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Expression of cyclooxygenase-2 in advanced stage ovarian serous carcinoma: Correlation with tumor cell proliferation, apoptosis, angiogenesis, and survival

Rouba Ali-Fehmi, MD,[a] Robert T. Morris, MD,[b] Sudeshna Bandyopadhyay, MD,[a] Mingxin Che, MD,[a] Veronica Schimp, DO,[b] John M. Malone Jr, MD,[b] Adnan R. Munkarah, MD[b,*]

Departments of Pathology[a] and Obstetrics and Gynecology, Division of Gynecologic Oncology,[b] Wayne State University School of Medicine, Detroit, Mich

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KEY WORDS
Cyclo-oxygenase–2
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Objective: Cyclo-oxygenase–2 seems to be involved at various steps in the processes of tumor progression. The objective of this study was to examine the relationship between cyclo-oxygenase–2 expression and tumor proliferation, apoptosis and angiogenesis in patients with advanced stage high-grade ovarian carcinoma.

Study design: Specimens from 118 patients with high-grade and advanced stage (III, IV) serous ovarian carcinoma were evaluated by immunohistochemistry for cyclo-oxygenase–2, Ki-67, vascular endothelial growth factor, and bcl-2 expression. Tumor microvessel density was assessed with CD34 immunostaining. We investigated the relationships between cyclo-oxygenase–2 expression and clinicopathologic characteristics, tumor angiogenesis (tumor microvessel density and vascular endothelial growth factor expression), and tumor proliferation and apoptosis. The effect of cyclooxygenase-2 expression on patient survival was determined.

Results: There was a significant positive correlation between cyclo-oxygenase–2 expression in tumor cells and markers of tumor proliferation and angiogenesis. In univariate survival analysis, high cyclo-oxygenase–2 and high Ki-67 expression showed a significant impact of on patient survival ($P < .001$). In multivariate regression analysis, only Ki-67 expression retained its significance as an independent poor prognostic factor (death hazard ratio, 2.0; 95% CI, 1.2-3.3; $P < .001$).

Conclusion: Expression of cyclo-oxygenase–2 correlates with tumor proliferation and tumor angiogenesis but not with apoptotic markers (bcl-2 expression) in high-grade, advanced-stage serous ovarian carcinoma.

* Reprint requests: Adnan R. Munkarah, MD, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Harper University Hospital & Wayne State University, 3990 John R, 4 Hudson, Suite 4922, Detroit, MI 48201.
E-mail: amunkara@med.wayne.edu

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Epidemiologic studies indicate that the use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a reduced risk of malignancies in the digestive tract.[1] The best known target of NSAIDs is the

Table I Patients' characteristics and COX-2 expression

Clinical features	N	COX-2 expression (n) Low	High	*P* value
Age				
<60 Y	47	14 (29.8%)	33 (70.2%)	.84
≥60 Y	71	23 (32.4%)	48 (67.6%)	
Race				
White	89	28 (31.5%)	61 (68.5%)	
Black	29	9 (31%)	21 (69%)	1.00
Stage				
III	97	32 (33%)	65 (67%)	.60
IV	21	5 (23.8%)	16 (76.2%)	

cyclo-oxygenase enzyme (COX), also referred to as prostaglandin endoperoxide synthase, a key regulatory enzyme in the prostaglandin/eicosanoid synthetic pathway. Two isoforms of COX have been identified, COX-1 and COX-2. COX-1 is expressed constitutively, whereas expression of COX-2 is not detectable in most healthy tissues but can be induced in response to cell activation by proinflammatory cytokines, growth factors, and tumor promoters.[2] Evidence from in vitro and in vivo studies suggests an important role for prostaglandins and their synthesizing enzyme COX-2 in carcinogenesis. COX-2 overexpression has been described in various malignancies including those of the colon, stomach, head and neck, endometrium, and cervix.[3-6] Exposure of various cancer cell lines to prostaglandins has been shown to induce COX-2 expression and increase cellular proliferation. Transfection of benign intestinal epithelial cells with COX-2–expressing vectors results in malignant transformation.[7] On the other hand, treatment of cancer cells with COX-2 inhibitors reduces cellular proliferation and induces apoptosis.[8] Tumor growth and progression necessitate not only cellular proliferation, but also the creation of new vasculature to supply cells with nutrients and oxygen. In vitro models for angiogenesis have shown that COX-2 expression plays a role in new vessel formation and that COX-2 inhibitors can reverse this effect.[9] There are emerging laboratory data that COX-2 expression may play a role in tumor angiogenesis.

The aim of this study was to investigate the relationship between COX-2 expression and molecular markers of proliferation, angiogenesis, and apoptosis in advanced stage, high-grade serous ovarian carcinomas. Markers selected for study were (1) Ki-67 expression to determine the tumor proliferative activity, (2) vascular endothelial growth factor (VEGF) expression and CD34 staining to evaluate tumor angiogenic activity and microvessel count, and (3) bcl-2 expression to study the level of apoptotic activity.

Material and methods

Patients who were diagnosed with epithelial ovarian cancer between 1993 and 1999 were identified according to the database files of the Division of Gynecologic Oncology and the Department of Pathology at Wayne State University. Patients with advanced-stage high-grade serous carcinoma who underwent primary surgery without previous chemotherapy were included in this study. From a retrospective review of medical records, the patient's demographic and surgical data were collected. Survival data were retrieved with the SEER database and the institution computerized clinical information system. Surgical staging was determined with the criteria that are recommended by the International Federation of Gynecology and Obstetrics (FIGO). Histologic type and grade were determined by 2 of the authors (R.A-F., M.C.), who used the World Health Organization criteria. After each case had been evaluated, 2 to 3 representative paraffin blocks were selected for study.

Immunohistochemical staining with antibodies to COX-2, CD34, bcl-2, and Ki-67 was performed; as our study was progressing, we introduced the use of micro tissue array for immunohistochemical and used it for VEGF with the same ovarian cancer tissue blocks that had been selected previously for the other stains. Sections were deparaffinized and subjected to immunohistochemical staining, with standard streptavidin-biotin-peroxidase techniques, with diaminobenzidine as the chromogen. In brief, 4- to 5-μ thick sections were antigen retrieved by steam treatment in a citrate buffer, quenched for 10 minutes with 3% hydrogen peroxide, preincubated with blocking serum at 1:20 in 2% bovine serum albumin/phosphate buffered saline solution (PBS) for 15 minutes at room temperature. After incubation with the primary antibodies, slides were rinsed with PBS, and the secondary antibody was applied at 1:500 in PBS for 30 minutes at room temperature. After rinses with PBS for 30 seconds, slides were incubated with streptavidin/peroxidase at 1:500 in PBS for 30 minutes at room temperature, then rinsed with PBS and incubated for 15 minutes in 0.06% diaminobenzidine and counter-stained with Harris modified hematoxylin (Fisher Healthcare, Hanover Park, Ill). The following antibodies were used for immunohistochemical staining: Bcl-2 (clone:124, 1:40 dilution, incubation for 2 hours; Dako Corp, Carpinteria, Calif), COX-2 (clone: H62, 1:100 dilution, incubation overnight; Santa Cruz Biotechnology, Inc, Santa Cruz, Calif), CD34 (clone: QBEnd/10, 1:20 dilution, incubation for 45 minutes; BioGenex Laboratories, San Ramon, Calif), Ki-67 (clone: MM1, 1:100 dilution, incubation for 2 hours; Vector Laboratories, Burlingame, Calif), VEGF (polyclonal, 1:100 dilution, incubation for 2 hours; Santa Cruz Biotechnology, Inc.).