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Expression of Interactive Genes Associated with Apoptosis and Their Prognostic Value for Ovarian Serous Adenocarcinoma*

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Abstract

Background. Malignant ovarian tumor is one of the leading causes of worldwide cancer death. It is usually characterized by insidious onset and late diagnosis because of the absence of symptoms, allowing ovarian cancer cases to progress rapidly and become unresectable. The tumor suppressor, p53, plays an important role in regulating cell cycles and apoptosis. p53 is regulated by several molecules, and it interacts with other apoptotic proteins.

Objectives. To compare the prognosis of ovarian serous carcinoma and evaluate the expression of DNA-PKcs, Akt3, GSK-3 β , and p53 in cancerous cells.

Material and Methods. DNA-PKcs, Akt3, GSK-3 β , and p53 expression levels were scored using immunohistochemistry staining of tissue samples from 132 women with ovarian serous adenocarcinoma. Expression was confirmed by real-time RT-PCR. Analyses were stratified by age, tumor grades, cancer stages and serum CA 125 levels.

Results. Significant differences in DNA-PKcs, Akt3, and p53 expression were observed between participants with different stages and tumor grades of ovarian serous adenocarcinoma. DNA-PKcs and p53 expression increased along with increasing tumor grade. Meanwhile, DNA-PKcs, Akt3, and p53 expression increased along with increasing cancer stage, and with a decrease in 5-year overall survival rate.

Conclusions. This study shows that elevated expression of DNA-PKcs, Akt3, and p53 in ovarian serous adenocarcinoma tissues are an indication of more advanced disease and worse prognosis (*Adv Clin Exp Med* 2016, 25, 3, 513–521).

Key words: epithelial ovarian cancer, DNA-PKcs, Akt3, GSK-3 β .

Malignant ovarian tumor is one of the leading causes of worldwide cancer death [1]. It is usually characterized by insidious onset and late diagnosis because of the absence of symptoms, allowing ovarian cancer cases to progress rapidly and become unresectable [2]. Cytoreductive surgery, fol-

lowed by adjuvant chemotherapy combined with platinum-based agents and taxane, is the standard treatment for ovarian carcinoma. Even with treatment, the 5-year overall survival (OS) rate for advanced carcinoma cases (stages III–IV) is 20–25%, and recurrence is relatively common [1, 3].

* This study was supported by a Biomedical Research Institute and Busan Cancer Center Grant (2014-24), Pusan National University Hospital, and by a grant from the National R&D Program for Cancer Control, Ministry for Health, Welfare and Family Affairs, Republic of Korea (0920050).

Ovarian carcinoma is considered to have a dynamic and variable pattern of cellular changes and a multistep process with underlying changes from normal ovarian cells to malignant cells, similar to other malignancies. Among these changes, alterations in the apoptotic process provide strong evidence of progressive conversion. Alteration of apoptosis can be observed in cells that live beyond their expected life cycle, indicating that they are malignant [4]. The tumor suppressor p53 is an important transcriptional factor that mediates cell growth arrest, senescence, and apoptosis in response to cellular damage such as stress, radical exposure, irradiation or need for DNA repair [5]. Cellular stress or damage stimulates p53 accumulation in the nucleus, resulting in the change of transcriptional function [6]. Among all the tumors that can arise in humans, about 50% are due to alterations in p53, and this alteration is known to be associated with poor prognosis and high rates of treatment failure or recurrence [7].

Several molecules are essential to the process of stabilizing p53 and interacting with each other in response to DNA damage. DNA-dependent protein kinase (DNA-PK) is known to signal the downstream effectors to carry out a response to DNA damage. As other molecules, Akt and GSK-3 become active by phosphorylation in response to DNA damage, and phosphorylated products are recognized by DNA-PK. Akt is also activated directly in response to DNA damage regardless of the phosphorylation of GSK-3 activities in response to its inactivation. On the other hand, down-regulation of Akt, which means the inhibition of phosphorylation, prevents phosphorylation of GSK-3 and reduces the accumulation of p53 in the cells [8].

The dynamics of interactions between p53 and these molecules suggest a potential strategy for activating p53 in tumors and may offer opportunities to evaluate tumor prognosis. Understanding these interactions can provide insights into molecular treatment strategies that allow patients to avoid surgery and chemotherapy. In this study, we focus on ovarian serous carcinoma and we evaluate the expression of DNA-PKcs, Akt3, GSK-3 β and p53 in cancerous cells and we compare the prognosis of ovarian serous adenocarcinoma based on patients' expression statuses.

Material and Methods

Patients and Tissue Samples

A total of 132 ovarian tissue samples from patients with ovarian serous carcinoma, collected from January 2005 to December 2008, were an-

alyzed; all patients provided informed consent to participate in the study. All samples were obtained from patients undergoing laparotomy for ovarian carcinoma at the Department of Obstetrics and Gynecology at Kosin University Hospital. Ethical approval for human-subject research was obtained from the Kosin Medical Center's Institutional Review Board. The inclusion criterion for enrollment was distinctive pathologic diagnosis of serous papillary ovarian carcinoma. Those who had concurrent second malignancies or prior malignancies within the previous five years (other than in situ or non-melanoma skin cancers) were excluded. Those who had received neoadjuvant chemotherapy before resection were also excluded to avoid the possible effects of such treatments on further histologic work up. Patients were excluded if they were human immunodeficiency virus-positive, had evidence of infection or had a concomitant autoimmune disease treated with immunosuppressive therapies.

For histologic examination, formalin-fixed and paraffin-embedded tumor sections obtained from the resected specimens were cut and stained with hematoxylin and eosin (H-E stain). An experienced gynecologic pathologist interpreted all tumor sections, provided a histologic diagnosis and confirmed tumor grades in a blinded manner. Tumor staging was defined according to the criteria set forth by the tumor-node-metastasis (TNM) classification system (seventh edition). For each case, clinical characteristics including age, TNM stage, and tumor grade were reviewed. The existence of tumor recurrence was observed for 5 years in a prospective manner. Patients were scheduled for their follow-up appointments at Kosin University Hospital's Department of Obstetrics and Gynecology. Follow-up for this study ended in December 2013. The median follow-up period was 48 months (range: 2–60 months).

Immunohistochemistry

4- μ m sections were consecutively cut from paraffin blocks and deparaffinized in xylene and rehydrated in a graded alcohol series. Immunostaining was performed using the BOND-MAX autostainer system (Leica Microsystems, Wetzlar, Germany). Antigen retrieval was performed using a microwave submerged in pH 6.0 citrate-phosphate buffer. The following primary antibodies were used: DNA-PKcs (1:250; Bethyl Laboratories, Montgomery, USA), Akt3 (1:30; Santa Cruz Biotechnology, Dallas, USA), GSK-3 β (1:80; Santa Cruz Biotechnology), and p53 (1:100; Dako, Glostrup, Denmark). The slides were counter-stained with Mayer's hematoxylin.

Immunohistochemistry Scoring

A single, experienced, board-certified pathologist without knowledge of patient characteristics blindly scored each tissue section for DNA-PKcs, Akt3, GSK-3 β , and p53 expression. Cells with nuclear staining were scored as positive cells. The proportion of cells that were positive for each of the above gene products was calculated. We used two categories to classify expression levels: < 10% of nuclear staining = low; $\geq$ 10% of nuclear staining = high (Fig. 1).

RNA Preparation and Quantitative Real-Time PCR

Total RNA was extracted from serous papillary ovarian carcinoma tissues using TaqMan reagent

(Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. DNA-PKcs, Akt3, GSK-3, and p53 gene expression levels were detected using a real-time PCR assay. Quantification of expression levels was performed in an ABI PRISM 7900HT Sequence Detection System (PE Applied Biosystems, Foster City, USA). PCT was based on the TaqMan fluorogenic detection system (PE Applied Biosystems) using a fluorogenic oligonucleotide probe designed to hybridize to the specific target sequence. The TaqMan probes were labeled at the 5' end with the fluorescent reporter dye, FAM (6-carboxyfluorescein) (R), and at the 3' end with the quencher dye, TAMRA (6-carboxytetramethylrhodamine) (Q). The sequences for gene-specific forward and reverse primers and the probes were designed using Primer Express 1.0 software (PE Applied Biosystems).

Fig. 1. Expression of DNA-PKcs, Akt3, GSK-3 β and p53 in ovarian serous adenocarcinoma cells stained by immunohistochemistry

The following primer sequences were used for real-time PCR:

DNA-PKcs (GenBank, Accession No. NM_006904)
forward, 5' CATGGAAGAAGATCCCCAGA 3'
reverse, 5' TGGGCACACCACTTTAACCAA 3'

Akt3 (GenBank, Accession No. NM_005465)
forward, 5' TAATACGACTCACTATTAGGGGT-
GCAAGGATTGTACACG 3'

reverse, 5' GATTTAGGTGACACTATAGGTT-
GCCAGGTCATCATAACG 3'

GSK-3 β (GenBank, Accession No. NM_002093)
forward, 5' GGTCTATCTTAATCTGGTGGTGG 3'
reverse, 5' TGGATATAGGCTAAACTTCGGAAC 3'

p53 mRNA (GenBank, Accession No. NM_000546)
forward, 5' AACAGTTCCTGCATGGGCGGC 3'
reverse, 5' AGGACAGGCACAAACACGCACC 3'

For assessment of the expression of each parameter, mRNA expression was scored based staining intensity: 0 = negative, 1 = weak, 2 = moderate, and 3 = strong. Focal expression intensity was also scored and this was added to the general intensity score: 0.5 = focally weak, 1.5 = focally moderate, and 2.5 = focally strong. Three well-trained and blinded observers read the slides and scored the mRNA expression of DNA-PKcs, Akt3, GSK-3 β and p53.

Statistical Analyses

All results are expressed as mean $\pm$ standard deviation. Kolmogorov-Smirnov's test was used to evaluate the normality of the distribution of the continuous data. Continuous variables were analyzed using the Student's *t*-test to compare the means of the groups. Comparisons between the groups were made using the χ^2 test for univariate analysis of categorical variables. The Kaplan-Meier test and the long-rank test were used to compare the OS rates between groups classified by immunohistochemistry scoring. SPSS v. 19.0 (IBM, Armonk, USA) was used for all analyses and we determined $p < 0.05$ to be our level of significance.

Results

Clinical Characteristics of Patients and Their Tumor Based on Immunohistochemistry Scoring

The patients' clinical characteristics are outlined in Table 1. All 132 cases of ovarian serous carcinoma were divided into two groups, according to expression levels of DNA-PKcs, Akt3, GSK-3 β and p53 (Fig. 1). DNA-PKcs was observed

Table 1. Clinical characteristics of 132 ovarian serous adenocarcinoma patients according to their scores after immunohistochemistry staining

Characteristics	DNA-PKcs expression			Akt3 expression			GSK-3 β expression			p53 expression		
	low (n = 90)	high (n = 42)	p-value	low (n = 79)	high (n = 53)	p-value	low (n = 63)	high (n = 69)	p-value	low (n = 75)	high (n = 57)	p-value
Age (years)	62 (17)	59 (21)	0.076*	61 (27)	63 (21)	0.105*	58 (23)	59 (31)	0.069*	61 (28)	59 (21)	0.126*
Grade			0.041**			0.217**			0.076**			0.000**
1-2	76 (84.4%)	29 (69.0%)		68 (86.1%)	37 (69.8%)		46 (73.0%)	59 (85.5%)		69 (92.0%)	36 (63.2%)	
3	14 (15.6%)	13 (31.0%)		11 (13.9%)	16 (30.2%)		17 (27.0%)	10 (14.5%)		6 (8.0%)	21 (36.8%)	
TNM stage			0.002**			0.030**			0.057**			0.000**
I - II	74 (82.2%)	24 (57.1%)		64 (81.0%)	34 (64.2%)		42 (66.7%)	56 (81.2%)		68 (90.7%)	30 (53.6%)	
III - IV	16 (17.8%)	18 (42.9%)		15 (19.0%)	19 (35.8%)		21 (33.3%)	13 (18.8%)		7 (9.3%)	27 (47.4%)	
CA 125 (unit/mL)	425 (238)	366 (212)	0.063*	456 (218)	412 (129)	0.045*	399 (158)	431 (316)	0.152*	486 (258)	312 (198)	0.031*
5-year OS rate	76.7%	61.9%	0.026***	78.5%	62.3%	0.034***	69.8%	75.4%	0.391***	77.6%	64.3%	0.049***

Values are mean (standard deviation). * - p-values by Student's *t*-test, ** - p-values by χ^2 test, *** - p-values by log-rank test, OS - overall survival.

through strong staining in 42 cases and through weak staining in 90 cases. Staining for Akt3 was strong in 53 cases and weak in 79 cases. Staining for GSK-3 β was strong in 69 cases and weak in 63 cases. Strong staining for p53 was observed in 57 cases and weak staining was observed in 75 cases. There was no difference in mean age between the groups. Tumor grade was significantly different between the high-expression group and low-expression group for both DNA-PKcs and p53 ($p = 0.041$ and < 0.001 , respectively). For each protein, the high-expression group was composed of a higher proportion of grade 3 cases compared to the low-expression group. There was no difference in the groups based on Akt3 and GSK-3 β expression. Regarding stage distribution, 98 patients had stage I or II cases while 34 patients had stage III or IV cases. Patients with high-expression of DNA-PKcs, Akt3 and p53 were significantly more likely than patients in the low-expression group to have a more advanced stage of cancer ($p = 0.002$, 0.030 and < 0.001 , respectively). On the other hand, the high-expression group of GSK-3 β had a lower distribution of stage III and IV cases, but not significantly so ($p = 0.057$). Serum CA 125 concentrations were significantly higher in the high-expression groups for Akt3 and p53 ($p = 0.045$ and 0.031 , respectively). However, serum CA 125 levels were not significantly different between the expression groups for DNA-PKcs and GSK-3 β .

DNA-PKcs, Akt3, GSK-3 β and p53 Expression Based on Quantitative Real-Time PCR

Fig. 2 shows mRNA expression of DNA-PKcs, Akt3, GSK-3 β and p53 in the ovarian serous adenocarcinoma tissues according to the expression

groups and as classified by immunohistochemistry scoring. The high-expression groups from immunohistochemistry staining demonstrated consistent mRNA expression patterns after semiquantitative assessment of real-time PCR analysis.

Prognosis Analysis

The 5-year OS rates are depicted in Table 1 and Fig. 3. The 5-year OS rates of the DNA-PKcs high-expression group and low-expression group were 61.9% and 76.7%, respectively. There was a significant difference in OS according to the expression groups (95% CI 0.75–0.95, $p = 0.026$). For the Akt3-expression analyses, the 5-year OS rates of the high expression group and low expression group were 62.3% and 78.5%, respectively, and this difference was significant (95% CI 0.86–0.96, $p = 0.034$). The 5-year OS rates of the GSK-3 β high-expression group and low-expression group were 75.4% and 69.8%, respectively. There was no significant difference in OS according to the two expression groups (95% CI 0.92–1.32, $p = 0.391$). Finally, the 5-year OS rates of the p53 high-expression group and low-expression group were 64.3% and 77.6%, respectively, and this difference was significant (95% CI 0.81–0.98, $p = 0.049$).

Discussion

This study investigated the expression of several apoptotic target molecules, including DNA-PKcs, Akt3, GSK-3 β and p53, for their prognostic value in cases of ovarian serous adenocarcinoma by analyzing whether there are associations between tumor stage and grade and expression levels. With the exception of GSK-3 β , all molecules were

Fig. 2. Relative levels of mRNA expression for genes involved in the p53 pathway as determined by real-time PCR: Two groups were classified by immunohistochemistry staining of DNA-PKcs, Akt3, GSK-3 β and p53. For each gene, mRNA expression was elevated in the high-expression group, while expression was suppressed in the low-expression group.

Fig. 3. Assessment of prognostic significance using Kaplan-Meier survival estimates and log-rank test

expressed at higher levels in the cancer cells with higher stages or grades. GSK-3 β did not have expression patterns that were meaningfully related to cancer severity. Indeed, in follow-up analyses, over-expression of DNA-PKcs, Akt3, and p53 resulted in lower 5-year OS rates within the group. This study is the first attempt to test the prognostic potential of DNA-PKcs, Akt3, and GSK-3 β at the same time for ovarian serous carcinoma.

All cells have a limited life span, and cell death occurs mainly as a result of passive necrotic processes or as part of an active process of programmed cell death, or apoptosis. Apoptosis itself plays an important role in one's whole life, from embryonic development to adult tissue homeosta-

sis [9]. Apoptosis is the most common mechanism by which the body eliminates unneeded or altered cells without local inflammation caused from the leakage of dead cell contents [10]. In normal cells, apoptosis starts in response to several developmental environments, such as decrease in the local concentration of particular tissue mediators or growth factors. With the pathologic environment, several stimuli include severe stress or damage to vital cellular components caused by ionizing radiation, heat shock, toxins, cell detachment from surrounding tissue, bacterial or viral infection, and/or oncogenic signaling, which results in the apoptotic tendency of the cells [11]. On the other hand, malignant tumors often exhibit defects in apoptotic

signaling pathways, resulting in tumor cell survival, even though they are under the pathologic condition [12].

Understanding apoptosis in cancer is essential because it not only gives insights into the pathogenesis of cancer but also offers clues for treatment and cure. In cancer cells, there is imbalance between cell proliferation and death, due to the abnormal intracellular signaling pathway. Alteration of apoptosis and apoptotic signals may occur at any stage of cell cycles, and any period of cell life, even in malignant cells [4]. For example, malignant cells come to have the power of over-proliferation, invasion, metastasis and recurrence as the result of genetic changes associated with apoptosis. As a result of transformation from the normal cells into malignant cells, the cells avoid death.

The p53 protein is a famous apoptotic molecule and tumor suppressor which is well-known to be associated with the pathogenesis of epithelial ovarian cancer [13]. In addition to the role as a tumor suppressor, p53 is also thought to be involved in cellular senescence and cell cycle arrest. p53 gene is located at the short arm of chromosome 17 (17p13.1) [4, 14]. Under stable conditions, the p53 protein is controlled at low cellular level by the proteasome degradation pathway. MDM2, an E3 ubiquitin ligase, is the most critical negative regulator for p53 at the proteasome degradation pathway [15]. On the other hand, under conditions with a broad variety of stress, including hypoxia, nutritional starvation, DNA damage, ribonucleotide depletion, and oncogene activation, p53 molecule is stabilized by post-translational modifications in the action of various enzymes. These enzymes include phosphatases, kinases, methylase, acetyltransferases, deacetylases, ubiquitin ligases, deubiquitinases, and SUMOylases [16].

p53 applies its function *via* transcriptional regulation of its target genes, primarily. It regulates cellular biological processes to maintain genomic integrity and prevent tumor formation by transcriptional regulation of target genes, including cell cycle arrest, cellular senescence, energy metabolism, anti-oxidant defense, apoptosis, and autophagy [17]. A recent study has reported that p53 also regulates apoptosis another way, through a transcription independent pathway. The study suggests that a fraction of the p53 protein translocates to mitochondria in response to stress, where p53 interacts with anti-apoptotic Bcl-xL and Bcl-2 to inhibit their functions, which results in the release of cytochrome C from the mitochondria, inducing apoptosis [18].

p53 has been thought to encourage autophagy by different mechanisms that may contribute to its role in tumor suppression. Autophagy plays a role

not only in tumorigenesis by maintaining genomic stability, but also tumor prevention in normal cells and tissues. Meanwhile, autophagy can promote tumor cell survival and tumor progression [19]. Interestingly, p53 has also been found to prevent autophagy under certain condition such as mutant forms of p53 [20]. The majority of p53 mutations associated with human cancers are missense mutations, which usually result in the full-length mutant p53 proteins. Though wild-type and mutant p53 alleles exist in heterozygous manner, mutant p53 can block the function of wild-type p53. However, p53 mutations are usually followed by a loss of heterozygosity in cancer cells, which means the loss or mutation of the normal wild-type p53 allele. Though wild-type p53 proteins are maintained at low levels through the proteasome degradation pathway in non-stressful circumstances, mutant p53 proteins usually accumulate and reach high levels in tumor cells [21].

In the present study, patients with high p53 expression, based on immunohistochemistry staining and real-time PCR, had poorer 5-year OS rate. Furthermore, the high-expression group was composed of a greater proportion of patients with advanced age, poorer tumor grade, and higher serum CA 125 levels, which are all well-known prognostic factors of epithelial ovarian malignancy. However, regardless of the clinical outcomes, more accumulation of p53 in the cancer cells may indicate a worse progression, even after optimal treatment. p53 can be used as a prognostic parameter during the early follow-up period, as well as a therapeutic target.

There are many molecules that interact with p53. In advance, DNA-PKcs, an enzyme in humans, belongs to the phosphatidylinositol 3-kinase-related kinase protein family. Its catalytic subunit is required for the DNA-repair pathway [22]. It has been suggested that DNA-PK controls the expression of p53 in response to DNA damage and appears to be essential for p53 to obtain DNA-binding activity. The DNA-PK protein product has been known to be associated with recognizing both single- and double-stranded breaks in DNA and binding to the damaged DNA [23]. As the Ku70 and Ku86 subunits of DNA-PK bind to damaged DNA, they induce the kinase activity of DNA-PKcs, which results in the phosphorylation of a wide variety of proteins acting in DNA damage circumstances, such as p53. This functional relationship between DNA-PK and p53 indicates that they may interact with each other and respond to certain types of DNA damage associated with malignant transformation. However, the direct pathway of DNA-PK in p53 phosphorylation and its important roles in the genesis of cancer remain unknown [24].

GSK-3 β has been known to play a role in certain intracellular signaling pathways, such as cellular proliferation, migration, immune responses, inflammation, glucose regulation and even apoptosis. In a study, GSK-3 β has been reported to be a key factor in oncological pathogenesis in some cancer types [25]. To understand the function of GSK-3 β associated with p53, the action of Mdm2 is essential. Mdm2 is dephosphorylated in response to cellular stress. Phosphorylated Mdm2 is vital for p53 degradation [26]. Some of these phosphorylation sites of Mdm2, serine-240 and serine-254 are phosphorylated or dephosphorylated by GSK-3 β . In response to cell damage, GSK-3 β is phosphorylated at the serine-9 site by depriving sites of Mdm2 of phosphorylation, which leads to its inactivation [27]. Inhibition of GSK-3 β increased the cellular level of p53, indicating that GSK-3 activity is required for p53 regulation. Consistent with this principle, overexpression of a mutation of GSK-3 β with the change of amino acid from the serine-9 site to an alanine can make the cells refractory to radiation-mediated inhibition, significantly as the result from the reduction of the accumulation of p53 in response to irradiation [28].

One of the kinases that directly phosphorylates GSK-3 β is Akt. The Akt family is also known to be involved in various biological processes, including cell proliferation, differentiation, apoptosis and tumorigenesis. Basic Akt is an inactive form. However, under the certain conditions that can make the kinase activated by phosphatidylinositol 3-kinases, phosphorylation of Akt may occur and become an active form. Though cytoplasmic Akt protein levels (in the form combined with PKB) were considerably higher than the level in the nucleus, it has been known that the significant part of Akt is localized in the nucleus. Nuclear Akt/PKB can be phosphorylated abruptly just after DNA damage, resulting in GSK-3 β phosphorylation. Down-regulation of Akt3 almost completely blocked phosphorylation of GSK-3 β even with the exposure of irradiation. In this manner, down-regulation of Akt3 may also reduce the accumulation of p53 in response to cell damage [8].

In this study, DNA-PKcs and Akt3 seem to have similar prognostic potential for ovarian serous adenocarcinoma, whereas GSK-3 β has very little. The DNA-PKcs high-expression group generally had more patients with poorer tumor grade and more advanced stages. Thus, it also had a very low 5-year OS rate. Unlike DNA-PKcs, Akt3 was associ-

ated with patients with a more advanced tumor stage and higher serum CA 125 concentration. There was also an indication of a lower 5-year OS rate. Thus, DNA-PKcs and Akt3 can be interpreted to act prognostically like p53. Our analysis with GSK-3 β staining resulted in findings that suggest it is not a meaningful prognostic indicator for ovarian serous adenocarcinoma. Given these outcomes, we hypothesize that DNA alterations in ovarian serous adenocarcinoma induce the up-regulation of DNA-PKcs and its kinase activity, which affect p53 expression directly and indirectly. The intracellular or extracellular conditions for up-regulation of DNA-PKcs induce Akt3. Akt has a positive effect on p53 expression, *via* MDM2, whereas over-phosphorylation of GSK-3 β reduces the need for producing GSK-3, resulting in lower expression of the molecule. Lower expression of GSK-3 β induces the accumulation of p53 in the cells. For these reasons, a higher expression of the p53 gene and the influenced expression of other possible interactive genes may be associated with the survival rate of epithelial ovarian cancer.

This study has some limitations. First, we did not exclude patients based on their p53 type (e.g. wild type or mutant type). Overexpressed p53 may be the mutant type, because the p53 wild type is fundamentally apoptotic after acute cell injury or double-stranded DNA damage. Furthermore, this study was not conducted under acute irritation conditions; rather it was conducted under chronic conditions after malignancy transformation. Second, the p53-inducing pathway in ovarian serous adenocarcinoma cases cannot be identified only with this study, because there are so many molecules involved in p53 action. Third, most of our samples happened to be in a relatively early cancer stage, so we cannot describe the prognosis of late stage ovarian serous adenocarcinoma sufficiently with these protein markers. A large-scale study is needed to try to replicate our findings.

To the best of our knowledge, this is the first study performed to explore the prognostic value of DNA-PKcs, Akt3 and GSK-3 β at the same time for cases of ovarian cancer. Also, we tried to reveal their prognostic potential and association with p53, a well-known cancer marker, using a prospective follow-up. In conclusion, special tissue staining patterns associated with DNA-PKcs and Akt3 may offer adjunctive clues for patients' survival prognosis, even in addition to the strength of routine p53 immunohistochemistry staining as a useful prognostic factor.

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Conflict of interest: None declared

Received: 21.11.2015

Revised: 13.01.2016

Accepted: 6.04.2016