Elevated Cyclooxygenase-2 Expression Is Associated with Altered Expression of p53 and SMAD4, Amplification of HER-2/neu, and Poor Outcome in Serous Ovarian Carcinoma

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ABSTRACT

Purpose and Experimental Design: Cyclooxygenase-2 (COX-2) is frequently expressed in human adenocarcinomas and inhibition of COX-2 suppresses tumor formation in various animal models of carcinogenesis. We analyzed expression of COX-2 protein in human serous ovarian carcinomas by immunohistochemistry (n = 442) and by Western blotting (n = 12) and COX-2 mRNA by reverse transcriptase PCR (n = 12). COX-2 immunoreactivity was correlated to clinicopathological variables and to expression of p53 and SMAD4 as detected by immunohistochemistry and to amplification of HER-2/neu as detected by in situ hybridization.

Results: COX-2 mRNA expression was detected in 75% (9 of 12) and COX-2 protein in 42% (5 of 12) of the serous ovarian adenocarcinoma specimens as detected by reverse transcriptase-PCR and Western blot analysis, respectively. Moderate to strong (elevated) immunoreactivity for COX-2 was detected in 70% (310 of 442) of the tumors. Elevated COX-2 expression associated with reduced disease-specific survival (P = 0.0011), high histological grade (P < 0.0001), residual tumor size > 1 cm (P = 0.0111), and age > 57 years (P = 0.0099). Tumors with altered immunostaining pattern for p53 or SMAD4 expressed more frequently elevated levels of COX-2 when compared with the tumors with normal staining pattern of these tumor suppressor genes (P < 0.0001 and P = 0.0004, respectively). In addition, elevated COX-2 expression associated with amplification of HER-2/neu oncogene (P = 0.0479).

Conclusions: Our results suggest that elevated expression of COX-2 associates with reduced survival in serous ovarian carcinomas and that expression of COX-2 may be induced in these tumors by loss of tumor suppressor genes such as p53 and SMAD4 and by amplification of HER-2/neu oncogene.

INTRODUCTION

Expression of cyclooxygenase-2 (COX-2) is elevated in a variety of human malignancies and in their precursor lesions (1–3). Inhibition of COX-2 activity or its genetic deletion leads to reduced number and size of intestinal polyps in mouse models of familial adenomatous polyposis (4, 5), and overexpression of COX-2 promotes mammary gland tumorogenesis in a transgenic mouse model (6). Furthermore, a COX-2-selective drug, celecoxib, reduced polyp burden in patients who suffer from familial adenomatous polyposis (7). Thus, COX-2 constitutes a rational target in chemoprevention.

Ovarian carcinoma exhibits several histological subtypes with different pathogenesis and outcome. Of the ovarian carcinomas, serous type is the most common, comprising >50% of the cases, and it has a relatively poor prognosis the 5-year survival being ~40% (8, 9). The aggressive nature of this disease is in accordance with the presence of higher frequency of cytogenetic changes than in other types of ovarian carcinoma (10, 11). The molecular pathogenesis of serous ovarian carcinoma is largely unknown, but altered expression of tumor suppressor gene p53 is frequent and confers poor prognosis (12–14). Furthermore, distal half of chromosome 18q is among the most frequently lost genomic regions in serous ovarian carcinoma (15–17). Interestingly, expression of tumor suppressor gene SMAD4, located at 18q21, is reduced in 30% of ovarian carcinomas (18). In respect of oncogenes, amplification or overexpression of HER-2/neu is found in ~30% of ovarian carcinomas, and it is associated with poor prognosis (18, 19).

It was recently reported that expression of COX-2 is elevated in ovarian cancer (20, 21). Moreover, elevated COX-2 expression has been reported to be an independent prognostic factor in ovarian cancer but that it did not associate with any other clinicopathological parameter, such as stage and grade (22). We have now studied expression of COX-2 protein in 442 serous ovarian carcinoma specimens using immunohistochemistry and have correlated the expression to prognosis, to clinicopathological parameters, and to expression of tumor suppressor genes p53 and SMAD4 and amplification of HER-2/neu oncogene. Our results show that elevated COX-2 expression correlates with reduced survival in patients who suffer from...
serous ovarian adenocarcinoma and that increased expression of
COX-2 may depend on loss of tumor suppressor gene activity
and increase in oncogene activity.

MATERIALS AND METHODS

Patients. The study consisted of 474 consecutive patients
treated for serous ovarian carcinoma at the Department of
Obstetrics and Gynecology of Helsinki University Central Hospital
between the years 1964 and 2000, and whose specimens could
be retrieved from the pathology files. The study was approved
by the local ethics committee. The histology was determined by
a gynecological pathologist and was verified by another pa-
thologist (R.B.). The clinical information of the patients was
extracted from the patient records and survival information from
the Population Register Center. The median age of ovarian
cancer patients was 57 years (range, 15–88 years) at the time of
diagnosis. The median follow-up time of patients alive at the
end of study period was 5.2 years (range, 0.4–36.1 years).
The patients were operated by gynecological oncologists, and the
clinical staging and grading were performed according to Fed-
eration of International Gynecology and Obstetrics. New treat-
ment regimens were adopted as follows: platinum-based chem-
otherapy at the beginning of the 1980s; radical surgery at the
end of the 1980s; and paclitaxel/platinum chemotherapy after
1996. The tumor samples for the study were obtained at primary
surgery before patients had received any chemotherapy.

Microarray Analysis. Tissue microarrays were con-
structed as described previously (23). In brief, tumor tissues
were embedded in paraffin, and 5-μm sections were cut from
each block and stained with H&E. A representative tumor area
was selected from H&E-stained sections of each tumor by our
pathologist (R.B.). Four tissue cores (diameter, 0.8 mm) were
obtained from each tumor block and replaced into recipient
paraffin block with a custom-built precision instrument
(Beecher Instruments, Silver Spring, MD). These samples were
cut with a microtome into 5-μm sections. The presence of
cancer cells was verified on H&E-stained sections.

Immunohistochemistry and in Situ Hybridization.
Specificity of the antibody and detailed immunohistochemistry
protocol have been described previously (24, 25). Specimens
were deparaffinized and antigen retrieved using microwave
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Immunohistochemistry and in Situ Hybridization.
Specificity of the antibody and detailed immunohistochemistry
protocol have been described previously (24, 25). Specimens
were deparaffinized and antigen retrieved using microwave
oven [(4 × 5 min in 700 W in 0.01 m sodium citrate buffer (pH
6)]. The slides were then immersed in 0.6% hydrogen peroxide
in methanol for 30 min to block endogenous peroxidase activity
and in blocking solution (1.5:100 normal horse serum in PBS)
for 15 min to block unspecified binding sites. Immunostaining
was performed with a COX-2-specific antihuman monoclonal
antibody (160112; Cayman Chemical Co., Ann Arbor, MI) in a
dilution of 1:200 (2.5 μg/ml) in PBS containing 0.1% sodium
azide and 0.5% BSA at room temperature for overnight.
The sections were then treated with biotinylated horse antimouse
immunoglobulin (1:200; Vector Laboratories, Inc., Burlingame,
CA) and avidin-biotin peroxidase complex (Vectastain ABCCom-
plex; Vector Laboratories, Inc.). The peroxidase staining was
visualized with 3-aminio-9-ethylcarbazole (Sigma Chemical Co.,
St. Louis, MO), and the sections were counterstained with
Mayer’s hematoxylin. To confirm the specificity of the staining,
12 histological sections with serous ovarian cancer and 6 non-
neoplastic ovarian samples were stained with and without hu-
man COX-2 control peptide (20 μg/ml; Cayman Chemical Co.)
for 1 h at room temperature before the staining procedure.
Immunostaining for p53 (n = 437) and SMAD4 (n = 433) of
the serous ovarian carcinomas has been described previously
(17). HER-2/neu gene amplification was assessed using chro-
mosomic in situ hybridization (n = 323) according to the method
of Tanner et al. (26).

Scoring. The intensity of staining was scored independ-
ently and in blinded manner by three investigators (T-L.E.,
B.v.R., and A.R.) from 474 serous ovarian carcinoma tissue
cores on the following scale: 0, no staining; 1, weak diffuse
cytoplasmic staining (may contain stronger intensity in <10%
of the cancer cells); 2, moderate granular cytoplasmic staining in
>10% of the cancer cells; and 3, strong granular cytoplasmic
staining in >50% of cancer cells. Only those tumors that ex-
hibited at least three tissue cores were included to the analysis
(442 of 474; 93%). All specimens with discordant scores were
reevaluated using a multiheaded microscope, and the consensus
score was used for additional analyses.

The immunoreactivity for p53 was scored according to the
intensity of nuclear staining (negative, weak, and moderate or
strong) and to the percentage of positively stained tumor cells
(<20, 20–49, and ≥50%). Tumors with weak immunostaining,
similar to that found in the surface epithelium of normal ovaries
and in normal serous epithelium of the fallopian tube, were
regarded as showing normal p53 expression. The majority
(94%) of these tumors had positive staining in <20% of tumor
cells. Two distinct patterns of altered p53 expression were
identified: completely negative p53 in which no staining was
found in any of the tumor cells and excessive p53 in which
>50% of tumor cells showed moderate or strong immunoposi-
tivity (44). Immunostaining for SMAD4 was scored altered
whenever the signal was absent or very weak, which was dif-
ferent from the staining observed in the surface epithelium of
normal ovaries and the general pattern of positive staining of the
tumor samples (17).

Western Blot Analysis. Tumor samples (50 mg) of 12
serous ovarian carcinoma specimens were crushed in 1 ml of
radioimmunoprecipitation assay buffer [150 mM NaCl, 1%
NP40, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, and
50 mM Tris (pH 8.0)] supplemented with Complete mini prote-
ase inhibitor mixture tablet (Boehringer, Mannheim, Germany)
by Fast Prep homogenization instrument (Qbiogene, Inc., Carls-
bad, CA) and centrifuged at 14,000 × g for 15 min. Protein
concentration was measured with BSA protein assay (Pierce,
Rockford, IL). Proteins (100 μg) were resuspended in sample
loading buffer [74 mM Tris-HCl (pH 6.8), 2% SDS, 12% gly-
cerol, 5% β-mercaptoethanol, and 0.015% bromphenol blue]
and separated by SDS-PAGE (12%). The proteins were trans-
ferred electrophoretically to Hybond-C extra nitrocellulose membranes
(Amersham, Buckinghamshire, United Kingdom). Nonspecific
binding was blocked by TBS-NP40, 5% low-fat dry milk solu-
tion, overnight at 4°C. For immunodetection, the membrane
was incubated with the mouse antihuman monoclonal COX-2 anti-
body (160112, 1:1000 dilution; Cayman Chemical Co.) with or
without COX-2 blocking peptide (360107; Cayman Chemical Co.)
for 1 h at room temperature. The membrane was then
washed three times in TBS-NP40 and incubated with the sheep
antimouse antibodies conjugated to hors eradish peroxidase (1: 2000 dilution). After four washes with TBS-NP40, COX-2 was visualized by enhanced chemiluminescence with Super Signal West Femto Maximum Sensitivity Substrate (Pierce) according to the manufacture’s protocol. Loading was controlled by goat antihuman β-actin antibody (1:500 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) with donkey antig o antibodies conjugated to horseradish peroxidase (1:2000 dilution; Santa Cruz Biotechnology) as the second antibody. The signals were visualized using Multilmage FC Light Cabinet (Promega, Madison, MI) and the digital imaging FluorChem 8800 software (α Innotech Corporation, San Leandro, CA).

RNA Isolation and Reverse Transcriptase-PCR. Tumor samples (100 mg) of 12 serous ovarian carcinoma specimens were crushed in 1 ml of TRIzol (Invitrogen, Carlsbad, CA) by Fast Prep instrument, after which RNA was purified by isopropanol precipitation after phenol/chloroform extraction. RNA concentration was determined by absorbance at 260 nm. Total RNA (1 µg) from 12 serous ovarian cancer samples was converted to cDNA with Moloney murine leukemia virus reverse transcriptase RNase H minus, RNasin (Promega), 2’deoxynucleotide 5’ triphosphates (Pharmacia Biotech), and random primers (Invitrogen) in a volume of 50 µl. The reverse transcriptase reaction mix of 5 µl was PCR amplified in reaction mixture of 45 µl that contained 2 units of Dy nazyme II polymerase and Dynazyme buffer (Finzymes, Espoo, Finland) and antisense and sense primers for COX-2 (0.5 µg) or glyceraldehyde-3-phosphate dehydrogenase (0.15 µg). The nucleotide sequences of the primer for COX-2 are as follows: 5′-TTCAAT GGAGATGTTGAGAATT-3′ (sense) and 5′-AGATCATCTC TGCCCTGAGTATCT-3′ (antisense); and for glyceraldehyde-3-phosphate dehydrogenase: 5′-CACCACATGGCAAATCTCATGGCAAC-3′ (sense) and 5′-TCATGACGAGGTCTACGTCCACC-3′ (antisense; Ref. 27). The PCR reaction mixture was heated to 94°C for 3 min followed by amplification for COX-2 by 40 and for glyceraldehyde-3-phosphate dehydrogenase by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. After the last cycle, the reactions were remained at 72°C for an additional 15 min. Amplified cDNAs were analyzed by 1.2% agarose gel electrophoresis and ethidium bromide staining. The amplified products were visualized under UV transillumination in Multi-Image FC Light Cabinet by using the digital imaging FluorChem 8800 software.

Statistical Analysis. For statistical analysis, COX-2 scores 0 and 1 were combined (low COX-2 expression), and scores 2 and 3 represented elevated COX-2 expression. The correlation between COX-2 staining intensity and clinically relevant and prognostic variables was assessed by using the χ2 test or by Fisher’s exact test. Probability of survival was estimated using the Kaplan-Meier method. Survival probabilities were compared between groups using the log-rank test or log-rank test for trend. Disease-specific survival time was defined as the time from primary surgery to death of the patient from ovarian cancer or to the end of the follow-up. Of the 442 patients with known COX-2 score, 244 (55%) died from ovarian cancer, 5 (1%) because of another cancer, and 32 (7%) because of another disease. The 37 deaths due to other causes than ovarian cancer were treated as censored cases, as well as those patients who were alive at the end of the follow-up (n = 161). Multivariate survival analysis was performed using the COX proportional hazards model, entering the following variables: COX-2 expression (score 0–1 versus 2–3); age (≤57 versus >57 years); grade (1, 2, and 3); stage (I, II, III, and IV); tumor size (≤10 versus >10 cm); and residual tumor size (≤1 versus >1 cm). All these data were available from 383 patients.

RESULTS

COX-2 Expression in Serous Ovarian Cancer Specimens as Detected by Reverse Transcriptase-PCR, Western Blot Analysis, and Immunohistochemistry. Expression of COX-2 mRNA was detected in 75% (9 of 12) and COX-2 protein in 42% (5 of 12) of the serous ovarian adenocarcinoma specimens as detected by reverse transcriptase-PCR and Western blot analysis, respectively. All tumors that expressed COX-2 protein were also positive for COX-2 mRNA. In Fig. 1, two positive specimens and two reverse transcriptase-PCR-negative samples are shown. The specificity of the antibody was confirmed by preadsorption with the antigenic peptide. This blocking procedure abolished the signal for COX-2 protein signal, thus confirming the specificity of the antibody (middle panel in Fig. 1B). Expression of COX-2 protein was analyzed in 442 serous ovarian carcinoma specimens using immunohistochemistry, of which 1% (n = 5) were negative (score 0) and 29% (n = 129) weakly (score 1), 48% (n = 211) moderately (score 2), and 22% (n = 99) strongly (score 3) positive. Moderate and strong (scores 2 and 3) cytoplasmic granular COX-2 immunoreactivity were defined as elevated expression and were seen in 70% of the tumors. Expression of COX-2 protein localized to the cytoplasm and to the perinuclear region of the cancer cells (Fig. 2), but stromal compartment was positive in only 7.9% (n = 35) of the tumors. The ovarian surface epithelium was negative or only weakly positive for COX-2 staining (Fig. 2).
Association of COX-2 Expression with Clinicopathological Parameters. Elevated expression of COX-2 associated with age $\geq 57$ years ($P = 0.0099$), high histological grade ($P < 0.0001$), and residual tumor size (maximal diameter of the tumor left in the patient at primary operation) $> 1$ cm ($P = 0.0111$). No correlation was found between COX-2 staining intensity and stage or size of the tumor (Table 1).

Association of COX-2 Expression with Disease-Specific Survival. Elevated COX-2 expression was associated with decreased disease-specific survival among the 442 serous ovarian cancer patients ($P = 0.0011$; Fig. 3A). When COX-2 expression was divided into three categories (score 0–1, score 2, and score 3), probability of survival was progressively reduced ($P = 0.0004$; Fig. 3B). In addition to COX-2, the variables that correlated with the disease-specific overall survival were age, histological grade, stage, tumor size, and residual tumor size (Table 2).

Multivariate Analysis. Multivariate analysis was performed to evaluate the independence of COX-2 expression as a
prognostic factor. In this analysis age (P = 0.0134), grade (P < 0.0001), stage (P < 0.0001), and residual tumor size (P < 0.0001) were identified as independent prognostic factors, but inclusion of tumor size (P = 0.8989) or COX-2 (P = 0.7439) did not add significant independent prognostic information.

**Correlation of COX-2 Expression with Expression of p53 and SMAD4 and with Amplification of HER-2/neu.** Elevated COX-2 expression was found more frequently in tumors with altered (completely negative or increased) p53 staining when compared with the tumors with normal (weak) p53 immunoreactivity (Table 3). Altered p53 staining was found in 38% (50 of 132) of COX-2 score 0–1, in 63% (130 of 208) of score 2, and in 81% (79 of 97) of score 3 (P < 0.0001). In respect of SMAD4, elevated COX-2 expression was also found more frequently in tumors with altered (negative or decreased) SMAD4 staining when compared with the tumors with normal SMAD4 immunostaining (Table 3). Altered SMAD4 staining was found in 22% (28 of 130) of COX-2 score 0–1, in 31% (63 of 206) of score 2, and in 58% (56 of 97) of score 3 (P < 0.0001). Tumors with combined

**Table 2** Five-year disease-specific survival of serous ovarian adenocarcinoma patients according to cyclooxygenase-2 (COX-2) immunoreactivity and to clinicopathological parameters

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>n (total)</th>
<th>n (%)</th>
<th>5-year survival (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2 expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (score 0–1)</td>
<td>132 (30%)</td>
<td></td>
<td>59.6 (50.9–68.4)</td>
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<tr>
<td>Elevated (score 2–3)</td>
<td>310 (70%)</td>
<td></td>
<td>44.6 (38.7–50.5)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
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<tr>
<td>≤57 years</td>
<td>238 (50%)</td>
<td></td>
<td>62.3 (55.9–68.7)</td>
<td>&lt;0.0001</td>
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<td>&gt;57 years</td>
<td>236 (50%)</td>
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<td>35.8 (29.1–42.4)</td>
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<tr>
<td>Histological grade</td>
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<td></td>
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<tr>
<td>I</td>
<td>181 (39%)</td>
<td></td>
<td>80.8 (74.8–86.7)</td>
<td>&lt;0.0001</td>
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<tr>
<td>II</td>
<td>121 (26%)</td>
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<td>35.5 (26.2–44.8)</td>
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<tr>
<td>III</td>
<td>164 (35%)</td>
<td></td>
<td>23.1 (16.0–30.2)</td>
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<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>I</td>
<td>101 (21%)</td>
<td></td>
<td>88.6 (81.9–95.3)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>64 (14%)</td>
<td></td>
<td>62.8 (50.3–75.2)</td>
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<tr>
<td>III</td>
<td>251 (53%)</td>
<td></td>
<td>38.9 (32.4–45.3)</td>
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<tr>
<td>IV</td>
<td>55 (12%)</td>
<td></td>
<td>8.5 (0–17.2)</td>
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<tr>
<td>Tumor size</td>
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<tr>
<td>≤10 cm</td>
<td>152 (33%)</td>
<td></td>
<td>54.5 (46.0–62.9)</td>
<td>&lt;0.0220</td>
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<tr>
<td>&gt;10 cm</td>
<td>315 (67%)</td>
<td></td>
<td>46.9 (41.1–52.7)</td>
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<tr>
<td>Residual tumor size</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
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<tr>
<td>≤1 cm</td>
<td>207 (49%)</td>
<td></td>
<td>74.6 (68.1–81.0)</td>
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<tr>
<td>&gt;1 cm</td>
<td>216 (51%)</td>
<td></td>
<td>21.3 (15.4–27.1)</td>
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</tr>
</tbody>
</table>

*a* Confidence interval.  
*b* Log-rank test or log-rank test for trend.

**Table 3** Association of elevated cyclooxygenase-2 (COX-2) expression with immunostaining for p53 and SMAD4 in serous ovarian adenocarcinoma

<table>
<thead>
<tr>
<th>Immunostaining</th>
<th>n (total)</th>
<th>Elevated COX-2/n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>437</td>
<td>96/178 (54%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td>59/67 (88%)</td>
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</tr>
<tr>
<td>Increased</td>
<td></td>
<td>150/192 (78%)</td>
<td></td>
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<tr>
<td>SMAD4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>433</td>
<td>184/286 (64%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Negative or decreased</td>
<td></td>
<td>119/147 (81%)</td>
<td></td>
</tr>
<tr>
<td>Normal p53 and normal SMAD4</td>
<td>76/143 (53%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal p53 and altered SMAD4</td>
<td>18/31 (58%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Altered p53 and normal SMAD4</td>
<td>108/143 (76%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Altered p53 and altered SMAD4</td>
<td>99/114 (87%)</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Normal HER-2</td>
<td>323</td>
<td>163/254 (64%)</td>
<td>=0.0013</td>
</tr>
<tr>
<td>Amplified HER-2</td>
<td></td>
<td>53/69 (77%)</td>
<td>=0.0264</td>
</tr>
</tbody>
</table>

*a* Fisher’s exact test.  
*b* Not significant.  
Versus normal p53 and normal SMAD4.  
Versus normal p53 and altered (negative or decreased) SMAD4.  
Versus altered (negative or increased) p53 and normal SMAD4.
defective expression of p53 and SMAD4 expressed more frequently elevated levels of COX-2 when compared with the tumors with only one of the tumor suppressor genes showing altered expression (Table 3). Elevated expression of COX-2 was found more frequently in tumors with amplification of HER-2/neu when compared with the tumors with normal HER-2/neu status ($P = 0.0479$; Table 3).

**DISCUSSION**

Our results provide evidence for COX-2 protein expression in a large series of consecutive cases of serous ovarian cancer specimens ($n = 442$). Elevated COX-2 expression was found by immunohistochemistry in 70% of the tumors, and it associated with reduced disease-specific survival ($P = 0.0011$). In accord with our data, elevated COX-2 expression has previously been detected in ovarian cancer (20–22). In addition to immunohistochemistry, we and others have detected COX-2 mRNA (22, 31) and the protein as detected by immunoblotting (29) in ovarian cancer specimens. We have detected COX-2 mRNA (22, 31) and the protein as detected by immunoblotting. Clearly the signal for COX-2 is weaker and 20–22). In addition to immunohistochemistry, we and others have detected COX-2 mRNA (22, 31) and the protein as detected by immunoblotting (29) in ovarian cancer specimens. We found expression of COX-2 mRNA in 9 of 12, Denkert et al., in 4 of 5 (22), and Shigemasa et al., in 26 of 36 (31) of ovarian adenocarcinoma specimens. Combined these data indicate that COX-2 mRNA is expressed in 72–80% of the ovarian carcinomas, which is consistent with our immunohistochemistry results (elevated expression in 70% of the tumors). It should be pointed out that COX-2 protein expression was strong in only 3 of 12 samples, weak in 2 of 12, and negative in 7 of 12 as detected by immunoblotting. Clearly the signal for COX-2 is weaker and less frequent than that observed in gastrointestinal tumors (2). To this end, it is not surprising that two reports have failed to detect COX-2 expression in a very limited number of ovarian cancer specimens (33, 34). Because COX-1 expression was reported to be expressed in ovarian cancer specimens by these two groups (33, 34), an explanation for these apparently discrepant data may depend on different relative amounts of COX-1 versus COX-2 gene products in ovarian cancer tissues due to sensitivity issues related to antibodies and methods of detection and/or because of different COX-enzyme expression patterns in different histological types of ovarian cancer. Indeed, we have published that very low COX-2 mRNA levels were detected in mucinous ovarian cancers when compared with gastric cancer specimens (35). In addition, our unpublished results indicate that frequency of COX-2 protein expression is relatively low in cancer cells derived from the mucinous type of tumors as detected by immunohistochemistry. All this indicates that the relative amount of COX-1 may be higher than that of COX-2 in ovarian carcinoma and that gastrointestinal tumors may express higher levels of COX-2 than ovarian tumors. However, thus far only expression of COX-2 but not that of COX-1 has been shown to associate with prognosis in ovarian cancer patients, indicating that assessing the expression levels of COX-2 protein is biologically meaningful.

The most frequent genetic alteration known in serous ovarian cancer is related to mutation of p53 tumor suppressor gene (12–14). The tubal (normal serous epithelium of Müllerian origin) staining pattern was regarded in our work as a reference of normal (wild-type) p53 expression. In carcinomas, two categories distinct from the normal expression were observed: excessive p53 (overexpression) and completely negative p53. Interestingly, completely negative p53 conferred as poor prognosis as excessive p53 when compared with tumors showing normal p53 expression (44). Traditionally, p53 overexpression by immunohistochemistry is thought to represent $TP53$ mutation, and p53 negative tumors are considered to carry wild-type $TP53$. However, high concordance of increased p53 protein is only seen with missense mutations, which result in protein that is resistant to degradation and has longer half-life than the wild-type counterpart. Other types of mutations (nonsense, insertion, deletion, and splice site aberrations) may result in truncated proteins that are functionally null but do not usually increase p53 protein stability. In addition to null mutations, the lost p53 immunostaining may be because of, for example, homozygous deletion or epigenetic silencing of the gene. In accordance with our results, one study reported the worst prognosis in stage III–IV ovarian carcinomas that showed null mutations of $TP53$ and lacking p53 immunostaining (36). Furthermore, our present data imply that p53 negative and p53 overexpressing tumors contain more often high expression of COX-2 than the normal low pattern of p53 expression. In addition to ovarian cancer, which has been found in our work and those of others (31), association of COX-2 expression and p53 immunostaining has previously been found in gastric and breast cancers (37, 38). Because the COX-2 gene has been shown to be induced in p53 defective cells and down-regulated by wild-type p53 (39, 40), there may exist a direct link between a defective p53 pathway and elevated levels of COX-2 expression in cancer cells.

In addition to p53, altered expression of another tumor
suppressor gene, SMAD4, has been detected in 28% of serous ovarian carcinomas (17). We found a positive correlation between elevated COX-2 expression and defective SMAD4 expression in the serous ovarian cancer specimens. It is not known whether SMAD4 can directly affect COX-2 expression. However, because tumor progression depends on cumulative genetic alterations, it is interesting to note that the frequency of COX-2 expression was higher in tumors with combined defect of p53 and SMAD4 when compared with the tumors with only one of the tumor suppressor genes affected. Elevated COX-2 expression was not restricted to p53 and SMAD4 aberrant tumors, and thus, other mechanisms are likely to be responsible for elevated COX-2 expression as well. In addition to tumor suppressor genes, function of several oncogenes is modified in ovarian cancer, which includes amplification and overexpression of HER-2/neu (18, 19). Overexpression of HER-2/neu induces COX-2 expression in mammary epithelial cells, and amplification of HER-2/neu correlated with COX-2 expression in breast cancer specimens (38, 41), but such association was not found in ovarian cancer as detected by immunohistochemistry (29). Our results show that elevated COX-2 expression in serous ovarian carcinoma correlates with amplification of HER-2/neu as detected by chromogenic in situ hybridization. It is interesting to note that combination of COX-2-selective drugs with inhibitors of HER-2/neu have provided an additive antitumor effect in experimental animal models (42, 43).

In conclusion, our results suggest that elevated expression of COX-2 associates with poor differentiation and with reduced survival in serous ovarian carcinomas. Expression of COX-2 associated with altered expression of tumor suppressor genes p53 and SMAD4 and with amplification of HER-2/neu oncogene, which may in part be responsible for induction of COX-2 expression in ovarian cancer.

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REFERENCES


Elevated Cyclooxygenase-2 Expression Is Associated with Altered Expression of p53 and SMAD4, Amplification of HER-2/neu, and Poor Outcome in Serous Ovarian Carcinoma
