Cox-2 immunohistochemical expression in epithelial ovarian carcinoma and platin sensitivity

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Summary

Purpose: The aim of the study was to assess whether COX-2 expression in epithelial ovarian carcinoma (EOC) tissue can distinguish between platin-sensitive and platin-resistant tumors. *Methods:* Clinical and histological data were obtained from medical records of EOC patients diagnosed between the years 1995 and 2005. Patients in complete clinical remission for > 6 months after discontinuation of first-line chemotherapy were considered to be platin-sensitive. Survival of ≤ 2 and > 5 years after diagnosis was considered as short- and long-term survival, respectively. Immunohistochemistry staining was performed on deparaffinized sections of tissue blocks obtained at first surgery. The intensity of staining and the percentage of stained cells was assessed by two pathologists blinded to clinical data and a scoring index was calculated. *Results:* Among 79 patients a positive stain (> 10% of cells stained) was observed in 61 (77.2%). No statistically significant association between distribution of platin sensitivity and immunohistochemical COX-2 staining parameters was observed, although the rate of long-term survival was significantly higher among platin-sensitive then among platin-resistant/unresponsive patients. *Conclusions:* Immunohistochemically determined COX-2 expression in EOC is not associated with platin sensitivity and survival.

Key words: COX-2 expression; Epithelial ovarian carcinoma; Platin sensitivity; Survival.

Introduction

Cyclooxygenases (COX) are enzymes that are necessary for converting arachidonic acid into prostaglandin endoperoxide (prostaglandin H2). The COX-2 gene has been characterized as an immediate-early gene associated with cellular growth and differentiation, and is involved in critical steps of tumor onset and progression [1, 2].

COX-2 is expressed in epithelial ovarian carcinoma (EOC) [3-17] as well as in tumors of low malignant potential [5, 9, 13] and benign ovarian tumors and ovarian tissue [5, 4 9]. The proportion of COX-2 expression in EOC, as assessed by immunohistochemistry, ranges from 31% [9] to about 85% [17]. A similar immunohistochemical staining pattern [13] and proportion of positivity [11] was reported in primary peritoneal carcinoma (PPC).

COX-2 expression has been reported in some studies to be associated with poor response to chemotherapy and outcome of ovarian carcinoma [3-5, 8-16] On the basis of this association several authors have suggested that it would be worthwhile to investigate whether the administration of selective COX-2 inhibitors combined with chemotherapy in ovarian carcinoma patients may improve the tumor chemosensitivity and the overall survival [5, 6, 15]. However the results concerning the use of such inhibitors (non-steroidal anti-inflammatory drugs (NSAIDs) for example, are conflicting. A population-based study from Denmark found that the standardized incidence ratio for ovarian cancer was reduced in women who were NSAID users [18] while in another study from the US no association could be detected between NSAID use and the risk of ovarian cancer [19].

EOC is one of the most common gynecological cancers; it is often diagnosed at an advanced stage and is the leading cause of death from gynecological malignancies. In most instances the treatment includes cytoreductive surgery followed by combination chemotherapy consisting of paclitaxel and platin. Although this adjuvant treatment regimen has a high overall response rate, some patients are chemoresistant and a large proportion of patients recur after initial complete clinical remission [20]. PPC behaves in a very similar manner [21].

The initial clinical response to platinum is a major determinant of outcome for EOC patients. In patients with persistent or recurrent disease after initial chemotherapy cure can not be achieved. The shorter the interval between first-line chemotherapy to recurrence, the lower the subsequent response rates to additional chemotherapy [22-25].

EOC and PPC patients who recur after complete initial remission are considered platinum-sensitive if the progression-free interval, i.e. the platin-free interval, is more than six months and platin-resistant if that free interval is less than six months.

The aim of the present study was to assess whether COX-2 expression in EOC and PPC tumor tissue obtained at the initial operation can distinguish between platin-sensitive and platin-resistant tumors.

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Material and Methods

Medical records of histologically proven EOC and PPC patients diagnosed during the 11-year period between January 1995 and December 2005 in our institution were located after Institutional Review Board approval and informed consent had been obtained. The patients initially underwent surgery for debulking and all patients, except those in Stage IAG1, completed six courses of adjuvant postoperative combination chemotherapy with paclitaxel (175 mg/m^2) and cisplatin (75 mg/m^2) or carboplatin (AUC 6).

The post treatment outpatient clinic follow-up included: assessment of the serum CA125 level performed shortly before the visit, questioning with regard to relevant symptoms and a physical examination by a gynecologic oncologist consisting of a general examination (palpation of lymph nodes in the supraclavicular and inguinal areas, palpation of the abdomen and liver and assessment of lower limb edema) and a pelvic examination (speculum inspection of the vagina and a vaginal and recto-vaginal bimanual examination).

Patients who after initial treatment were asymptomatic, had no findings on physical examination and with serum CA125 levels within normal limits, were considered to be in complete clinical remission. Patients were considered to have persistent or recurrent disease when at least one of these criteria was abnormal. During the entire study period computed tomography (CT) was performed when clinically necessary and during the second half of the study period it was also routinely performed at termination of initial treatment.

Patients were categorized as being platinum-sensitive if they remained in complete clinical remission for more than six months after the end of first-line chemotherapy, and platinum-resistant if after initial complete clinical remission recurrent disease was diagnosed six months or less after the discontinuation of first-line chemotherapy. Patients with disease that persisted or progressed during treatment were categorized as unresponsive.

Short-term survival was considered when the patient died within two years after diagnosis and long-term survival was considered when the patient survived, with or without disease, more than five years after diagnosis.

Differences between groups were analyzed by the chi-squared test or Fisher's exact test when appropriate.

Immunohistochemistry

Tumor tissue biopsies from primary tumors were obtained at first surgery in all cases. Tissue specimens were fixed in formalin and paraffin-embedded according to standard procedures. Immunohistochemistry was performed on deparaffinized 4µ sections of paraffin-embedded tissue blocks. The detection of COX-2 was done using rabbit monoclonal antibody to human cyclooxigenase-2 (Dako Denmark) diluted to a titer of 1:50, and a modified labeled streptavidine technique, run on an automated system (Ventana Autostainer Nexes, Tucson, AZ, USA). 3,3'-diaminobenzidin was used as the chromogen. All sections were counterstained with Mayer's hematoxylin.

Analysis of all stained tissue sections was performed by two pathologists by means of light microscopy counting 10 high power fields (x400) with a minimum of 1,000 cells counted per slide. The pathologists were blinded to clinical data and the follow-up course of the malignancy. Differences in staining interpretation occurred in seven (9.3%) cases and were reconciled following appropriate discussion between the pathologists.

The staining was assessed by both pathologists according to two parameters: the subjectively evaluated intensity of staining and the proportion of cytoplasmic stained cells. Staining of more than 10% of the cells was considered positive. The staining intensity was graded on a 0 to 3 scale, in which 0 represents no detectable staining, and 1, 2 and 3 represent faint, intense and very intense staining, respectively.

A scoring index was than calculated by multiplying the intensity grade by the percentage of stained cells. The score was considered low when the index was equal or less than 1 and high when it was more than 1.

Sections of colon carcinoma known to express COX-2 served as positive controls.

Results

During the study period 82 histologically proven EOC and eight PPC patients were diagnosed. Given the similarities between the two tumors, EOC and PPC patients were combined for the purpose of analysis. Of these 90 patients the tissue blocks of 11 were not available. The remaining 79 (87.8%) patients comprised the study group.

The mean age of the study group patients was 60.9 (± 10.3). Other selective characteristics of the patients are presented in Table 1.The majority of the patients were in the 50-69 age group, had elevated preoperative CA125 serum levels, had Stage III serous carcinomas of high histological grade and were platin-sensitive.

Short-term survival was observed in 13 (16.4%) and long-term survival in 24 (30.4%) patients. Among the patients with short-term survival, one (7.7%) was platin-

Table 1. — *Selected characteristics of the study group patients*.

Characteristics	No.	%
Total	79	100.0
Age distribution		
< 50	14	17.7
50-69	44	55.7
70+	21	26.6
Pretreatment CA125 (U/mL)		
< 35	7	8.9
36-1000	42	53.2
1000+	26	32.9
Unknown	4	5.0
Stage at diagnosis		
I	13	16.5
II	10	12.6
III	53	67.1
IV	3	3.8
Histological type		
Serous	57	72.2
Endometrioid	13	16.5
Mucinous	5	6.3
Other	4	5.0
Grade		
1	7	8.9
2	17	21.5
3	44	55.7
Not recorded	11	13.9
Platin		
Sensitive	58	73.4
Resistant	13	16.5
Unresponsive	8	10.1

sensitive and among those with long-term survival all (100.0%) were platin-sensitive. This difference was statistically highly significant ($p < 0.001$)

A positive stain was observed in 61 (77.2%) patients. Table 2 presents platin sensitivity according to immunohistochemical staining parameters, i.e. staining positivity, staining intensity and scoring index. Since the distribution of resistant and unresponsive tumors according to immunohistochemical staining parameters was similar (not shown) they were combined for the purpose of analysis. No statistically significant differences between platin-sensitive and platin-resistant/unresponsive patients were found according to immunohistochemical staining parameters. There was also no association between imunohistochemical staining parameters and long- and short-term survival (Table 3).

Table 2. — *Platin sensitivity according to immunohistochemical staining parameters*.

	Platin sensitivity Resistant/				
	Sensitive		Unresponsive		
I. staining[1]	No.	%	No.	%	*p*
Total	58	100.0	21	100.0	
Stain					NS
Positive	44	75.9	17	80.9	
Negative	14	24.1	4	19.1	
Intensity grade					NS
0	14	24.2	4	19.1	
1	10	17.2	4	19.1	
2, 3	34	58.6	13	61.8	
Score					NS
Low	37	63.8	14	66.7	
High	21	36.2	7	33.3	

[1]I = immunohistochemical; NS = Not significant.

Table 3. — *Distribution of survival length according to immunohistochemical staining parameters*.

	Survival				
	Short		Long		
I. staining[2]	No.	%	No.	%	*p*
Total	13	100.0	24	100.0	
Stain					NS
Pos.	9	69.2	18	75.0	
Neg.	4	30.8	6	25.0	
Intensity					NS
0	4	30.8	6	25.0	
1	4	30.8	6	25.0	
2, 3	5	38.4	12	50.0	
Score					NS
Low	4	30.8	11	45.8	
High	9	69.2	13	54.2	

[1]I. staining = immunohistochemical staining; NS = Not significant.

Discussion

Our proportion of positive COX-2 immunohistochemical staining (77.2%) of EOC tissue is in the range of that reported by others [9, 17].

As could have been expected, the rate of long-term survival was significantly higher among platin-sensitive than among platin-resistant/unresponsive patients. Nevertheless we found no statistically significant differences between platin sensitive and platin resistant/unresponsive EOC patients according to immunohistochemical staining parameters namely staining positivity, intensity of staining and scoring index. In support of this result is a study that found that both cisplatin-sensitive and cisplatin-resistant cell lines equally expressed COX-2 protein when assessed by Western blot analysis [26].

Table 4. — *Studies evaluating the association between ovarian cancer prognostic variables, survival and COX-2 expression*.

Author (Reference number)	Residual Histologic	Age	Stage	Grade	Disease	Type	Survival
Denkert C. *et al*. [5]		–			–		+
Ferrandina G. *et al*. [6]		–			–		–
Ferrandina G. *et al*. [7]		–	–	–	–		–
Shigemasa K. *et al*. [9]		–	–		–	+	–
Erkinheimo T.L. *et al*.[10]		+		+	+		+
Khalifeh I. *et al*. [11]							+
Seo S.S. *et al*. [12]			+	+	+	+	+
Singhal P.K. [13]		–		+	–	+	–
Raspollini M.R. *et al*. [14]							+
Raspollini M.R. *et al*. [15]							+
Raspollini M.R. *et al*. [16]							+
Ozel E. *et al*. [17]		–	–	–		–	–

Association present +; Association absent –; Blank spaces: association not specified or not relevant.

To the best of our knowledge the present study is the first attempt to correlate COX-2 expression in EOC with platin sensitivity according to the conventionally defined criteria for platin sensitivity and platin resistance [25]. Distinction between these two categories is of great importance since it may help to identify patients prior to institution of postoperative treatment who will not respond to initial platin-based chemotherapy and who therefore might be candidates for adjuvant treatment with other agents.

Our finding is in contrast to other studies that reported a high correlation between COX-2 expression and response to chemotherapy [6, 7, 15, 16] and that the association between COX-2 positivity and chemoresistance was retained in multivariate analysis. However, in these investigations chemoresistant patients comprised those who did not respond to the initial first-line chemotherapy. Such patients were allotted by us to the unresponsive group.

The results of studies dealing with the association between COX-2 expression survival and prognostic factors are inconsistent (Table 5). Many studies reported that COX-2 expression in EOC is associated with reduced survival [5, 10-12, 14-16]. Similar to us, Raspollini *et al*. [16] also compared long- and short-term survivors, namely 23 patients living with no evident disease five years after primary treatment to 18 patients who had died of progression of disease no later than two years. In contrast to our results they found that COX-2 overexpression was an independent predictor of survival in univariate and multivariate analyses.

However, in several other studies no correlation between COX-2 expression in EOC and survival was

found [6, 13, 17]. The lack of association between COX-2 staining parameters and survival of less than two years and more than five years observed in the present study is thus in line with these reports.

COX-2 expression in EOC has also been reported in some studies to be associated with poor prognostic factors such as age > 57 years [10] residual tumor status [10, 12] stage, presence of ascites [12] and high histological tumor grade [10, 12]. Yet other investigations found that immunohistochemical staining did not differ in distribution according to these prognostic factors [5-7, 9, 13]. Contradictory results have even been obtained in the same study. Thus, in one study that reported a negative association between COX-2 expression with survival, no association to EOC prognostic factors was observed [5]. In others that found an association between COX-2 expression and chemoresistance there was also no association with important prognostic factors [6, 7].

With regard to histological type, in some studies no association with COX-2 expression was found [6, 7, 17]. Others reported higher COX-2 expression in serous carcinomas [13] or non mucinous carcinomas [12] while in one, a high association with endometrioid tumors [9] was found.

The present study reflects the contradictory results in the literature. The conflicting results of COX-2 immunohistochemical staining in EOC studies are possibly attributable to differences in staining technique, in staining assessment and population differences.

The main weakness of the present study is the small sample size in each subcategory and this may be the reason for the lack of association between COX-2 immunohistochemical staining parameters and platin sensitivity and survival. We are also aware that our criteria for complete clinical remission after initial surgery and completion of chemotherapy in EOC patients are not absolutely precise. It is well known that these criteria, i.e. absence of symptoms, lack of findings on physical examination and CA125 levels in the normal range, do not indicate absence of disease. Such patients may have small or microscopic residual disease although even imaging procedures such as computerized tomography or positron emission tomography, are normal as well [27-29]. Therefore some of our patients in the complete remission group may have actually been unresponsive to chemotherapy.

Nevertheless our study seems to indicate that in EOC there is no association between COX-2 expression and platin sensitivity and survival.

The clinical value of COX-2 expression assessment remains to be elucidated.

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