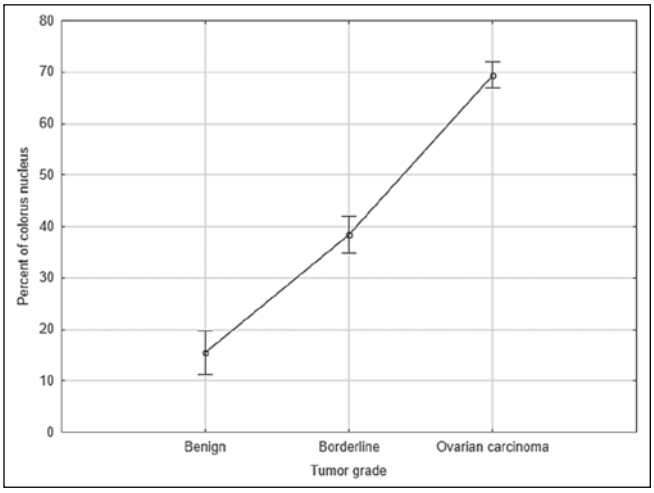

Figure 2. The percentage of stained nuclei depending on the malignancy stage of mucous tumors.

of survivin and response to platinum/cyclophosphamide was not observed [14]. Another team reported a significantly higher ratio of remission after Taxol treatment in a group of patients with low survivin expression in the tumor. However, only cytoplasmic expression of survivin was detected in this experiment [28].

Our findings seem to support the hypothesis that the level of survivin allows to predict a more aggressive prognosis, although some data indicate the absence of any relationship between the expression of survivin and prognosis for certain solid tumors [5]. Also, a directly proportional correlation between high level of survivin in the nucleus and good prognosis in stomach and bladder neoplasms was found [29, 30]. It cannot be excluded that biological and clinical significance of survivin expression is associated with tissue specificity.

Survivin may play a dual role, depending on its cellular location. It has no anti-apoptotic abilities in the cytoplasm because it does not inhibit caspase activity. There is no definitive evidence that survivin-expressing nucleus is a factor promoting cell proliferation [31, 32]. Experiments on different cell lines showed that survivin inhibits defect formation during cell division and suppresses proliferation. Some clinicopathological studies revealed a positive correlation between the expression of survivin in the nucleus and various parameters of growth factors in certain cancers. These results indicate that survivin expression is an adverse prognostic factor.

In conclusion, the presence of this protein may be a diagnostic and a prognostic marker. It can also become a new potential target in treating certain types of neoplasms, at least in the early stages of the disease. Perhaps in the near future survivin will be a new cancer marker in routine diagnostic assays, improving the detection of early-stage neoplasms. It can be a step towards introducing a proper treatment and, above all, extending the life of many patients.

Conclusions

1. Survivin expression is strongly related to the degree of malignancy and tumor differentiation.
2. Survivin expression is higher in serous neoplasms than in mucinous neoplasms.

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