Journal of BUON 12: 99-104, 2007
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ORIGINAL ARTICLE

Relationship of serum levels of tumor markers with tissue expression of gene products in ovarian carcinoma

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Summary

Purpose: *The aim of this prospective study was to determine serum levels and tissue expression of CA125, CA 15-3, p53, HER-2 and nm23 tumor markers, which are used in the detection and follow up of patients with ovarian carcinoma.*

Patients and methods: *19 patients with malignant and benign ovarian tumors were included in this study. Serum levels of CA125, CA 15-3 and p53 tumor markers were detected in preoperative and postoperative blood samples using ELISA technique. Tissue expression of p53, HER-2 and nm23 were examined using immunohistochemistry.*

Results: *All serum tumor markers were elevated in patients with ovarian carcinoma. Serum level of CA 15-3 was increased in patients with ovarian carcinoma (median 48.33 U/ml, normal range 0-36), while it was normal in patients with benign ovarian tumors (median 20.67 U/ml; p >0.05). CA125 serum values were strikingly increased in ovarian carcinoma (median 264.16 IU/ml, normal range 0-35) and benign ovarian tumors (median 119.59 IU/ml; p <0.05). Serum levels of p53 in patients with ovarian carcinoma were increased (median 0.69 U/ml, normal range 0-0.50) compared to patients with benign tumors (0.32 U/ml; p <0.05). Histological HER-2 overexpression was detected in 7 cases, including 4 with strong (score 3+ and 2+) and 3 with weak or no HER-2 expression (score 1+ and 0) in ovarian carcinoma tissue; in benign tumors HER-2 overexpression was detected in 1 case (p >0.05). Strong overexpression of p53 was detected in 3 cases with malignant and none with benign tumors (p >0.05); and strong overexpression of nm23 was detected in 5 cases with malignant and 2 with benign tumors (p >0.05).*

Conclusion: *Serum levels of CA125, CA 15-3 and p53 are strikingly increased, as well as the expression of HER-2 and p53 in carcinomatous tissue. Detection and analysis of multiple tumor-specific markers in serum and tissue can give useful clinical information for the management of ovarian carcinoma and can also improve the sensitivity and specificity of these markers.*

Key words: benign ovarian tumors, oncogenes, ovarian carcinoma, serum tumor markers, tissue tumor markers

Introduction

Ovarian carcinoma is the leading cause of cancer death in women. It ranks sixth in incidence after breast, lung, colon, rectal and endometrial cancer. In female genital pathology it is third, after cervix and corpus uteri carcinoma [1,2]. The peak incidence of ovarian cancer is at the 62nd year of life.

Prognosis of ovarian carcinoma depends on the clinical and histopathological examination. The most strong prognostic factor is the stage of disease at the time of diagnosis, based on the AJCC (American Joint Committee on Cancer) and FIGO staging classifications [3].

No efficient method for screening and detection of early-stage ovarian cancer exists [4].

Received 21-11-2006; Accepted 19-12-2006

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Multiple genetic changes including activation of proto-oncogenes and inactivation of tumor suppressor genes are involved in the development of this malignancy [5].

Alterations of oncogenes such as K-ras, c-erbB-2/HER-2/neu and c-myc, and of tumor suppressor genes such as p53, BRCA1 and BRCA2, play an important role in etiopathogenesis. Allelic losses of specific chromosomes, deletions and mutations are mechanisms that activate/inactivate these genes [6,7].

One of the most important tumor suppressor genes is p53, localized on the chromosome 17p. It has been shown that one of the pathways through which the p53 gene controls the cell cycle is by transactivating p21WAF1/CIP1, a cyclin-dependent kinase (cdk) inhibitor. By inhibiting cdks, p21WAF1/CIP1 blocks the G1 to S-phase transition in the cell cycle so that cells cannot synthesize new genetic material and divide, and the genome repair system may be activated. Mutation of p53 tumor suppressor gene, with resultant overexpression of mutant p53 protein, occurs in 50% of all malignancies [8]. This multimarker can be detected in cancers of the breast, lung, colon, ovary, liver, esophagus, urinary bladder, etc. [9].

Numerous independent studies confirmed the clinical relevance of the detection of overexpression of the human epidermal growth factor receptor $p185^{HER2}$. HER-2 /neu is a 185-kDa glycoprotein and a transmembrane receptor with tyrosine kinase activity [10,11]. Overexpression of the HER-2/neu oncogene occurs in approximately 30% of breast and ovarian cancers. In most studies, HER-2/neu overexpression indicates increase of mitotic index and correlates with poor survival and poor response to treatment.

A gene located in chromosome 17, called nm23 (non metastatic), encodes for NDPK enzymes, which participate in cell cycle control. During the nm23 gene identification studies it was confirmed that there is a 8-membered gene family (H1-H8) with the same function and structurally very similar [12]. In literature they are also known as NDPK-A and B, nm23H1-H8, NME-1 and 2 (expressed in nonmetastatic cells). nm23 can be found as nonmutated (normal) and mutated type, which inhibits cell cycle differentiation and control [13,14].

Serum levels of tumor markers, such as CA125, CA 19-9, CEA (carcinoembryonic antigen), CA 15-3, AFP (alfa-fetoprotein), hCG (human chorionic gonadotropin), LDH (lactate dehydrogenase) etc. are of a great help in diagnosis and response to therapy in ovarian cancer.

CA125, or carbohydrate antigen, is a high-molecular weight antigenic glycoprotein. It is a highly sensitive but not specific antigen, because it can be raised in conditions and diseases such as endometriosis, adenomyosis, pelvic infections, menstrual bleeding, polycystic ovaries, pregnancy (especially first trimester), myomas, liver cirrhosis, systemic lupus erythematosus, pneumonia, pericarditis, etc. It can be found in various concentrations on cell surfaces originated from the celomic epithelium: peritoneum, pleura, pericardium, endosalpings, endometrium, and endocervix. Serum levels of CA125 are of great help in the diagnosis of epithelial malignant ovarian tumors [15]. It is known that the levels of this marker are increased in more than 80% of the patients with epithelial nonmucinous ovarian cancer. Also it can be useful in assessing the response to therapy, in the patients' follow up and in the confirmation of persistent or relapsed disease [16,17].

CA 15-3 (MUC-1, mucin breast carcinoma) is a cell membrane glycoprotein which can be found in various malignant and normal cells [18]. Apart from breast cancer, serum levels of CA 15-3 can be elevated in ovarian, colon, lung and liver carcinoma. Many studies have shown that follow up of serum levels of this tumor marker can complement CA125 in ovarian cancer evaluation.

Patients and methods

Nineteen patients with ovarian tumors were included in this prospective study which was conducted at the Clinic of Obstetrics and Gynecology, Clinical Centre, in Kragujevac, Serbia. After exploratory laparotomy and pathological study of the ovarian tumor, patients were divided into two groups: group I - pathologically verified benign ovarian tumor; and group II-pathologically verified malignant tumor (Table 1).

Serum levels of CA125 (normal up to 36 IU/ml),

Table 1. Histology of ovarian tumors in 19 patients

Histology	*Patients, n*
Benign	
Benign cystic teratoma	3
Mucinous cystadenoma	2
Serous cystadenoma	1
Malignant	
Mucinous adenocarcinoma	6
Serous adenocarcinoma	2
Endometrioid adenocarcinoma	2
Metastatic to ovary	3

CA 15-3 (normal up to 35 IU/ml) and p53 (normal up to 0.50 U/ml) tumor markers were detected in preoperative and postoperative (one month after the operation) blood samples using commercial ELISA techniques (Breast Cancer Antigen CA15-3 Enzyme Immunoassay Test Kit, DRG International Inc, USA; Cancer Antigen CA125 Enzyme Immunoassay Test Kit, DRG International Inc, USA; and Cancer Antigen p53 Enzyme Immunoassay Test Kit, Bender Med. Systems, USA).

Oncogene and tumor suppressor gene expression was detected in frozen sections of tumor tissue after surgery, using immunohistochemistry.

Statistics

Statistical analysis of the results was conducted using the commercial program pack SPSS (version 10.0, SPSS Inc.). Tests used in analysis were Kolmogorov-Smirnov test, Student's t-test, and chi-square test. Normally distributed values were tested using t-test for small depended and independed samples, Wilcoxon test for related samples with nonparametric distribution and Mann-Whitney U-test and Kruskal-Wallis test were used for comparison between two and more nonparametric groups of values. p-values <0.05 were considered statistically significant.

Results

Serum level of the tumor markers

Figure 1 shows tumor marker CA 15-3 results subdivided according to tumor nature (benign or malignant). At the time of diagnosis the serum levels of CA 15-3 in patients with cancer were twice as high compared with women having a benign tumor (malignant preoperative samples: 48.33 ± 75.24 U/ml; benign preoperative samples: 20.67 ± 22.95 U/ml). However, this difference was not statistically significant (Kruskall Wallis: $x^2 = 1.917$, df = 2, p = 0.383; Mann Whitney: p = 0.279).

Pre and postoperative serum levels showed no significant difference in the group with malignant tumors (t-test for small dependable samples: p = 0.178; Wilcoxon test: p = 0.263). The same not statistically significant results were obtained by comparing pre and postoperative serum levels in the group with benign tumors.

Figure 2 shows greater sensitivity for CA 125. The obtained values in the group with malignant tumors (preoperative: 264.16 ± 177.94 IU/ml; postoperative: 136.29 ± 168.41 IU/ml) showed statistically significant difference (t-test: p = 0.032; Wilcoxon test: p = 0.036). These patients had 9-fold higher values of CA 125 (264.16 IU/ml) compared with the upper limit of normal (35.0 IU/ml). The group with benign tumors had pre and postoperative values 119.59 IU/ml and 114.57 IU/ml, respectively. Comparison of serum preoperative levels between benign and malignant tumors groups showed borderline difference (t-test and Mann Whitney test: p = 0.05).

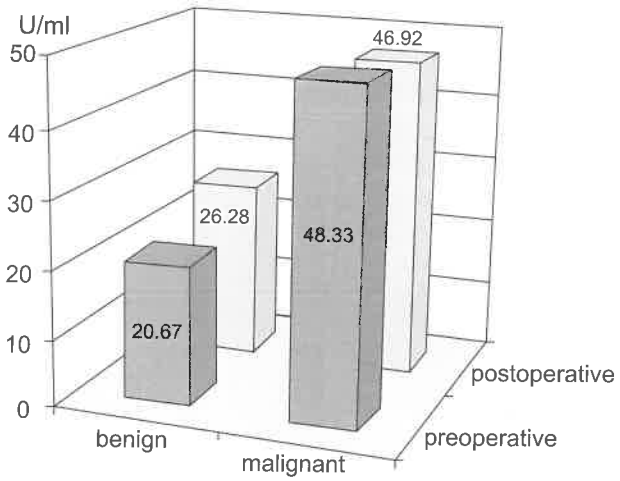


Figure 1. Pre and postoperative serum levels of CA 15-3 in benign and malignant ovarian tumors.

Figure 3 depicts the comparison of the preoperative serum values of p53 between the benign and malignant tumors groups, which shows statistically significant difference (t-test: p = 0.041; Mann Whitney test: p = 0.037).

In the group of malignant ovarian tumors, the

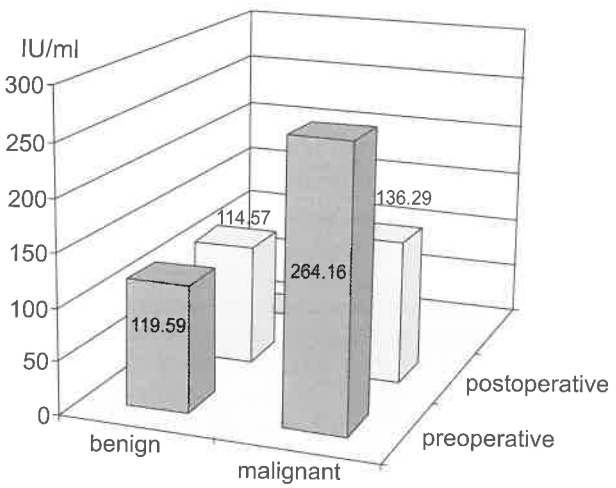


Figure 2. Pre and postoperative serum levels of CA 125 in benign and malignant ovarian tumors.

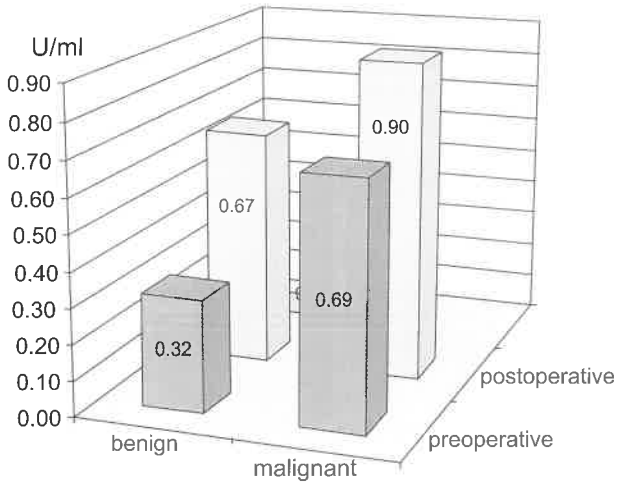


Figure 3. Pre and postoperative serum levels of p53 in benign and malignant ovarian tumors.

median serum value of this marker was 0.69 ± 0.48 U/ml. Postoperatively this value increased to 0.90 ± 1.04 U/ml but without reaching statistical significance (t-test: p = 0.479; Wilcoxon test: p = 0.327).

Analysis of preoperative (0.32 ± 0.16) and postoperative (0.67 ± 0.26) serum levels in the group of benign tumors showed statistically significant difference (t-test: p = 0.032; Wilcoxon test: p = 0.043).

Tissue immunohistochemical results of the oncogenes and tumor suppressor genes expression

The intensity of the membrane staining for HER -2 was heterogeneously distributed. HER-2 overexpression was detected in 7 cases, including 4 with strong (score 3+ and 2+) and 3 with weak or no HER-2 expression (score 1+ and 0).

Strong HER-2 expression was obtained in 57.14% of ovarian carcinoma cases and in 33.33% of benign ovarian tumors (x^2, p=0.43) (Table 2).

Strong p53 expression was found in 42.86% of the examined malignant tumors, and in none of the benign tumors (x^2, p=0.253).

Immunohistochemical examination of nm23 gave results which correlated with the results taken for HER-2 expression. Strong nm23 expression was present in 71.43% of the samples of malignant tumors and in 66.67% of benign tumors (x^2, p=0.663).

Correlation analysis showed that the intensity of the expression of the tested markers gave statistically significant match between the findings HER-2 and nm23 (r = 0.804; p = 0.005).

Table 2. Strong immunohistochemical gene expression in malignant and benign tumors

Tumor marker	*Malignant Patients, n (%)*	*Benign Patients, n (%)*	*p-value*
HER-2	4/7 (57.14)	1/3 (33.33)	0.430
p53	3/7 (42.86)	0/3 (0)	0.253
nm23	5/7 (71.43)	2/3 (66.67)	0.663

Discussion

Measurement of serum tumor markers appears to reflect the total tumor burden in the body and represents the summation of numerous subclinical disease forms.

Tumor cells display on their surface protein molecules which cannot be found in normal cells, or they can but in markedly decreased numbers. These molecules are called tumor-associated antigens or tumor markers. Measurement of the serum level of tumor markers can provide relevant data about the development of ovarian carcinoma, diagnosis, prognosis, and response to therapy [19,20]. Up until now no tumor marker with adequately high sensitivity exists in order to be used as routine screening, so their usage refers only to monitoring response to therapy and disease follow up [21].

CA 15-3 is most commonly used in the early detection of breast cancer. In more than 75% of the cases the CA 15-3 serum level is increased, indicating the presence of breast cancer, and can precede the clinical and radiological signs of the disease by more than 6 months [22]. This tumor marker is a membrane glycoprotein, mucin type, and both malignant and normal cells of many organs (breast, lung, ovary, pancreas) secrete it. The results of this study showed increase of the serum level in ovarian carcinoma patients, but without statistical significance. Patients with benign ovarian tumors had normal values. This survey showed that the determination of the serum level of this marker has no diagnostic value, for in the tested group of patients it showed very low sensitivity. It is possible that testing CA 15-3 in larger numbers of patients may show higher sensitivity.

CA 125 shows high sensitivity in ovarian cancer, being often used in routine practice. Numerous studies have confirmed the importance of the determination of the serum level of this marker, even with the slightest presence of an adnexal tumor, and with the transvaginal Doppler sonography they constitute an irreplaceable diagnostic tool [17]. The results of this study are in accordance with those found in the relevant literature [23-25]. Patients with benign tumors had very increased preoperative values. In malignant tumor patients, the

difference comparing the normal values and the benign tumor group of patients was highly significant, and the median values of CA 125 serum marker were almost 10 times higher than the normal values. Furthermore, the deviation of CA 125 serum level from the normal range was even 655% times increased. The reason of this better sensitivity of CA 125 is most likely due to the fact that this tumor marker is being produced by malignant cells of epithelial origin, and also from proliferating mesothelial cells [26].

In the literature the transcription factor p53 is reported as overexpressed in more than 50% of all malignant tumors. The accumulation of the mutant p53 protein in the malignant cells can lead to the creation of anti-p53 autoantibodies in the serum and the other body fluids, which can be detected by the ELISA technique. Many studies have been conducted, measuring the p53 serum levels of autoantibodies in various tumors such as lung, urinary bladder, prostate and breast cancer [27,28]. There are few studies which have examined the serum levels of p53 in patients with ovarian carcinoma [29-31]. In the present study, a significant increase in the median serum values of p53 was found in cancer patients: over 37% of them had higher serum values than the upper limit of normal. Serum p53 levels were normal in women with benign tumors. The increase of p53 serum levels in the postoperative samples is very interesting, although there was not statistically significant difference.

The tissue expression of p53 was determined on frozen sections with immunohistochemistry. Positive findings were confirmed in 42.86% of the tested malignant tumors, and none of the benign tumors. These results correlate to a large extent with the results of serum p53 determination [32,33].

HER-2 oncogene is localized on chromosome 17. Its oncoprotein is a transmembrane tyrosine kinase receptor and belongs to the EGFR family of the receptors [34]. It is normally expressed in decreased levels on all cells of the body. Over 90% of the relevant studies clearly indicate that overexpression of HER-2 is connected with bad prognosis, and 71% of these studies confirm HER-2 as an independent prognostic factor [35-39]. Furthermore, evidence suggests that HER-2 can be also a predictive factor by which the success of a certain therapy can be presumed.

The results of our study mostly match those of the relevant literature: strong overexpression was present in 57% of the ovarian carcinoma tissues and in 33% of the benign ovarian tumors. However, considering the fact that immunohistochemistry was carried out in a very small sample of patients, the percent marked as positive (+3) was not statistically significant.

Immunohistochemical testing of the nm23 marker gave results which correlate with the results of HER-2 expression. nm23 expression was high in 71.43% of the malignant and in 66.67% of the benign tumors. Although highly sensitive this marker cannot help in the differential diagnosis of benign and malignant tumors.

Conclusions

The tumor markers specific for ovarian carcinoma were increased in the patient group of malignant tumors:

1) All of the examined tumor markers (CA 125, CA 15-3 and p53) were increased in patients with ovarian carcinoma, but CA 125 has shown the highest sensitivity.

2) The intensity of the tissue expression of HER-2, p53 and nm23 was positive in approximately 50% of the total number of malignant tumor samples, and these results correlate well with the data from the relevant literature.

3) The tissue expression of p53 and its level in the serum were well correlated.

4) The results of clinical studies suggest that for early diagnosis and adequate follow up of ovarian cancer it is necessary to use not only CA 125, but also other markers such as CA 15-3 and p53, which can give more information about the behavior of the malignant disease.

Nevertheless, none of the numerous diagnostic and prognostic parameters which are used today is perfect. The basic reason for that is the fact that the biological characteristics of the tumors under examination often do not give the real picture of the disease. Bonding of clinical, diagnostic and prognostic tests may give more help in solving this situation.

References

1. Berg JN, Lampe JG. High risk factors in gynecologic cancer. Cancer 1981; 48: 429-441.
2. Averette HE, Hoskins W, Nguyen HN et al. National survey of ovarian carcinoma: A patient care evaluation study of the American College of Surgeons. Cancer 1993; 71: 1629-1638.
3. Yarbro JW, Page DL, Fielding LP, Partridge EE, Murphy GP. American Joint Committee on Cancer prognostic factors consensus conference. Cancer 1999; 86: 2436-2446.
4. NIH Consensus Development Conference on Ovarian Cancer: Screening, treatment, and follow-up. Gynecol Oncol 1994; 55: S173.
5. Kanjuh V (Ed). General tumor pathology. Redakcija Medicinskog Podmlatka, Beograd, 1968 (in Serbian).
6. Gayther SA, Warren W, Mazoyer S et al. Germline mutation

of the BRCA1 gene in breast and ovarian cancer families provides evidence for genotype-phenotype correlation. Nat Genet 1995; 11: 428-433.
7. Suzuki M, Saito S, Saga Y et al. Mutation of K-RAS proto-oncogene and loss of heterozygosity on 6q27 in serous and mucinous ovarian carcinomas. Cancer Genet Cytogenet 2000; 118: 132-135.
8. Harris C, Hollstein M. Clinical implications of the p53 tumor suppressor gene. NEJM 1993; 18: 1318-1327.
9. Pieretti M, Powell DE, Gallion HH, Case EA, Conway PS, Turker MS. Genetic alterations on chromosome 17 distinguish different types of epithelial ovarian tumors. Hum Pathol 1995; 26: 393-397.
10. Paik S, Hazan R, Fisher ER et al. Pathologic findings from the national surgical adjuvant breast and bowel project: prognostic significance of erbB-2 protein overexpression in primary breast cancer. J Clin Oncol 1990; 8: 103-109.
11. Gusterson B, Machin L, Gullick W et al. Immunohistochemical distribution of c-erbB-2 in infiltrating and in situ breast cancer. Int J Cancer 1988; 42: 842-849.
12. Simone G, Falco G, Caponio MA et al. nm23 expression in malignant ascitic effusions of serous ovarian adenocarcinoma. Int J Oncol 2001; 19: 885-890.
13. Wu X, Zhang Z, Shi D. Nucleoside diphosphate kinase/nm23H1 expression in ovarian epithelial carcinoma: retroperitoneal lymphatic metastasis and survival. Zhonghua Yi Xue Za Zhi 1998; 78: 275-277.
14. Kapitanovic S, Spaventi R, Vujsic S et al. nm23-H1 gene expression in ovarian tumors-a potential tumor marker. Anticancer Res 1995; 15: 587-590.
15. Barrios CH, de Guedes Neto E, Monteggia P, Fuhrmeister F, Basso A, Siqueira DP. Value of CA 125 as a tumor marker in ovarian carcinomas. Rev Assoc Med Bras 1995; 41: 329-332.
16. Crombach G, Zippel HH, Wurz H. Experiences with CA 125, a tumor marker for malignant epithelial ovarian tumors. Geburtshilfe Frauenheilkd 1985; 45: 205-212.
17. Menon U, Talaat A, Rosenthal AN et al. Performance of ultrasound as a second line test to serum CA125 in ovarian cancer screening. Br J Obst Gynecol 2000; 107: 165-169.
18. Gang Y, Adachi I, Ohkura H et al. CA 15-3 is present as a novel tumor marker in the sera of patients with breast cancer and other malignancies. Gan To Kagaku Ryoho 1985; 12: 2379-2386.
19. Stearns V, Yamauchi H, Hayes DF. Circulating tumor markers in breast cancer: accepted utilities and novel prospects. Breast Cancer Res Treat 1998; 52: 239-259.
20. Hayes DF, Isaacs C, Stearns V. Prognostic factors in breast cancer: current and new predictors of metastasis. J Mammary Gland Biol Neoplasia 2001; 6: 375-379.
21. Lamerz R. Clinical relevance of tumor markers. Wien Klin Wochenschr 1989; 101: 464-472.
22. Pujol H. Importance of early detection of metastases. Impact on survival and quality of life. Nucl Med Biol 1994; 21: 303-315.
23. Nikolic B, Mitrovic A, Lazic J. Meaning of CA 125 in making therapy decision in adnexal inflammatory tumors. Bosn J Basic Med Sci 2006; 6: 3-6.
24. Erdemoglu E, Kamaci M, Ozen S, Sahin HG, Kolusari A. Ovarian hemangioma with elevated CA125 and ascites mimicking ovarian cancer. Eur J Gynaecol Oncol 2006; 27: 195-196.
25. Terauchi F, Onodera T, Nagashima T et al. Sclerosing stromal tumor of the ovary with elevated CA125. J Obstet Gynaecol Res 2005; 31: 432-435.
26. Kandylis K, Vassilomanolakis M, Baziotis N et al. Diagnostic significance of the tumor markers CEA, CA 15-3 and CA 125 in malignant effusions in breast cancer. Ann Oncol 1990; 1: 435-438.
27. Crawford LV, Pim DC, Bulbrook RD. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. Int J Cancer 1982; 30: 403-408.
28. Mack U, Ukena D, Montenarh M, Sybrecht GW. Serum anti-p53 antibodies in patients with lung cancer. Oncol Rep 2000; 7: 669-674.
29. Terauchi F, Okamoto A, Nagashima T et al. Clinical significance of p21 (WAF1/CIP1) and p53 expression in serous cystadenocarcinoma of the ovary. Oncol Rep 2005; 14: 363-368.
30. Berker B, Dunder I, Ensari A, Cengiz SD, Simsek E. Prognostic significance of apoptotic index and bcl-2 and p53 expression in epithelial ovarian carcinoma. Eur J Gynaecol Oncol 2002; 23: 505-510.
31. Harlozinska A, Bar JK, Montenarh M, Kartarius S. Relations between immunologically different p53 forms, p21(WAF1) and PCNA expression in ovarian carcinomas. Oncol Rep 2002; 9: 1173-1179.
32. Chang K, Ding I, Kern FG, Willingham MC. Immunohistochemical analysis of p53 and HER-2/neu proteins in human tumors. J Histochem Cytochem 1991; 39: 1281-1287.
33. Kupryjanczyk J, Bell DA, Yandell DW, Scully RE, Thor AD. p53 expression in ovarian borderline tumors and stage I carcinomas. Am J Clin Pathol 1994; 102: 671-676.
34. Navolanic PM, Steelman LS, McCubrey JA et al. EGFR family signaling and its association with breast cancer development and resistance to chemotherapy. Int J Oncol 2003; 22: 237-252.
35. Ross JS, Fletcher JA. The HER-2/neu oncogene: prognostic factor, predictive factor and target for therapy. Semin Cancer Biol 1999; 9: 125-138.
36. Verri E, Guglielmini P, Puntoni M et al. HER2/neu oncoprotein overexpression in epithelial ovarian cancer: evaluation of its prevalence and prognostic significance. Clinical study. Oncology 2005; 68: 154-161. Epub 2005, Jul 11.
37. Hogdall EV, Christensen L, Kjaer SK et al. Distribution of HER-2 overexpression in ovarian carcinoma tissue and its prognostic value in patients with ovarian carcinoma: from the Danish MALOVA Ovarian Cancer Study. Cancer 2003; 98: 66-73.
38. Riener EK, Arnold N, Kommoss F, Lauinger S, Pfisterer J. The prognostic and predictive value of immunohistochemically detected HER-2/neu overexpression in 361 patients with ovarian cancer: a multicenter study. Gynecol Oncol 2004; 95: 89-94.
39. Nielsen JS, Jakobsen E, Holund B, Bertelsen K, Jakobsen A. Prognostic significance of p53, HER-2, and EGFR overexpression in borderline and epithelial ovarian cancer. Int J Gynecol Cancer 2004; 14: 1086-1096.