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PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) IN OVARIAN CARCINOMA AND ITS RELATION TO LYMPH NODE METASTASIS AND PROGNOSIS

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Objective: To investigate expression of proliferating cell nuclear antigen (PCNA) in ovarian epithelial cancer and its relation to lymph node metastasis, outcome of second-look laparotomy (SLL) and prognosis. Methods: Monoclonal antibody PC10 was used to stain PCNA in archival paraffin-embedded tissues. Results: PC10 immunostaining was performed successfully in all 74 primary and 31 intraperitoneal metastatic tumors. The expression levels of PCNA were significantly increased in 31 metastatic tumors compared with their primary tumor from the same patients (7.94 vs 6.89, P=0.042). The expression levels was more elevated in bilateral than in unilateral ovarian cancer, but it was not associated with lymph node metastasis, clinical stage, histological grade and subtype. In 28 patients with stage III ovarian cancer undergone SLL, the mean immunoreactive score (IRS) of PCNA of the primary tumor was significantly higher in patients with negative SLL than in those with positive SLL (7.59 vs 6.10, P=0.03). Since chemotherapy was performed following surgical debulking, negative SLL more frequently seen in patients with high PCNA expression might suggest better chemotherapeutic sensitivity due to higher proliferation fraction of tumor cell. Univariate analysis of survival indicated that the overall survival was inversely associated with the level of PCNA expression, while multivariate analysis with Cox's model showed that independent prognostic factors were the residual tumor after primary debulking (P<0.001) and clinical stage (P<0.05), followed by PCNA expression (P=0.09). Conclusion: The expression of PCNA may be useful in predicting the patients' prognosis, but is not correlated with lymph node metastasis.

Key words: Ovarian neoplasm, Proliferating cell nuclear antigen, Lymphatic metastasis, Prognosis

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It is suggested that abnormal regulation of cell cycle and uncontrollable proliferation resulted in tumor generation and development. The levels of proliferation may reflect malignancy of human neoplasm. Proliferating cell nuclear antigen (PCNA) is one of antigen, which is associated with cell cycle, and often is regarded as index of cell proliferation. Ovarian cancer is characterized with rapidly proliferating and poor prognosis. But it is not clear that correlation of biologic behavior to PCNA. The aim of our work is to evaluate the levels of PCNA expression in ovarian carcinoma. And also try to determinate the relationship between PCNA expression and lymph node metastasis (LNM), prognosis and outcome of second-look laparotomy (SLL).

MATERIAL AND METHODS

Patients: The study included 74 patients with primary ovarian epithelial carcinoma in our hospital from 1987 to 1995. Their average age was 51 years

(19-63). According to histological classification of WHO, there were 40 serous, 8 mucous, 7 endomatrial adenocarcinoma and 19 other epithelial carcinoma. The patients were divided into stage I (5/74), stage II (12/74), stage III (53/74) and stage IV (4/74) by the criterion of International Federation of Gynecology and Obstetrics (FIGO) in 1988. Those patients were selected by following conditions: undergoing a thoughtful staging laparotomy, the status of retroperitoneal lymph node confirmed by lymphadenectomy or sampling surgery, and no use of chemotherapy before acquiring tumor sample for this study. There were 28 patients received SLL after primary debulking surgery and chemotherapy.

PCNA measurement: Of 74 patients studied, 74 samples from primary tumor of each patient and 31 samples from intraperitoneal metastatic tumor were selected. Fresh tissue was fixed by Forman in 24 hours, then embedded with paraffin. Five-micrometer paraffin tissue continuous sections were mounted on 10 piece of glass slides, one for routine HE staining and the other slides for immunohistochemical staining. Immunohistochemical staining for PCNA was performed by avidin-biotin-peroxidase complex (ABC) technique. It involved sequential application of diluted anti-PCNA (PC10) IgM monoclonic antibody (DAKO Corp., 1:500), biotinglated goat anti-mouse IgM (Vector Lab., 1:200) and peroxidase-labeled streptavidin (Vector Lab., 1: 100). Antigen was visualized by yielding a brown staining in the nucleus of PCNA-positive cells. Considering both staining intensity (SI) and positive cells percent (PP), immunoreactive score (IRS) was applied for the levels of PCNA expression, IRS equals the product of SI and PP.[1] SI value with 0, 1, 2 and 3 stands for negative, weak, middle and strong reaction, and PP value with 0,1, 2, 3 and 4 means that the amount of positive cell account for 0, <10%, 11-50%, 51-80% and >80%, respectively.

Statistical analysis: All patients studied were followed up for median time 913 days (106-3, 239). Mean values of PCNA were compared by F test. The Kaplan-Meier survival curves, log-rank test and Cox proportional hazards model were used for survival analysis (SAS 6.11 software package).

RESULTS

Clinical feature of PCNA expression

All primary tumors in this cohort of 74 women were found positive staining for PCNA with an average IRS of 6.48. With respect to IRS value, 74 patients were divided into 3 groups as following: 38% of patients were of IRS value of 1-4 (grade I), 15% IRS 5-8 (grade II), and 47% IRS 8-12 (Grande III). The IRS of PCNA expression in patients with bilateral ovaries invaded (7.56) was significantly higher than that with unilateral ovary invaded (5.76, P=0.028). Of 31 patients with both primary and intraperitoneal metastatic tumor sample measured, the expression of PCNA was significantly elevated in 31 intraperitoneal metastatic tumors compared with their primary tumor from same patient (6.89 vs 7.94, P=0.042). However the expression levels of PCNA were not associated with patients age, histologic grade, subtype, and stage.

Relationship between PCNA expression and LNM

Forty-one (55.4%) of 74 patients studied were confirmed pathologically having lymphonode metastasis (LNM). But the average IRS (6.98) of PCNA expression in patients with LNM was not significantly different from that without LNM (6.40, P=0.31).

Prognostic value of PCNA expression

The PCNA expression was analyzed with respect to overall survival. The survival of patients with grade I, II and III of IRS value are displayed in Figure 1. According to univariate analysis, there was a tendency for patients with lower survival rates to exhibit higher grade of ISR (P=0.033). Also, the survival of patients was significantly associated with stage, LNM, or amount of residual disease after primary debulking surgery. However, multivariate analysis of Cox model indicated that independent factors of prognosis only included residual tumor (P<0.001), stage (P=0.012), followed by PCNA expression (P=0.09).

PCNA expression: Relation to results at SLL

In this study, there were 28 patients of stage III with a residual of the largest diameter 2-3 cm after primary debulking. Those patients experienced 6-8 cycles of systemic chemotherapy with a scheme of cisplatinum, adriamycin and cyclophoramide. They underwent SLL, for no evidence of disease was found by physically examination and image examination

such as CT, Ultrasonic. There was significantly different average IRS of PCNA expression between 16 patients with negative SLL (7.59) and 18 patients with positive SLL (6.10, P=0.03).

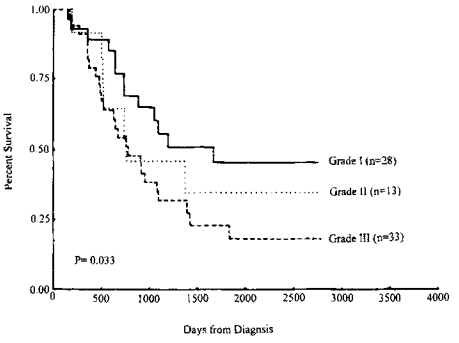

Fig. 1. Patients survival by IRS trisect

DISCUSSION

Overproliferation maybe cause generation of cancer. And proliferation may also response for tumor invasion and metastasis. So that, the levels of proliferation in some tumor can reflect the extent of malignancy of the human tumor. PCNA or cyclin, is a 36 kDa accessory protein to DNA polymerase- δ that is located in the cell nucleus. It is necessary during replication and repair of DNA main chain, and normal cell cycle. PCNA begins to present in later G1-phase, peaks at S-phase, then declines in G2-phase, but it does not present in M-and G0-phase. So PCNA is considered as one of indexes of cell proliferation, which also includes S-phase fraction (SPF) by flow cytometric measure, Thymidin, BrdU or Ki-67 labeling index. PCNA expression has been proved to related with those proliferating indexes. A significantly advantage to PCNA immunostaining in this study is that utilizing anti-PC10 monoclonic antibody specific for proliferation antigen and sensitive to paraffin section. In addition, using IRS for measurement of PCNA expression is more objective.

It was reported that the increased expression of PCNA in patients with gastric, bladder carcinoma and lymphoma suggested their poor outcome.[2] Gao et al[3,4] observed that the expression levels of PC10 in serous and mucous ovarian carcinoma escalate from benign, borderline to malignance tumors. Thomas et al[5] also found that the positivity of PC10 was 91.8% in patients with stage I and stage III, IV, and was lower in patients with borderline tumors. In addition, the lower levels of PCNA, the longer survival in patients with optimal debulking (44 ms vs. 22 ms, P=0.017). In the present study, we demonstrated that PC10 expression levels in tumor cell were strongly inverse associated with the patients survival. However, when we analyzed the relationship PCNA expression and LNM, we didn't found any correlated, although proliferation may relate to ovarian cancer cell spread to peritoneum for PCNA expression in those samples from intraperitoneal metastatic tumor was higher than in that from primary tumor. This would suggest that lymph node metastasis needed other active mechanism besides proliferation.

Conte et al[6,7] determined the thymidine labeling index in 74 patient with epithelial ovarian cancer who were entered into two consecutive randomized trails of platium-containing regimen. They found no correlation between the thymidine labeling index and patient's survival. Of note, a higher response rate to chemotherapy was detected in those patients with more rapidly proliferating tumors. Hartmann et al[8] reported that 5-year survival rates in patients with lower and higher than median of PCNA index were respectively 15% and 40% (P=0.003). There were statistically different PCNA indexes between 25 patients with positive SLL (24.3) and 22 patients with negative SLL (37.5, P=0.02), They considered that the patients with higher PCNA expression were sensitive to chemotherapy, so that their survival were improved. It was also reported that the levels of PCNA expression of ovarian cancer were decreased after chemotherapy. Contrarily, Gadducci et al[9] studied retrospectively 20 patients who underwent SLL after chemotherapy with platium-based regiment, and they found that the patients with high and low PCNA expression had respectively 0% and 36% complete pathological remission (CPR). In 28 patients with stage III of this study, who had similar residual tumor and chemotherapy, the patients with negative SLL presented higher PCNA expression than the positive SLL. It is implied that the tumor with high PCNA could be better sensitive to chemotherapy, so that the residual tumor vanished easily. However, all patients in the study presented the tendency that PCNA expression was inversely related to survival due to receiving variable regiments. It is suggested that PCNA expression reflected both the malignancy and the sensitivity to chemotherapy in human ovarian epithelial carcinoma. Our further works is to confirm

this initial conclusions.

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