REVIEW ARTICLE

CONTRIBUTION OF IMMUNOHISTOCHEMISTRY IN PROGNOSTIC ASSESSMENT OF EPITHELIAL OVARIAN CARCINOMA – REVIEW OF THE LITERATURE I.

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Summary: Epithelial ovarian carcinoma is worldwide the sixth most common female cancer, and this malignancy carries the highest mortality among all gynecological cancers. The high mortality is due mostly to the fact that the tumor is frequently diagnosed late, in advanced stage, as the early disease is often asymptomatic and no effective screening methods are available. The most important prognostic factors in ovarian carcinoma are the stage, size of residual tumor following surgery, presence of ascites, age and the general condition of the patient, tumor histology, and, in patients with early disease, also the grade of the tumor. Large number of studies on prognostic and predictive factors in epithelial ovarian carcinoma has been published, often with contradictory results. The most intensely studied prognostic factors are those for expression of hormonal receptors, for tumor proliferation activity (mainly by antigen Ki-67 and topoisomerase IIα), the markers of apoptosis (p53, p21, mdm2, bcl-2 and other proteins), or other oncoproteins (particularly HER-2/neu).

Key words: *Epithelial Ovarian Carcinoma; Prognosis; Immunohistochemistry; Steroid Receptors; Proliferation activity; HER-2/neu; Apoptosis*

Introduction

Epithelial ovarian cancer (EOC) is the sixth most common cancer in women worldwide. In Western countries, ovarian cancer is the fifth most common malignancy and ranks fourth in cancer mortality (24). EOC carries the highest mortality among all gynecological malignancies. The high mortality is due mostly to the fact that the tumor is frequently diagnosed late, in advanced stages (III, or even IV) as the early stages are often asymptomatic, and no effective screening methods are available. The average 5-year survival is around 40%, in patients with advanced disease only 10–20% (24,43).

In the absence of preventable etiologic factors or effective tools for screening, the only possible means of improving survival currently lies with the optimal management of patients after initial diagnosis (6). In the early 1990s, the Gynecologic Oncology Group (GOG) demonstrated that age, performance status, stage, tumor grade, tumor cell type (histology), presence or absence of ascites, size and number of residual lesions after primary cytoreduction surgery, and administration of cisplatin-based chemoterapy are of prognostic significance in advanced EOC (19,32).

There has been considerable effort directed at identifying factors that accurately predict outcome in patients with advanced EOC. A variety of putative prognostic factors have been reported, but their independent prognostic significance remains unclear. Immunohistochemistry has been widely used in the search of such markers. In this review four groups of potential prognostic factors are discussed:

1. steroid receptors expressed by tumor cells,
2. cell growth kinetics in ovarian carcinoma assessed by examination of proliferation activity of the tumor cell (mainly by antigen Ki-67 and topoisomerase IIα),
3. the expression of oncoprotein HER-2/neu,
4. the expression of markers of apoptosis (p53, p21, mdm2, bcl-2 and other proteins).

1. Steroid receptors

Progesterone and estrogen receptors are important hormones secreted by the ovary and acting through specific receptors. The interaction between steroid hormones and their respective receptors (estrogen receptor, ER and progresterone receptor, PR) are thought to play an important role in the process of carcinogenesis in gynecologic cancers as well as other primary tumors. Steroid hormone receptor status is of primary importance in breast carcinoma, but has also been shown to be a prognostic indicator in endometrial and prostate carcinomas. Tumor expression of ER and/or PR, as well as their pattern of combinations

(ER+/PR+, ER+/PR-, ER+/PR-, ER-/PR-) have been identified as predictive factors for response to endocrine treatment (28).

In contrast to breast carcinoma, limited, inconclusive and conflicting data regarding the prognostic significance of ER and PR expression are available for EOC, and clinical value of determining steroid hormone receptors in this malignancy is controversial. Earlier work (23,39,41) was performed using DCC method (dextran-coated charcoal). DCC detection of estrogen receptor, however, has been found to give falsely positive results if surrounding benign tissue expresses this receptor, or falsely negative results have been obtained in cases of receptors masked by endogenous estrogen. Today, immunohistochemistry is considered the method of choice because it allows an exact assignment of ER and PR expression to tissue components of interest (28).

Some of recent studies found expression of PR to be an independent indicator of favorable prognosis in EOC (1, 14,15,28). Münstedt et al. demonstrated that the favorable course of PR+ ovarian carcinoma relates primarily to the subgroup ER-/PR+ expressing tumors. This tumor phenotype was associated with superior prognosis compared to tumors with other steroid hormone receptors combinations, and ER-/PR+ tumors were associated with lower volume of ascites, less advanced tumor stage and lower tumor grade, reflecting a less aggressive tumor biology. The favorable effect of the ER-/PR+ phenotype was retained in multivariate analysis (28).

Although there is no single explanation for the effect of steroid hormone receptor expression on prognosis, two hypotheses have been proposed. Estrogen-responsive cells efficiently repair DNA and avoid apoptosis, leading to clonal expansion and drug resistance (30). On the other hand, progesterone promotes cell differentiation and apoptosis, and stimulation of PR inhibits DNA synthesis and cell division (29). These mechanisms may explain why patients with ER+/PR- tumors have the worst and those with ER-/PR+ tumors the best prognosis (28).

However, other studies did not confirm these results (12,44). Our study on 96 patients with ovarian carcinoma found prognostic significance of expression of PR, and ER-/PR+ phenotype of tumors only in univariate, but not in multivariate analyses (44).

2. Proliferation activity of the tumor cell

The proliferation index showed the correlation with the prognosis and other known clinicopathologic features in several primary tumors, including lung and breast carcinomas, and lymphomas. The number of proliferating cells can be determined using a variety of methods, but many of these methods have significant technical limitations. In particular, DNA flow cytometry has been widely used in EOC. However, for DNA index only a single cell suspension is required, and the tissue architecture is lost and not evaluable (9).

Immunohistochemistry, using monoclonal antibodies on formalin-fixed paraffin-embedded archival material has been widely used. Ki-67 and topoisomerase IIα are most frequently evaluated immunohistochemically detectable proliferation antigens. Ki-67 is a nuclear non-histone protein expressed in cells in G1, S, G2, and M cell cycle phases, but absent from quiescent cells in G0. Type II DNA topoisomerases are nuclear enzymes that play a crucial role in DNA replication. They catalyze the relaxation of supercoiled DNA and separate intertwined DNA duplexes in an ATP-dependent process, through the generation of a double-stranded nick on the DNA during trascription. Topoisomerase IIα is expressed during the G1, S, G2, and M phases cell cycle (13).

Studies on DNA content and cell proliferation in EOC have yielded conflicting results regarding the prognostic significance of these parameters. Garzetti et al. (9) and Huettner et al. (20) found that malignant ovarian neoplasm had higher median percentage of Ki-67 staining than borderline and benign tumors, and they observed a significant relationship between the Ki-67 index and disease-free survival that was independent of histologic grade and stage. Similarly, Goff et al. (12), Rölke et al. (36), Sengupta et al. (38), and Kaern et al. (21) found that Ki-67 is marker that is expressed differently between the short- and long-term survivors. High cellular proliferative activity was associated with poor outcome (21). On the other hand, other studies did not confirm relationship between proliferation activity and EOC prognosis (5,34,44).

Gotlieb et al. tried to evaluate topoisomerase IIα compared to Ki-67 expression as a marker for tumor behavior and for prognosis in EOC. Ki-67 expression was more frequent in short-term survivors compared to long-term survivors, but the difference was less prominent than with topoisomerase IIα. Specificity and sensitivity as prognostic factors was 88.2% and 93.8% for topoisomerase IIα, compared to 55.6% and 88.2% for Ki-67 (13). Similar results were also reported by van der Zee et al. (45).

3. Oncoprotein HER-2/neu (c-erb-2)

The HER-2/neu oncogene encodes a transmembrane glycoprotein that is member of the class I receptor tyrosine kinase family, which includes the epidermal growth factor, HER-2/neu, HER-3 and HER-4 (16). The HER-2/neu oncogene is located on chromosome 17 and is not activated by a point mutation, but through amplification and over expresion of the wild-type gene (16,27).

Amplification of the HER-2/neu oncogene may be observed in 20–30 % of cases in a wide spectrum of neoplastic disorders (e.g. breast, salivary glands, or lung carcinomas), and HER-2 over-expression is correlated with a poor prognosis (22,27). In clinical practice, the monoclonal anti-HER-2/neu antibody transtuzumab (Herceptin) has been used as the first molecular targeted biologic agent for patients with HER-2/neu over-expressing metastatic breast carcinoma and, more recently, in adjuvant setting (42).

Similarly, many studies in EOC have reported on the association between HER-2/neu expression and outcome (3, 5,16,17,22,27,31,37). Some earlier studies reported that HER-2/neu over-expression was a poor prognostic factor (3,37), but later studies reported that HER-2/neu expression had no relationship with prognosis (5,16,17,22,27,37). Thus, no definitive conclusion has been reached as to the relationship between HER-2/neu expression and prognosis.

Different rates of HER-2/neu expression result from different methods and techniques of detecting HER-2/neu and different criteria for evaluating HER-2/neu expression. First, methods of detecting HER-2/neu expression include immunostaining HER-2/neu expression (with various antibodies) and the direct detection of amplification of the HER-2/neu gene such as fluorescent in situ hybridization (FISH). Second, the scoring system that used in studies was different. In recent studies staining for determining HER-2/neu protein expression was scored on a scale 0, +1, +2 and +3 according to the HercepTest (HercepTest, DAKO, Glostrup, Denmark), and +2 and +3 were regarded as over-expression. This scoring system was developed in 1997, and HercepTest has been approved by the United States Food and Drug Administration as a standardized diagnostic kit to detect HER-2/neu. Therefore, recent studies have reported more reliable results than previous studies (42).

Most studies examined relationship between HER-2/neu expression and prognosis in various histological types of ovarian carcinoma, but not in a specific histological type. The examination of the relationship between HER-2/neu expression and outcome should focus on a prognostic importance of histological type and considered to the stage of cancer. Tanabe et al. limited their study to clear cell carcinoma that is chemotherapy-resistant EOC variant of poor prognosis and found neither association between HER-2/neu expression and outcome, nor association between HER-2/neu over-expression and the stage or lymph node metastasis. This study demonstrated that HER-2/neu expression in this histological type was not a prognostic risk factor (42).

4. Markers of apoptosis

Proto-oncogenes and tumor suppressor genes play a critical role in normal cell growth and in tumorigenesis. Proto-oncogenes normally stimulate differentiation and proliferation, but when altered, promote malignant transformation. In contrast, tumor suppressor genes inhibit cell division and/or promote cell death, and inactivation causes loss of the normal negative control of cell growth. Genetic alterations thus drive the transformation of normal cells into highly malignant clones (31).

The **p53** tumor suppressor gene is the most frequently mutated gene in human cancer that plays a critical role in the regulation of cell cycle and apoptosis. Mutations of p53 have been found in approximately 40–80% of EOC cases (35). The p53 gene resides at a critical "crossroads" that modifies diverse cellular functions. In the event of injury, human cells are dependent on a functional p53 for DNA repair, or, if the damage is irreparable, for apoptosis. The p53 protein, acting as a transcription factor, activates or alternatively represses the transcription of genes leading to the expression of specific elements necessary for the inhibition of cell growth and the induction of apoptosis. The absence of functional p53 thus leads to deregulated cellular proliferation. Mis-sense mutations of the p53 gene result in protein that have longer half-life than their wild-type counterparts and are resistant to degradation. As a result, these mutations appear to give rise to p53 over-expression by immunohistochemical techniques with a variety of different antibodies. Null mutation (insertion, deletion, splice site aberration, and nonsense) result in a truncated protein product that cannot bind DNA or induce apoptosis. Such mutation generally do not result in increased p53 stability, and the truncated protein is often undetected by conventional immunohistochemical techniques (40).

Several lines of evidence have elucidated a major role of functional p53 gene for enhancing therapeutic response to chemotherapy or radiation. Mutant p53 therefore may directly decrease tumor cell sensitivity to chemotherapeutic agents and promote the emergence of drug resistant population of cancer cells (38, 40).

The role of p53 protein in EOC is contentious, and there is a number of studies with contradictory results. Several studies have identified p53 protein as an adverse prognostic factor for survival in EOC (18,31,33,40). Other studies have suggested that alterations in p53 expression in ovarian cancer affect sensitivity to chemotherapy (35,38). In contrast, there are a number of studies that suggest that p53 expression has no prognostic value in EOC (8,25).

The aim of study Dogan et al. was to investigate the prognostic significance of p53 nad mdm2 protein expression in EOC (7). **Mdm2** gene is a proto-oncogene that encodes a nuclear protein that negatively regulates the trascriptional activating function of p53. In addition, mdm2 protein can sequester the p53 protein. Thus, over-expression of mdm2 protein results in an effect similar to the mutational inactivation of p53. It was found that mdm2 expression predicts response to chemotherapy, and co-expression of p53 and mdm2 proteins was also related to poor outcome (7). However, Mano et al. did not confirm this in multivariate analysis (26).

Protein **p21$^{waf1/cip1}$** is a cyclin-dependent kinase inhibitor that is usually induced through a p53 related pathway. p21$^{waf1/cip1}$ has been shown to be integral to control of the cell cycle after DNA damage. Up-regulation of p21$^{waf1/cip1}$ by p53 is integral to sustaining G2 arrest after DNA damage. In cells without functional p53, p21$^{waf1/cip1}$ can be up-regulated by the activation of protein kinase C. Although p21$^{waf1/cip1}$ has been studied in EOC, the role of this protein as a prognostic indicator is still controversial (11,38). Some studies confirm the importance of the combination of p21 and p53 staining in determining EOC prognosis.

Expression of p53 protein in the absence of $p21^{waf1/cip1}$ expression was a better marker of poor prognosis than either p53 or $p21^{waf1/cip1}$ expression status alone (2,11,46).

Proteins of the **Bcl-2** family are critical regulators of the apoptotic pathway. Certain members of the family promote apoptosis (e.g. Bax, Bad, Bcl-X_S) while others have an anti-apoptotic function (Bcl-2, Bcl-X_L). The ratio of pro- and anti-apoptotic members, such as Bax and Bcl-2 is critical in the inhibition or induction of apoptosis (38). A variety of tumors, including EOC, resistant to anticancer drugs express Bcl-2, suggesting that Bcl-2 may protect cancer cells from programmed cell death induced by a variety of antitumor agents, including cisplatin (26). Mano et al. found that Bcl-2 protein may represent a possible predictor of response to chemotherapy. Multivariate analysis revealed that Bcl-2 protein is significant independent prognostic factor (26), but other studies did not confirm this observation (4,38). Geisler et al. found that Bcl-2 protein itself is not an independent prognostic indicator, but the combination of p53 and Bcl-2 can independently predict survival (10).

Conclusion

Immunohistochemicaly detectable prognostic and predictive factors in EOC have recently been widely covered in the literature, specifically the expression of steroid receptors by tumor cells, the assessment of cell growth kinetics by examination of proliferation activity of the tumor, the expression of oncoprotein HER-2/neu, and the expression of markers of apoptosis. A number of studies have been reported, often with contradictory results. However, no single immunohistochemically detectable marker has been so far identified in EOC that would provide reliable and reproducible prognostic or predictive information.

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References

1. Akahira J, Inoue T, Suzuki T, Ito K, Konno R, Sato S, Moriya T, Okamura K, Yajima A, Sasano H. Progesterone receptor isoforms A and B in human epithelial ovarian carcinoma: immunohistochemical and RT-PCR studies. Br J Cancer 2000;83:1488–94.
2. Bali A, O'Brien PM, Edwards LS, Sutherland RL, Hacker NF, Henshall SM. Cyclin D1, p53, and p21Waf1/Cip1 expression is predictive of poor clinical outcome in serous epithelial ovarian cancer. Clin Cancer Res 2004;10:5168–77.
3. Berchuck A, Kamel A, Whitaker R, Kerns B, Olt G, Kinney R, Soper JT, Dodge R, Clarke-Pearson DL, Marks P, et al. Overexpression of HER-2/neu is associated with poor survival in advanced epithelial ovarian cancer. Cancer Res 1990; 50:4087–91.
4. 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;23:505–10.
5. Camilleri-Broet S, Hardy-Bessard AC, Le Tourneau A, Paraiso D, Levrel O, Leduc B, Bain S, Orfeuvre H, Audouin J, Pujade-Lauraine E. HER-2 overexpression is an independent marker of poor prognosis of advanced primary ovarian carcinoma: a multicenter study of the GINECO group. Ann Oncol 2004; 15:104–12.
6. Chi DS, Liao JB, Leon LF, Venkatraman ES, Hensley ML, Bhaskaran D, Hoskins WJ. Identification of prognostic factors in advanced epithelial ovarian carcinoma. Gynecol Oncol 2001;82:532–7.
7. Dogan E, Saygili U, Tuna B, Gol M, Gurel D, Acar B, Koyuncuoglu M. p53 and mdm2 as prognostic indicators in patients with epithelial ovarian cancer: a multivariate analysis. Gynecol Oncol 2005;97:46–52.
8. Fallows S, Price J, Atkinson RJ, Johnston PG, Hickey I, Russell SE. P53 mutation does not affect prognosis in ovarian epithelial malignancies. J Pathol 2001; 194:68–75.
9. Garzetti GG, Ciavattini A, Goteri G, De Nictolis M, Stramazzotti D, Lucarini G, Biagini G. Ki67 antigen immunostaining (MIB 1 monoclonal antibody) in serous ovarian tumors: index of proliferative activity with prognostic significance. Gynecol Oncol 1995;56:169–74.
10. Geisler JP, Geisler HE, Miller GA, Wiemann MC, Zhou Z, Crabtree W. p53 and bcl-2 in epithelial ovarian carcinoma: their value as prognostic indicators at a median follow-up of 60 months. Gynecol Oncol 2000;77:278–82.
11. Geisler HE, Geisler JP, Miller GA, Geisler MJ, Wiemann MC, Zhou Z, Crabtree W. p21 and p53 in ovarian carcinoma: their combined staining is more valuable than either alone. Cancer 2001;92:781–6.
12. Goff BA, Muntz HG, Greer BE, Tamimi HK, Gown AM. Oncogene expression: long-term compared with short-term survival in patients with advanced epithelial ovarian cancer. Obstet Gynecol 1998;92:88–93.
13. Gotlieb WH, Goldberg I, Weisz B, Davidson B, Novikov I, Kopolovic J, Ben-Baruch G. Topoisomerase II immunostaining as a prognostic marker for survival in ovarian cancer. Gynecol Oncol 2001;82:99–104.
14. Harding M, Cowan S, Hole D, Cassidy L, Kitchener H, Davis J, Leake R. Estrogen and progesterone receptors in ovarian cancer. Cancer 1990;65:486–91.
15. Hempling RE, Piver MS, Eltabbakh GH, Recio FO. Progesterone receptor status is a significant prognostic variable of progression-free survival in advanced epithelial ovarian cancer. Am J Clin Oncol 1998;21:447–51.
16. Hengstler JG, Lange J, Kett A, Dornhofer N, Meinert R, Arand M, Knapstein PG, Becker R, Oesch F, Tanner B. Contribution of c-erbB-2 and topoisomerase IIalpha to chemoresistance in ovarian cancer. Cancer Res 1999;59:3206–14.
17. Hogdall EV, Christensen L, Kjaer SK, Blaakaer J, Bock JE, Glud E, Norgaard-Pedersen B, Hogdall CK. 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.
18. Hogdall EV, Kjaer SK, Blaakaer J, Christensen L, Glud E, Vuust J, Hogdall CK. P53 mutations in tissue from Danish ovarian cancer patients: from the Danish „MALOVA" ovarian cancer study. Gynecol Oncol 2006;100:76–82.
19. Hoskins WJ, Bundy BN, Thigpen JT, Omura GA. The influence of cytoreductive surgery on recurrence-free interval and survival in small-volume stage III epithelial ovarian cancer: a Gynecologic Oncology Group study. Gynecol Oncol 1992; 47:159–66.
20. Huettner PC, Weinberg DS, Lage JM. Assessment of proliferative activity in ovarian neoplasms by flow and static cytometry. Correlation with prognostic features. Am J Pathol 1992;141:699–706.
21. Kaern J, Aghmesheh M, Nesland JM, Danielsen HE, Sandstad B, Friedlander M, Trope C. Prognostic factors in ovarian carcinoma stage III patients. Can biomarkers improve the prediction of short- and long-term survivors? Int J Gynecol Cancer 2005;15:1014–22.
22. Kim YT, Kim JW, Lee JW. c-erbB-2 oncoprotein assay in ovarian carcinoma and its clinical correlation with prognostic factors. Cancer Lett 1998;132:91–7.
23. Kommoss F, Pfisterer J, Thome M, Schafer W, Sauerbrei W, Pfleiderer A. Steroid receptors in ovarian carcinoma: immunohistochemical determination may lead to new aspects. Gynecol Oncol 1992;47:317–22.
24. Kurman RJ. Blaustein's Pathology od the Female Genital Tract. Fifth Edition. Springer-Verlag, New York, Berlin, Heidelberg, 2002:791–904.
25. Laframboise S, Chapman W, McLaughlin J, Andrulis IL. p53 mutations in epithelial ovarian cancers: possible role in predicting chemoresistance. Cancer J 2000;6:302–8.
26. Mano Y, Kikuchi Y, Yamamoto K, Kita T, Hirata J, Tode T, Ishii K, Nagata I. Bcl-2 as a predictor of chemosensitivity and prognosis in primary epithelial ovarian cancer. Eur J Cancer 1999;35:1214–9.
27. Medl M, Sevelda P, Czerwenka K, Dobianer K, Hanak H, Hruza C, Klein M, Leodolter S, Mullauer-Ertl S, Rosen A, et al. DNA amplification of HER-2/neu and INT-2 oncogenes in epithelial ovarian cancer. Gynecol Oncol 1995; 59:321–6.
28. Münstedt K, Steen J, Knauf AG, Buch T, von Georgi R, Franke FE. Steroid hormone receptors and long term survival in invasive ovarian cancer. Cancer. 2000; 89:1783–1791.
29. Murdoch WJ. Perturbation of sheep ovarian surface epithelial cells by ovulation: evidence for roles of progesterone and poly(ADP-ribose) polymerase in the restoration of DNA integrity. J Endocrinol 1998;156:503–8.
30. Murdoch WJ, Van Kirk EA. Oestradiol inhibits spontaneous and cisplatin-induced apoptosis in epithelial ovarian cancer cells: relationship to DNA repair capacity. Apoptosis 1997;2:478–84.
31. 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;14:1086–96.
32. Omura GA, Brady MF, Homesley HD, Yordan E, Major FJ, Buchsbaum HJ, Park RC. Long-term follow-up and prognostic factor analysis in advanced ovarian car-

cinoma: the Gynecologic Oncology Group experience. J Clin Oncol 1991;9: 1138–50.
33. Ozalp SS, Yalcin OT, Basaran GN, Artan S, Kabukcuoglu S, Minsin TH. Prognostic significance of deletion and over-expression of the p53 gene in epithelial ovarian cancer. Eur J Gynaecol Oncol 2000;21:282–6.
34. Reitmaier M, Rudlowski C, Biesterfeld S, Rath W, Schroder W. Comparative studies on the biological significance of the marker for proliferation Ki-67-Antigen and PCNA in primary ovarian carcinoma. Zentralbl Gynakol 2000; 122:361–7.
35. Reles A, Wen WH, Schmider A, Gee C, Runnebaum IB, Kilian U, Jones LA, El-Naggar A, Minguillon C, Schonborn I, Reich O, Kreienberg R, Lichtenegger W, Press MF. Correlation of p53 mutations with resistance to platinum-based chemotherapy and shortened survival in ovarian cancer. Clin Cancer Res 2001; 7:2984–97.
36. Röhlke P, Milde-Langosch K, Weyland C, Pichlmeier U, Jonat W, Loning T. p53 is a persistent and predictive marker in advanced ovarian carcinomas: multivariate analysis including comparison with Ki67 immunoreactivity. J Cancer Res Clin Oncol 1997;123:496–501.
37. Rubin SC, Finstad CL, Wong GY, Almadrones L, Plante M, Lloyd KO. Prognostic significance of HER-2/neu expression in advanced epithelial ovarian cancer: a multivariate analysis. Am J Obstet Gynecol 1993;168:162–9.
38. Sengupta PS, McGown AT, Bajaj V, Blackhall F, Swindell R, Bromley M, Shanks JH, Ward T, Buckley CH, Reynolds K, Slade RJ, Jayson GC. p53 and related proteins in epithelial ovarian cancer. Eur J Cancer 2000;36:2317–28.
39. Sevelda P, Denison U, Schemper M, Spona J, Vavra N, Salzer H. Oestrogen and progesterone receptor content as a prognostic factor in advanced epithelial ovarian carcinoma. Br J Obstet Gynaecol 1990;97:706–12.
40. Shahin MS, Hughes JH, Sood AK, Buller RE. The prognostic significance of p53 tumor suppressor gene alterations in ovarian carcinoma. Cancer 2000; 89:2006–17.
41. Slotman BJ, Nauta JJ, Rao BR. Survival of patients with ovarian cancer. Apart from stage and grade, tumor progesterone receptor content is a prognostic indicator. Cancer 1990;66:740–4.
42. Tanabe H, Nishii H, Sakata A, Suzuki K, Mori Y, Shinozaki H, Watanabe A, Ochiai K, Yasuda M, Tanaka T. Overexpression of HER-2/neu is not a risk factor in ovarian clear cell adenocarcinoma. Gynecol Oncol 2004;94:735–9.
43. Tavasolli A, Devilee P. Pathology and Genetics of Tumours of the Breast and Female Genital Organs. In: World Health Organization Classification of Tumours. IARC Press, 2003, Lyon, 117–145.
44. Tomsova M, Melichar B, Sedlakova I, Nova M. Prognostic markers in ovarian carcinoma – retrospective study. Cesk Patol 2005;41:51–9.
45. van der Zee AG, Hollema H, Suurmeijer AJ, Krans M, Sluiter WJ, Willemse PH, Aalders JG, de Vries EG. Value of P-glycoprotein, glutathione S-transferase pi, c-erbB-2, and p53 as prognostic factors in ovarian carcinomas. J Clin Oncol 1995; 13:70–8.
46. Werness BA, Freedman AN, Piver MS, Romero-Gutierrez M, Petrow E. Prognostic significance of p53 and p21(waf1/cip1) immunoreactivity in epithelial cancers of the ovary. Gynecol Oncol 1999;75:413–8.

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