RESEARCH COMMUNICATION

Lack of Prognostic Significance of HER-2/neu in Early Epithelial Ovarian Cancer

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Abstract

A total of 74 patients with apparent early stage epithelial ovarian cancer who underwent exploratory laparotomy at King Chulalongkorn Memorial Hospital or other hospitals and were referred for further treatment, were evaluated. Formalin fixed paraffin-embeded ovarian tissue specimens were collected and immuno-stained with HER-2/neu antibodies for compariosn with clinicopathologic data after median follow up of 46 months (range 3 – 83 months). The prevalence of HER-2/neu overexpression in these patients was 10.2%. No significant correlation between HER-2/neu overexpression and clinicopathological parameters (stage, ascites, capsular rupture, capsular adherence, histological subtype and histological grade) was found. Disease free survival and overall survival did not statistically differ between those with lesions positive or negative for HER-2/neu overexpression.

Key Words: HER-2/neu - early ovarian cancer - immunohistochemistry

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Introduction

Epithelial ovarian carcinomas are responsible for the death of more than all other gynecological malignancy. Most of them are detected in an advanced stage of the disease approximately in 80% of the patients (Ozols et al.,2000; Disaia and Creasman,2002), and are usually referred to the cancer center at the beginning of the treatment. Early stage ovarian cancer can practically be operated because of localizing nature of the disease, and truly localized disease (stage IA, grade1-2) is curable by surgery alone (Monga et al., 1991; Trimbos et al.,1991). However, half of the early stage ovarian cancer patients in Thailand, operated in the tertiary care hospital or even in the university hospital, were referred for treatment with inadequate staging.

Identification of prognostic factors such as substage, ascites or positive peritoneal cytology, capsular rupture, capsular adherence or excrescences, histological subtype and histological grade (Soper,1994; Ozols et al.,2000), may be key to successive treatment for patients in the past. However, even among women with early stage disease, long term survival rates lower than 70% has been reported (Soper,1994). The reason of which is because there is yet no unequivocal support for a survival benefit from any form of adjuvant therapy in early ovarian cancer (Hogberg et al.,2001). Eventhough complete surgical evaluation had been attempted, most randomized multi-center control trial (Bolis et al.,1995; Trope et al.,2000) in early stage epithelial ovarian cancer was still inconclusive as regards to the question of adjuvant chemotherapy. More recently, markers associated with functional tumor differentiation (such as tumor ploidy, expression of oncogenes and abnormal forms of suppressor genes) have been evaluated for prognostic importance in order to help identified truly high risk patients who deserved restaging operation or adjuvant treatment.

Tyrosine kinases comprise the largest family of oncogenes. These genes are divided into two classes, receptor and nonreceptor kinases, depending on their structure and function. Structural similarity of epidermal growth factor (EGF) and the HER-2/neu tyrosine kinase have lent to the hypothesis that overexpression of this protein may involved in the signal transduction for growth factor and may result in malignant transformation process. The gene-encoding EGF is called erbB , and the EGF gene that encodes HER-2/neu is called erbB-2.

Although a number of studies reported impaired clinical outcomes in patients with HER-2/neu overexpression (Slamon et al.,1989; van Dam et al.,1994; Berchuck,1999), others failed to demonstrate the relation between the course of the disease and HER-2/neu overexpression (Fajac et al.,1995; van der Zee et al.,1995). Works by Slamon(1989) as well as Berchuck(1990) have demonstrated that HER-2/neu is overexpressed in approximately one third of ovarian cancers in comparison to normal ovarian epithelium. Berchuck(1990) and Felip (Felip et al.,1995), conduct the studied in advanced stage epithelial ovarian carcinoma, found that the patients who had overexpression of this protein also had more chance to have persistent disease at second-look laparotomy and had drastically reduced median survival, in comparison

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to those patients who had normal tumor HER-2/neu expression. Among the asian countries, reports on HER-2/nue overexpression was between 25%and 32% (Yang et al.,1998;Seki et al.,2000) and worsening outcome remained obscure. Most of the studies above had studied on advanced stage disease or all stage with a small number of patients in each stage. In early stage epithelial ovarian cancer, the studies of HER-2/neu overexpression seem to have no strong prognostic significant (Rubin et al.,1994). However, the study in early stage disease still limit by the patient number and number of the reports.

This study is intended to investigate the prevalence of HER-2/neu overexpression in the apparent early stage ovarian cancer, which are still problematic in referral centers, and correlate it with clinicopathological characteristics and prognostic outcomes.

Materials and Methods

Retrospective reviews of patients with apparent early stage epithelial ovarian carcinoma who underwent surgery at Department of Obstetrics and Gynecology, King Chulalongkorn Memorial Hospital between January 1995 to December 1999 have been studied. Patients who were operated elsewhere and hence referred for postoperative treatment at the department were also reviewed. Patients who were diagnosed to have early stage epithelial ovarian cancer were: 1) tumor was confined to ovary or ovaries and 2) tumor was confined to true pelvis according to the surgeon operative finding. Patients who had intra-abdominal disease or advanced metastasis, those who did not have pathological block of primary specimens, those who did not have proper operative record and those who did not complete postoperative adjuvant therapy (if they should receive) were all excluded.

Out of the 110 patients who were treated at King Chulalongkorn Memorial Hospital with the diagnosis of apparent early stage epithelial ovarian cancer, 74 patients were recruited into the study. The operative notes of every patient and their pathologic slides were reviewed for staging according to the criteria of 1985 FIGO classification. Fifty-eight patients were operated at King Chulalongkorn Memorial Hospital and 16 patients were operated at other hospitals and referred for further treatment. All patients were followed-up every four weeks in the first year after surgery and then every eight weeks thereafter. We have followed up patients until December, 31 2004. This study was approved by the Research Ethics Committee of Faculty of Medicine.

Table 1. Association of HER-2/neu Expression and Clinicopathologic Variables

Variables	No.	No. HER-2/neu immunostain (%) Negative	Positive	*P* Value
FIGO stage				
I	62 (83.8%)	43 (69.4%)	19 (30.6%)	P > 0.05
II	12 (16.2%)	8 (66.7%)	4 (33.3%)	
Grade				
1	34 (45.3%)	25 (73.5%)	9 (26.5%)	
2	22 (29.3%)	14 (63.6%)	8 (36.4%)	P > 0.05
3	18 (24.0%)	12 (66.7%)	6 (33.3%)	
Histology				
Serous	6 (8.1%)	5 (83.3%)	1 (16.7%)	
Mucinous	18 (24.3%)	14 (77.8%)	4 (22.2%)	
Clear cell	22 (29.7%)	12 (54.5%)	10 (45.5%)	P > 0.05
Endometrioid	21 (28.4%)	17 (81.0%)	4 (19.0%)	
Mixed type	7 (9.5%)	3 (42.9%)	4 (57.1%)	
Tumor rupture				
Spontaneous	9 (12.2%)	5 (55.6%)	4 (44.4%)	
Accidental	39 (52.7%)	25 (64.1%)	14 (39.5%)	P > 0.05
None	26 (35.1%)	21 (80.8%)	5 (19.2%)	
GOG Risk group				
Low risk	20 (27.0%)	17 (85.0%)	3 (15.0%)	P > 0.05
High risk	54 (73.0%)	34 (63.0%)	20 (37.0%)	
Adhesion				
Yes	45 (60.8%)	31 (68.9%)	14 (31.1%)	P > 0.05
No	29 (39.2%)	20 (69.0%)	9 (31.0%)	
Size				
≤ 10 cm	23 (31.1%)	17 (73.9%)	6 (26.1%)	P > 0.05
> 10 cm	51 (68.9%)	34 (66.7%)	17 (33.3%)	
Recurrent				
Yes	12 (16.2%)	8 (66.7%)	4 (33.3%)	P > 0.05
No	62 (83.8%)	43 (69.4%)	19 (30.6%)	
Resection				
Completely	65 (87.8%)	46 (70.8%)	19 (29.2%)	P > 0.05
Incomplete	9 (12.2%)	5 (55.6%)	4 (44.4%)	
Ascites				
Yes	19 (25.7%)	11 (57.9%)	8 (42.1%)	P > 0.05
No	55 (74.3%)	40 (72.7%)	15 (27.3%)	

Immunohistochemistry

Overexpression of c-erbB-2 was determined by immunohistochemistry using a streptavidin technique. Thin, 3-micrometer slices of paraffin embedded specimens were deparaffinized in a routine manner. After the paraffin was removed, the section were treated for 30 minutes with 0.3% hydrogen peroxide in methanol to block endogenous peroxidase. For antigen retrieval, the sections were placed in coplin jars containing 10 mM citrate buffer, pH 6.0 and, heated in microwave on high for 2 minutes, followed by 5 minutes at medium low. In order to eliminate nonspecific staining, the section were incubated with normal horse serum for 30 minutes at room temperature. After washing, the section were incubated at room temperature for 30 minutes with antiHER-2/neu (rabbit anti-human c-erbB-2 oncoprotein, Dako) then washed 3 times with phosphate-buffered saline solution(PBS), and applied with 1:500 Swine Anti-rabbit IgG for 45 minutes at room temperature. Being washed three times with buffer solution, all section were incubated with streptavidin for 40 minutes at room temperature and PBS washing. Finally, the sections were incubated with 3,3' diamenobenzydine, counterstained with hematoxyline, dried and then applied with coverslips. The slides were scored by a gynecologic pathologist, who was blinded to the clinical data at the interpretation. Expression was scored using the following standard semiqualitative scale developed for HER2: 0 indicates less than 10% of cells exhibiting any level of staining; 1+ denotes more than 10% of cells with barely perceptible light membranous rimmed staining that may not totally encircle the cell membrane; 2+ refers to more than 10% of cells with light-moderate membranous rimmed staining that totally encircles the cell membrane; and 3+ reflects more than 10% of cells with moderate-strong membranous rimmed staining that totally encircles the cell membrane. Because of the uncertain biologic significance of cytoplasmic HER2 staining, only membrane staining was considered positive.(Figure 1) Staining area of more than 10% with 2+ and 3+ intensity were considered HER-2/neu overexpression according to standard semiquantitative scale developed for HER2 (Bookman et al., 2003). Gene amplification or gene expression (such as fluorescence *in situ* hybridization[FISH]) was not specifically evaluated in this study.

Statistical analysis

Overexpression of HER-2/neu and Clinicopathological characteristics were correlated using Chi-square or Fisher exact test. Survival was measured in months from the date of surgery to the date of death or the date of last follow-up. Progression free survival was defined as the time from the date of surgery to the date of first evidence of tumor progression or recurrence. The survival time was plotted by standard life table method (Kaplan-Meier) (Kaplan and Meier,1958) and compared by log rank test (Mantel-Hanzel) SPSS (version 11.5) software. A p-value of < 0.05 was considered significant.

Results

The mean age of patient studies was 46.31 years with a range of 24 to 67. The distribution of patients by stage, histologic grade, tumor cell type and completeness of surgical staging is shown in Table 1. The median follow up was 46 months (range 3 – 83 months).

Out of 58 patients underwent exploratory laparotomy at King Chulalongkorn Memorial Hospital, 29 patients (50%) had been adequately staged (primary tumor reduction with peritoneal cytology evaluation and omentectomy and pelvic and paraaortic lymph node palpating or sampling), compared to 6 out of 16 patients (37.5%) referred from other hospitals. All the patients that inadequately staged were counseling about risk and benefit of repeat operation and adjuvant treatment.

Twenty nine patients out of thirty nine (74.4%), who were inadequately staging, received adjuvant treatment and 10 patients (25.6%) had been observed. In adequately staging group, 29 out of 35 patients (82.9%) received adjuvant therapy and 6 patients (17.1%) were followed up only. In the incomplete staging group, 3 patients had progressive disease and 2 patients had recurrent disease. Compared to the complete staging group, 1 patient had progressive disease and 6 had recurrent disease.

Twelve out of seventy four patients (16.2%) had either disease progression (5.4%) or relapse (10.8%) and all of these patients died of disease at the time of analysis. The median time to progression was 7 months (ranged 1-40 months).

Twenty three cases (31.1%) had positive for immunostaining for HER-2/neu which considered

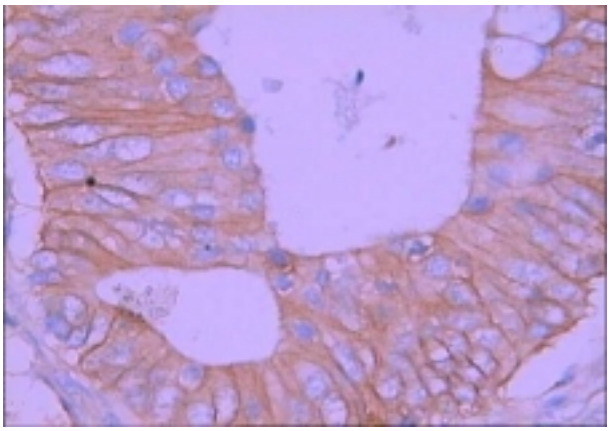

Figure 1. Positive Membranous HER-2/neu Overexpression

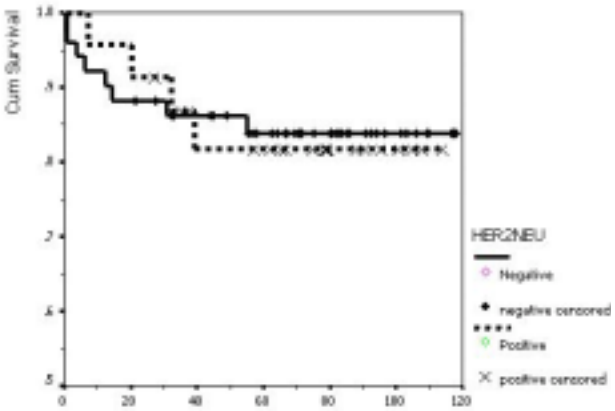

Figure 2. Survival Analysis According to HER-2/neu Overexpression

overexpression. We examined the relationship between HER-2/neu overexpression and clinicopathological characteristics of this apparent early stage epithelial ovarian cancer (Table 1) and found no correlation between stage, histological type, tumor grade, tumor rupture or adhesion, tumor size, recurrence or progression, residual tumor and HER-2/neu overexpression.

Disease free and overall survival was not different significantly in HER-2/neu overexpression versus normal HER-2/neu expression (Figure 2).

Discussion

The advent of modern technique of molecular biology has allowed various kind of gene to be studied. A long-term goal in the study of molecular genetics of a particular tumor type is to catalogue specific genes that are affected by mutations and the relative order in which they are affected, and, ultimately, to use the molecular blueprint to improve methods of diagnosis, prognostication, and treatment.

HER-2/neu was first identified as the oncogene associated with the development of neuroblastoma in rats exposed to ethylnitrosourea in utero (Padhy et al.,1982). The mechanism in which HER-2/neu is oncogenic believed to be a single point mutation in the membrane-spanning region (Bargmann et al.,1986). In human malignancies, however, HER-2/neu amplification and overexpression, rather than point mutation, have been noted (Di Fiore et al.,1987). In the identification process, immunohistochemical technique, one of which was used to study expression of protooncogene, allows us to observe HER-2/neu expression at a cellular level. The study of Slamon et al.(1989) show the near-perfect concordance between gene amplification and strong immunohistochemical staining on tumor specimens. However, other methods in which tissue homogenates are used to measure levels of DNA, RNA and protein all are subject to error due to differences in the proportion of cancer cells relative to stromal elements in tumor samples. Although, there are still conflicting data about whether measure gene amplification and protein overexpression would associate with response to treatment compare to measure phosphorylated HER2 level (Gordon et al., 2006). On the other hand, scoring of HER-2/neu expression by immunohistochemical assessment is less easily reproducible and inexpensive.

Ovarian cancer was one of the first malignancies in which amplification/ expression of HER-2/neu was felt to have prognostic significance. The percentage of patients with ovarian cancer having HER-2/neu overexpression ranged between 5.9% (Felip et al.,1995) to 40% (Leng et al.,1997). This wide range of prevalence may be explained by variation in the assessment of HER-2/neu activation by immunohistochemical or other methods. These methods differ in significant details, including primary antibodies, frozen versus paraffin-embedded tissue, detection techniques, and criteria for interpretation.

Prevalence of HER-2/neu overexpression in early stage of the disease was 20% in prior report (Rubin et al.,1994) and 10.2% in this study. The correlation between HER-2/neu overexpression and other prognostic variables is not clear. Like other authors (Rubin et al.,1994; Fajac et al.,1995; van der Zee et al.,1995), we found no correlation between overexpression and FIGO stage, histologic type, histologic grade or other prognostic factors. The lower rate of recurrent cases in this study might hinder its correlation. As we know, only 12 patients (16.2%) in our study showed disease progression or recurrence compare to 15 patients (37%) in the study by Rubin et al. (1994). There were too limited in the number of events to consider overexpression of HER-2/neu as a prognostic factor in early epithelial ovarian cancer. Recent studies showed correlation between overexpression and stage (Kacinsky et al.,1992; Seidman et al.,1992; Felip et al.,1995). These studies found a marked relationship between more advanced tumors at the time of diagnosis and protein overexpression, which needed further studies in our patient population.

Although some articles (Huettner et al.,1992; Kacinsky et al.,1992) suggested endometrioid, clear cell or high-grade carcinomas have the highest levels of HER-2/neu protein expression, our results did not reach statistical significance.

In our study, we found no correlation between survival time and protein overexpression (Figure 2). This result are similar to those obtained by Rubin et al.(1994) and Kacinsky et al.(1992) In contrast, Slamon et al.(1989), Berchuck et al.(1990) and Felip et al.(1995) found shorter survival times in patients with HER-2/neu overexpression. However, the need to assess meticulously the peritoneal cavity and retroperitoneal lymph nodes in the patients with apparent early-stage disease in order to obtain completeness of staging is still an important part of the operative procedure.

The predictive value of HER-2/neu in ovarian cancer remains to be determined. Preclinical data have shown a synergistic effect on cell kill between antibodies directed against HER-2/neu and platinum compound (Hancock et al.,1991). The mechanism that causes in the synergy remains to be established, although it appears that antibodies against HER-2/neu may interfere with the formation of platinum adducts. The Gynecologic Oncology Group has initiated an evaluation of anti-HER2 antibody in recurrent or refractory ovarian cancer for patients with a primary peritoneal carcinoma (Gynecologic Oncology Group Protocol 160). The study showed 2+/3+ HER2 expression on immunohistochemistry study of 11.4%. The overall response rate to Anti-HER2 Antibody was 7.3%, included one complete and two partial responses (Bookman et al., 2003). Subsequently, it is planned that additional studies will be performed with anti-HER2 antibody together with chemotherapy to determine if synergy can be shown either with platinum compounds, paclitaxel, or the combination in patients with recurrent ovarian cancer. Moreover, there are HER targeting agents in the trial which show promising outcome in the near future (Gordon et al., 2005; Gordon et al., 2006).

In conclusion, this is the first report about the prevalence of HER-2/neu overexpression in apparent early stage epithelial ovarian cancer in Thailand. The prevalence of HER-2/neu overexpression is 10.2%. We could not

demonstrate the association of HER-2/neu overexpression and clinicopathological factors and survival outcome in this study.

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