Cyclooxygenase-1 Is Overexpressed in Multiple Genetically Engineered Mouse Models of Epithelial Ovarian Cancer

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Abstract

Cyclooxygenases-1 and -2 (Cox-1 and Cox-2) are two distinct isoforms that catalyze the conversion of arachidonic acid to prostaglandins. The role of Cox-2 in a variety of cancers is well recognized, but the contribution of Cox-1 remains much less explored. We have previously shown that human epithelial ovarian tumors have increased levels of Cox-1, but not Cox-2. We also observed that Cox-1 is highly expressed in a mouse model of epithelial ovarian cancer (EOC), which lacks p53 but overexpresses *c-myc* and *K-ras* or *c-myc* and *Akt*. More importantly, a Cox-1-selective inhibitor, SC-560, attenuates EOC growth. In the present investigation, we used various genetically engineered mouse models of EOC to determine whether Cox-1 overexpression is unique to specific genetic and oncogenic alterations or is widespread. These models include: (*a*) deletion of both *p53* and *Rb*, (*b*) induction of the transforming region of SV40 under the control of Mullerian inhibitory substance type II receptor, or (*c*) activation of K-Ras in the absence of Pten locally in the ovarian surface epithelium. We found that these three models, which produce spontaneous EOC, also show up-regulated expression of Cox-1, but not Cox-2. The results provide further evidence that Cox-1 overexpression is common in various models of EOC. Thus, Cox-1 serves as a potential marker of EOC and is a possible target for the prevention and/or treatment of this deadly disease. (Cancer Res 2006; 66(5): 2527-31)

Introduction

The genetic and molecular mechanisms underlying ovarian cancer remain largely unknown, and treatment options for patients with advanced disease are limited. Epithelial ovarian cancers (EOC) originating from ovarian surface epithelial (OSE) cells comprise 90% of ovarian cancers (1, 2). EOCs are morphologically and biologically heterogeneous, causing difficulty in defining molecular events underlying the disease development and progression (1). Research primarily focusing on colorectal cancer has provided strong evidence that nonsteroidal anti-inflammatory steroids (NSAID) are effective in both cancer prevention and treatment of established tumors (3). NSAIDs interfere with prostaglandin biosynthesis by inhibiting cyclooxygenases-1 and -2 (Cox-1 and Cox-2), the enzymes that catalyze the rate-limiting step in prostaglandin biosynthesis from arachidonic acid. These drugs encumber colorectal tumor growth primarily by attenuating Cox-2 activity, although there is a possibility that other non-Cox-2 targets are also affected. In addition, Cox-2 is overexpressed in a variety of extracolonic cancers, and selective Cox-2 inhibitors show potent antineoplastic effects *in vivo* in preclinical models of various solid malignancies (2). However, epidemiologic studies examining whether NSAIDs prevent or delay development of ovarian cancers remain inconclusive (reviewed in refs. 3–5). The question is further exacerbated by many published reports of Cox-2 expression in ovarian cancer rather than Cox-1 (reviewed in refs. 4, 5). Many of these studies used immunologic techniques to differentiate between Cox-2 and Cox-1, or did not examine Cox-1 expression. The nonspecificity of many commercially available Cox-2 antibodies provoked us to use multiple approaches to examine Cox isoform expression in EOC. We previously showed that Cox-1, but not Cox-2, is highly expressed in human EOC (4), and in a mouse EOC model lacking p53 but overexpressing *c-myc* and *K-ras* or *c-myc* and *Akt* (5, 6). Other groups have also recently questioned the importance of Cox-2 in ovarian tumorigenesis (7, 8). In recent years, several independent laboratories have developed mouse models of EOC employing strategies for genetic or functional inactivation of tumor suppressor genes and/or activation of oncogenes specifically in the epithelial compartment of the ovary (9–11). In this study, we used these various mouse models to examine the expression of Cox isoforms to determine whether Cox-1 overexpression is common to EOC arising from various manipulations of tumor suppressor genes and/or oncogenes.

Materials and Methods

Genetically engineered mouse models of EOCs. EOCs used in the present investigation were induced either by (*a*) inactivation of p53 and Rb, (*b*) induction of activated *K-ras* in the absence of Pten, or (*c*) induction of the transforming region of SV40 T antigen (TAg) under transcriptional control of a portion of the murine Mullerian inhibiting substance type II receptor (MISIIR) gene promoter locally in the OSE as previously described (9–11). These are existing mouse models that are maintained independently in the laboratories of the investigators who generated them (9–11). Our previous experiments using *AdCre* and *Rosa26STOPfloxPLacZ* mice showed that recombination occurs in 60% to 80% of cells (10). Using microdissection-PCR assay, it was also shown that all neoplastic cells lack wild-type alleles of *p53* and *Rb* from the earliest morphologically detectable lesions (10).

Tumors were graded based on the established histopathologic criteria. The diagnosis of well and poorly differentiated neoplasms reflects the degree of structural and cytologic atypia as well as their proliferative activity as described in previous publications (9–12).

RT-PCR. Total RNA was isolated with Trizol according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). After DNase

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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treatment (Ambion, Austin, TX), 1 μg of total RNA was reverse transcribed with Superscript II or Superscript III (Invitrogen). PCR was done as previously described (13, 14). The primers for Cox-1 were 5′-AGGAGATGGCTGCT-GAGTTGG-3′ (sense) and 5′-AATCTGACTTTCTGAGTTGCC-3′ (antisense) and those for Cox-2 were 5′-ACACACTCTATCACTGGCACC-3′ (sense) and 5′-TTCAGGGAGAAGCGTTTGC-3′ (antisense).

Hybridization probes. *cDNA* clones for *Cox-1* and *Cox-2* have been previously described (15). In brief, a *cDNA* fragment encoding Asn_{82}-Gln_{360} was used to generate the *Cox-1* probe, whereas a *cDNA* fragment encoding Met_1-Gln_{270} including 55 nucleotides at the 5′-untranslated region was used to generate the *Cox-2* probe. For *in situ* hybridization, sense and antisense ^{35}S-labeled *cRNA* probes were generated using Sp6 and T7 polymerases, respectively.

***In situ* hybridization.** *In situ* hybridization was done as previously described (14). Sections hybridized with sense probes did not exhibit any positive signals and served as negative controls. Furthermore, day 1 and day 4 pregnant mouse uterine sections were used as positive controls for *Cox-2* and *Cox-1*, respectively (ref. 16; Supplemental Fig. S1).

Western blot analysis. Tissue samples were prepared as previously described (5). After measuring protein concentrations, extracts (20 μg protein) were boiled for 5 minutes in SDS sample buffer. The samples were run on 10% SDS-PAGE gels under reducing conditions and transferred onto nitrocellulose membranes. The membranes were blocked with 10% milk in TBST, and probed with Cox-1 (1:1,000, kindly provided by David DeWitt, East Lansing, MI) or Cox-2 (1:30,000, Cayman, Ann Arbor, MI) for 16 hours at 4°C (5, 17). After washing, blots were incubated in peroxidase-conjugated donkey anti-goat IgG or donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Protein signals were detected using chemiluminescent reagents (Amersham, Piscataway, NJ). The antibodies to Cox-1 and Cox-2 are highly specific as determined by Western blot analysis of pregnant mouse uterine extracts obtained from *Cox-1*$^{-/-}$ and *Cox-2*$^{-/-}$ mice (Supplemental Fig. S2).

Immunohistochemistry. Immunohistochemistry was done as previously described (5). In brief, formalin-fixed paraffin-embedded tumor sections (6 μm) were subjected to immunostaining using Cox-1 or Cox-2 antibodies, as described above. After deparaffinization and hydration, sections were subjected to antigen retrieval by autoclaving in 10 mmol/L sodium citrate solution (pH 6.0) for 15 minutes. A Histostain-Plus kit (Zymed, San Francisco, CA) was used to visualize the antigen; reddish-brown deposits indicate sites of positive immunostaining.

Primary culture of OSE cells and gene array. Primary culture of OSE cells was done as previously described (10). In brief, individual ovaries were dissected and digested in DMEM/F12 (Ham's) medium containing collagenase-dispase at 5% CO_2 for 1 hour. After brief washing, the collected OSE cells were placed in 12-well plates, covered with 0.1% gelatin, maintained in Ham's medium supplemented with 5% fetal bovine serum, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 10 ng/mL epidermal growth factor, 500 ng/mL hydrocortisone, 5 μg/mL insulin, 5 μg/mL transferrin, and 5 ng/mL sodium selenite. The cells were passaged and exposed to 200 multiplicity of infection of either *AdCMVlacZ* or *AdCMVCre* for 2 hours to establish OSE control or knockout cells, respectively. Treated cells at the indicated passages after infection were used for microarray studies using Affymetrix mouse GeneChip U74Av2.

Results and Discussion

Cox-1 is expressed in ovarian cancer arising from surface epithelium lacking *p53* and *Rb*. We evaluated differential expression of Cox isoforms in mouse EOC generated by deleting *p53* and *Rb* in OSE cells of mice carrying *p53*$^{floxP/floxP}$/*Rb*$^{floxP/floxP}$ genes by local delivery of adenovirus-mediated Cre (*AdCMVCre*). These mice primarily develop well-differentiated (39%) and poorly differentiated (45%) serous epithelial neoplasms, although some develop mixed types (10). We analyzed Cox-1 and Cox-2 expression in five independent EOC samples. Analysis of RT-PCR results shows that *Cox-1* mRNA levels are high in well-differentiated serous epithelial neoplasms, whereas levels of *Cox-2* mRNA are low to undetectable. However, both *Cox-1* and *Cox-2* mRNAs are detected in poorly differentiated neoplasms (Fig. 1*A*). This pattern is also reflected at the protein level as determined by Western blotting (Fig. 1*B*). We next examined cell-specific expression of *Cox-1* and *Cox-2* in tumor sections by *in situ* hybridization (Fig. 1*C*). The expression of *Cox-1* is high in all tumor samples, whereas *Cox-2* expression is low to undetectable, except for some focal expression in poorly differentiated neoplasms. Immunohistochemistry also detected high levels of Cox-1 protein with low levels of Cox-2 in poorly differentiated neoplasms (Fig. 1*D*). Collectively, these results provide evidence that Cox-1 and not Cox-2 is primarily expressed in EOC originating from OSE missing *p53* and *Rb*.

A microarray study comparing global gene expression between *p53*$^{floxP/floxP}$/*Rb*$^{floxP/floxP}$ OSE cells (controls) or cells lacking both *p53* and *Rb* genes after local delivery of *AdCMVCre* found that *Cox-1* expression is >50-fold higher in cells missing both genes when compared with control OSE cells. In contrast, *Cox-2* expression was insignificant when compared with intact OSE cells (Fig. 2*A*). Comparative RT-PCR results of these samples more or less followed the pattern of microarray data (Fig. 2*B*). This

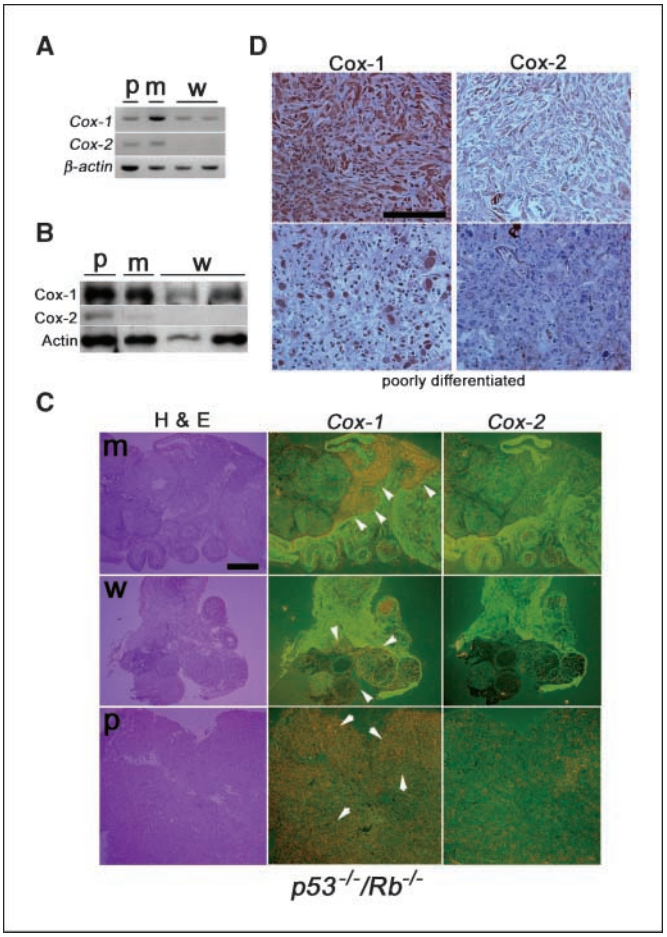

Figure 1. Differential expression of Cox-1 and Cox-2 in ovarian tumors induced by inactivation of *p53* and *Rb* in the surface epithelium. Results from representative samples. *A,* RT-PCR results. *β-Actin* is a housekeeping gene. *B,* Western blot analysis. Actin serves as a control. *C, in situ* hybridization results. *Arrowheads,* sites of higher signal intensity. H&E staining; *bar,* 500 μm; *p,* poorly differentiated; *w,* well-differentiated; *m,* mixed. *D,* immunohistochemistry. *Red deposits,* sites of positive immunostaining; *bar,* 200 μm.

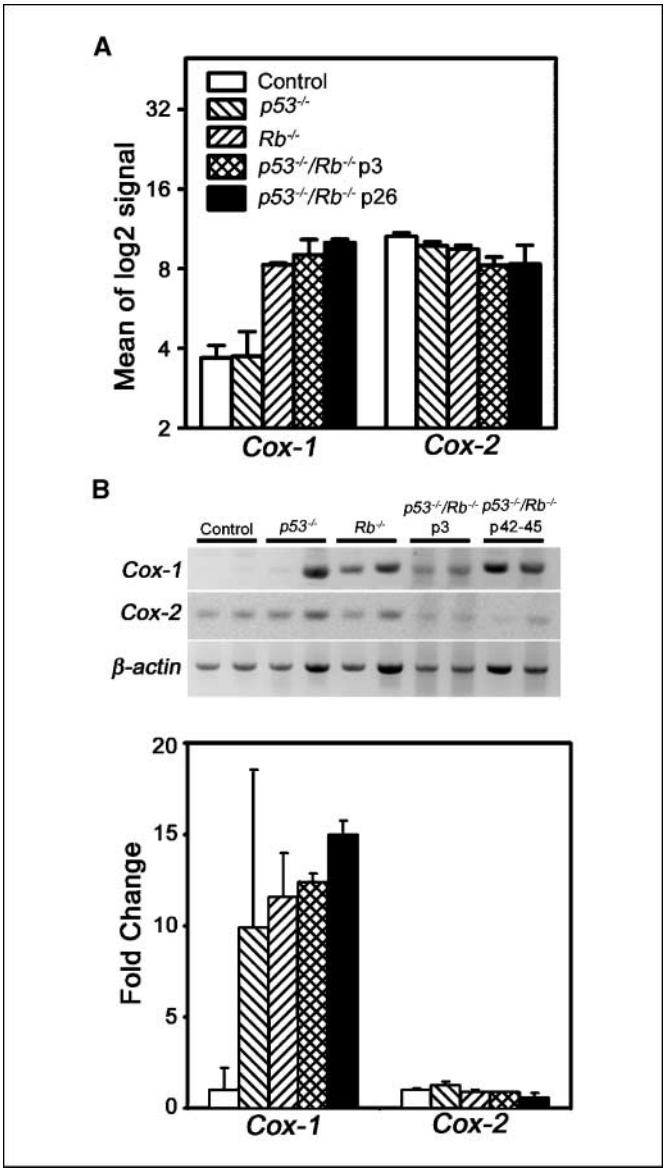

Figure 2. *A,* comparative microarray analysis of *Cox-1* and *Cox-2* expression in OSE. OSE cells carrying $p53^{floxP/floxP}$, $Rb^{floxP/floxP}$, or $p53^{floxP/floxP}/Rb^{floxP/floxP}$ were treated with either *AdCMVCre* or *AdCMVLacZ* recombinant adenovirus resulting in $p53^{-/-}$, $Rb^{-/-}$, $p53^{-/-}/Rb^{-/-}$, or control cells, respectively. Cells were collected after 3 passages ($p53^{-/-}$, $Rb^{-/-}$, $p53^{-/-}/Rb^{-/-}$ p3 and control) and after 26 passages ($p53^{-/-}/Rb^{-/-}$ p26) of *AdCMVCre* infection. *Cox-1* gene (mean of log2 signal ± SE): 3.69 ± 0.42 ($p53^{floxP/floxP}/Rb^{floxP/floxP}$ *LacZ*, control), 3.73 ± 0.89 ($p53^{-/-}$), 8.28 ± 0.11 ($Rb^{-/-}$), 9.03 ± 1.29 ($p53^{-/-}/Rb^{-/-}$ p3), and 10.05 ± 0.31 ($p53^{-/-}/Rb^{-/-}$ p26). Pair-wise comparison of test samples versus control ($n = 3$) by *t* test yielded two-tailed $P = 0.9658$ ($p53^{-/-}$, $n = 3$), 0.0005 ($Rb^{-/-}$, $n = 3$), 0.0169 ($p53^{-/-}/Rb^{-/-}$ p3, $n = 3$), and 0.0017 ($p53^{-/-}/Rb^{-/-}$ p26, $n = 2$). *Cox-2* gene (mean of log2 signal ± SE): 10.59 ± 0.34 ($p53^{floxP/floxP}/Rb^{floxP/floxP}$ *LacZ*, control), 9.79 ± 0.34 ($p53^{-/-}$), 9.49 ± 0.31 ($Rb^{-/-}$), 8.24 ± 0.62 ($p53^{-/-}/Rb^{-/-}$ p3), and 8.35 ± 1.48 ($p53^{-/-}/Rb^{-/-}$ p26). Pair-wise comparison of test samples versus control ($n = 3$) by *t* test yielded two-tailed $P = 0.1661$ ($p53^{-/-}$, $n = 3$), 0.0751 ($Rb^{-/-}$, $n = 3$), 0.0291 ($p53^{-/-}/Rb^{-/-}$ p3, $n = 3$), and 0.1557 ($p53^{-/-}/Rb^{-/-}$ p26, $n = 2$). *B,* RT-PCR results of *Cox-1* and *Cox-2* of representative RNA samples used for microarray analysis. *β-actin* is a housekeeping gene.

observation is consistent with the findings that almost 100% of $p53^{floxP/floxP}/Rb^{floxP/floxP}$ mice develop neoplasms after deletion of these genes in the OSE by local delivery of *AdCMVCre* (10). These results suggest that expression of Cox-1, but not Cox-2, is elevated in the early stages of serous EOC.

***Cox-1* is expressed in ovarian cancer arising from the surface epithelium expressing *LSL-K-ras*$^{G12D/+}$ in the absence of *Pten*.** Dinulescu et al. have recently shown that endometrioid epithelial ovarian carcinomas, as characterized by metaplastic squamous differentiation and notable glandular morphology, develop after activation of *K-ras* with simultaneous deletion of *Pten* in the OSE in *LSL-K-ras*$^{G12D/+}$/*PTEN*$^{floxP/floxP}$ mice by local delivery of adenovirus-mediated *Cre* (11). Using this model, we examined whether endometrioid EOCs differentially express Cox isoforms in eight independent samples. *In situ* hybridization and Western blotting were done in these well-differentiated tumor specimens. Again, we observed abundant expression of *Cox-1* with low to undetectable *Cox-2* expression. Once again, *Cox-2* expression was spotty and restricted to small areas (Fig. 3*A*). The results of Western blotting on Cox-1 and Cox-2 protein levels are consistent with their mRNA expression (Fig. 3*B*). These results suggest that not only serous, but also endometrioid EOCs, express higher levels of Cox-1, but not Cox-2.

***Cox-1* is expressed in EOC derived by expression of the SV40 TAg in the ovaries of mice.** Connolly et al. have previously shown that female mice expressing SV40 TAg under the transcriptional control of the *MISIIR* gene promoter (*TgMISIIRTAg*) develop poorly differentiated ovarian tumors (9). We also used this model to examine differential expression of Cox-1 and Cox-2 in 11 poorly differentiated independent samples. Using RT-PCR, we found that *Cox-1* expression was higher in all tumors, whereas that of *Cox-2* was low to undetectable (Fig. 4*A*). Western blot analysis showed similar results (Fig. 4*B*). We also examined cell-specific expression of *Cox-1* and *Cox-2* in tumor sections by *in situ* hybridization (Fig. 4*C*). Again, the expression of *Cox-1* was high in all tumor samples. In contrast, *Cox-2* expression was low to undetectable, except for minor focal expression. These results suggest that EOC originating from an oncogenic insult also abundantly express Cox-1, but not Cox-2.

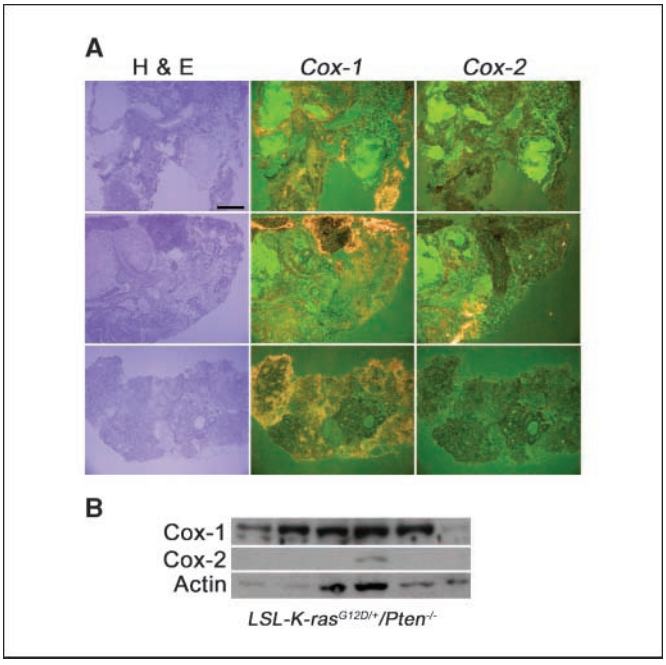

Figure 3. Differential Cox-1 and Cox-2 expression in endometrioid ovarian tumors induced by inactivation of *Pten* and activation of *K-ras* in the surface epithelium. Results of representative samples. *A, in situ* hybridization results. H&E staining; *bar,* 500 μm. *B,* Western blot analysis. Actin serves as a control.

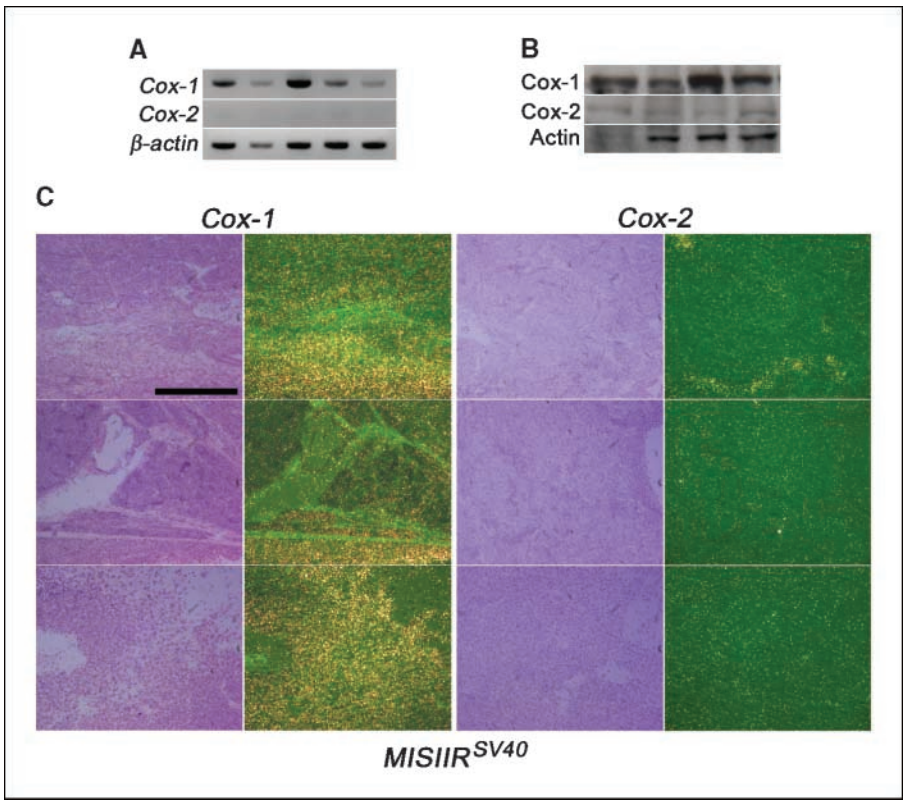

Figure 4. Differential Cox-1 and Cox-2 expression in ovarian tumors induced by overexpressing SV40 T antigen in the surface epithelium under the direction of a MISIIR promoter. Results of representative samples. *A,* RT-PCR results. *β-Actin* is a housekeeping gene. *B,* Western blot analysis. Actin serves as a control. *C, in situ* hybridization results. H&E staining, *bar,* 250 μm.

The highlight of the present investigation is that Cox-1 is the predominant isoform expressed at high levels in a variety of mouse models of EOC. The present results are exciting, and these models are clinically relevant because Cox-1 is also overexpressed in human EOC (4). The current realization that Cox-1 could be as important as Cox-2 in certain normal and pathologic situations provides new insight concerning the role of Cox-derived prostaglandins in pathophysiology (4, 5, 15). Indeed, we have shown previously that Cox-1-derived prostaglandins, particularly prostaglandin E_2, stimulates the expression of proangiogenic factors in human OVCAR3 cells which is inhibited by a Cox-1-selective inhibitor, SC-560 (4). More recently, we have shown that Cox-1-derived prostacyclin stimulates cell proliferation with attenuation of apoptosis in a mouse model of EOC. More importantly, SC-560 inhibited tumor growth in this model (5). It is also interesting to note that Cox-1-derived prostaglandin E_2 stimulates cell motility during zebrafish gastrulation (18).

Mutation or loss of tumor suppressor genes, amplification of growth-stimulatory factors, and/or suppression of death signaling pathways cause increased susceptibility to various cancers, including breast and ovarian cancers in women (19). For example, mutations in several tumor suppressor genes including *p53, BRCA1*, or *BRCA2*, and/or activating mutations or amplification of proto-oncogenes such as *c-myc, K-ras*, and *Akt* are considered contributors to human EOC. Therefore, the mouse models of EOC in which specific tumor suppressor genes were deleted with amplification of oncogenes serve as relevant models to better understand the etiology and progression of EOC. Our previous and present work showing predominant expression of Cox-1, not Cox-2, in EOC of both humans and in four different mouse models places the Cox-1 isoform as an important candidate for further investigation.

Although a wealth of information is available regarding regulation of Cox-2 in pathophysiologic situations, very little is known regarding the regulation of Cox-1 expression. Traditionally, *Cox-1* is considered a housekeeping gene. There is now evidence that two Sp1 sites in the human *Cox-1* promoter direct its constitutive expression in human umbilical vein endothelial cells (8). A role for histone deacetylase is also implicated in regulating *Cox-1* promoter activity in astrocytes (20). We have previously shown that *Cox-1* expression is influenced by estrogen and/or progesterone in the mouse uterus (16). However, it is not yet clear how Cox-1 is overexpressed in EOC. Our preliminary data suggests that an inhibitor of histone deacetylase influences *Cox-1* expression in mouse EOC cells *in vitro*. It is possible that chromatin remodeling is important for Cox-1 expression in EOC, which is currently under investigation in our laboratory.

Nonetheless, the present investigation forms the basis for the independent laboratories to examine the efficacy of Cox-1 inhibition or dual inhibition of both Cox-1 and Cox-2 to prevent and/or treat EOC growth in these mouse models. Because Cox-1 expression is heightened in different types of EOC in mouse models, this isoform may serve as a potential marker for the early detection of ovarian cancer and could be a promising target for EOC treatment.

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References

1. Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC. Ovarian surface epithelium: biology, endocrinology, and pathology. Endocr Rev 2001;22:255–8.
2. Nikitin AY, Hamilton TC. Modeling ovarian cancer in the mouse. In: Mohan RM, editor. Research Advances in Cancer. Kerala: Global Research Network; 2005. p. 49–59.
3. Gupta RA, Dubois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer 2001;1:11–21.
4. Gupta RA, Tejada LV, Tong BJ, et al. Cyclooxygenase-1 is overexpressed and promotes angiogenic growth factor production in ovarian cancer. Cancer Res 2003; 63:906–11.
5. Daikoku T, Wang D, Tranguch S, et al. Cyclooxygenase-1 is a potential target for prevention and treatment of ovarian epithelial cancer. Cancer Res 2005;65:3735–44.
6. Orsulic S, Li Y, Soslow RA, Vitale-Cross LA, Gutkind JS, Varmus HE. Induction of ovarian cancer by defined multiple genetic changes in a mouse model system. Cancer Cell 2002;1:53–62.
7. Kino Y, Kojima F, Kiguchi K, Igarashi R, Ishizuka B, Kawai S. Prostaglandin E2 production in ovarian cancer cell lines is regulated by cyclooxygenase-1, not cyclooxygenase-2. Prostaglandins Leukot Essent Fatty Acids 2005;73:103–11.
8. Yang WL, Roland IH, Godwin AK, Xu XX. Loss of TNF-α-regulated COX-2 expression in ovarian cancer cells. Oncogene 2005;24:7991–8002.
9. Connolly DC, Bao R, Nikitin AY, et al. Female mice chimeric for expression of the simian virus 40 TAg under control of the MISIIR promoter develop epithelial ovarian cancer. Cancer Res 2003;63:1389–97.
10. Flesken-Nikitin A, Choi KC, Eng JP, Shmidt EN, Nikitin AY. Induction of carcinogenesis by concurrent inactivation of p53 and Rb1 in the mouse ovarian surface epithelium. Cancer Res 2003;63: 3459–63.
11. Dinulescu DM, Ince TA, Quade BJ, Shafer SA, Crowley D, Jacks T. Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer. Nat Med 2005;11:63–70.
12. Nikitin AY, Connolly DC, Hamilton TC. Pathology of ovarian neoplasms in genetically modified mice. Comp Med 2004;54:26–8.
13. Zhang J, Goorha S, Raghow R, Ballou LR. The tissue-specific, compensatory expression of cyclooxygenase-1 and -2 in transgenic mice. Prostaglandins Other Lipid Mediat 2002;67:121–35.
14. Tranguch S, Cheung-Flynn J, Daikoku T, et al. Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation. Proc Natl Acad Sci U S A 2005;102:14326–31.
15. Wang H, Ma WG, Tejada L, et al. Rescue of female infertility from the loss of cyclooxygenase-2 by compensatory up-regulation of cyclooxygenase-1 is a function of genetic makeup. J Biol Chem 2004;279: 10649–58.
16. Chakraborty I, Das SK, Wang J, Dey SK. Developmental expression of the cyclo-oxygenase-1 and cyclo-oxygenase-2 genes in the peri-implantation mouse uterus and their differential regulation by the blastocyst and ovarian steroids. J Mol Endocrinol 1996;16:107–22.
17. Rouzer CA, Kingsley PJ, Wang H, et al. Cyclooxygenase-1-dependent prostaglandin synthesis modulates tumor necrosis factor-α secretion in lipopolysaccharide-challenged murine resident peritoneal macrophages. J Biol Chem 2004;279:34256–68.
18. Cha YI, Kim SH, Sepich D, Buchanan FG, Solnica-Krezel L, Dubois RN. Cyclooxygenase-1-derived PGE_2 promotes cell motility via the G-protein-coupled EP4 receptor during vertebrate gastrulation. Genes Dev 2006; 20:77–86.
19. Wenham RM, Lancaster JM, Berchuck A. Molecular aspects of ovarian cancer. Best Pract Res Clin Obstet Gynaecol 2002;16:483–97.
20. Taniura S, Kamitani H, Watanabe T, Eling TE. Transcriptional regulation of cyclooxygenase-1 by histone deacetylase inhibitors in normal human astrocyte cells. J Biol Chem 2002;277:16823–30.