Transitional Cell Hyperplasia and Carcinomas in Urinary Bladders of Transgenic Mice with Keratin 5 Promoter-Driven Cyclooxygenase-2 Overexpression

Russell D. Klein,[1] Carolyn S. Van Pelt,[2] Anita L. Sabichi,[3] Jorge dela Cerda,[3] Susan M. Fischer,[4] Gerhard Fürstenberger,[5] and Karin Müller-Decker[5]

[1]Departments of Human Nutrition and Internal Medicine, Ohio State University, Columbus, Ohio; Departments of [2]Veterinary Medicine and Surgery and [3]Clinical Cancer Prevention, University of Texas M.D. Anderson Cancer Center, Houston, Texas; [4]Department of Carcinogenesis, University of Texas M.D. Anderson Cancer Center, Smithville, Texas; and [5]Research Group Eicosanoids and Tumor Development, Deutsches Krebsforschungzentrum, Heidelberg, Germany

Abstract

The inducible form of cyclooxygenase (COX), COX-2, is up-regulated in many epithelial cancers and its prostaglandin products increase proliferation, enhance angiogenesis, and inhibit apoptosis in several tissues. Pharmacologic inhibition and genetic deletion studies showed a marked reduction of tumor development in colon and skin. COX-2 has also been strongly implicated in urinary bladder cancer primarily by studies with nonselective COX- and COX-2-selective inhibitors. We now show that forced expression of COX-2, under the control of a keratin 5 promoter, is sufficient to cause transitional cell hyperplasia (TCH) in 17% and 75% of the heterozygous and homozygous transgenic lines, respectively, in an age-dependent manner. TCH was strongly associated with inflammation, primarily nodules of B lymphocytes; some T cells and macrophage infiltration were also observed. Additionally, transitional cell carcinoma was observed in ~10% of the K5.COX-2 transgenic mice; no TCH or transitional cell carcinoma was observed in wild-type bladders. Immunohistochemistry for vascular proliferation and vascular endothelial growth factor showed significant increases above that in wild-type urinary bladders. Our results suggest that overexpression of COX-2 is sufficient to cause hyperplasia and carcinomas in the urinary bladder. Therefore, inhibition of COX-2 should continue to be pursued as a potential chemopreventive and therapeutic strategy. (Cancer Res 2005; 65(5): 1808-13)

Introduction

The prostaglandin G/H synthases, cyclooxygenase-1 (COX-1) and COX-2, are key enzymes in the synthesis of prostaglandins from arachidonic acid. COX-1 is expressed in nearly all tissues and is thought to be responsible for the low levels of prostaglandins needed for tissue maintenance and homeostasis. COX-2 is not expressed in most tissues under normal conditions, but expression is rapidly induced by growth factors or agents that cause tissue irritation or inflammation (1). COX-2 is expressed in many cancers (2, 3), and prostaglandins have been reported to increase proliferation, enhance angiogenesis, promote invasion, and inhibit apoptosis and differentiation. Genetic deletion or chemical inhibition of COX-2 in mice results in a decrease in several types of tumors (4–7). Transgenic overexpression of COX-2 in mice results in the spontaneous development of mammary tumors in multiparous mice (8), induces proliferative epithelial lesions in virgin mammary glands (9), and enhances dimethlybenz(*a*)anthracene-induced skin carcinogenesis (10).

There is strong evidence that COX-2 expression and activity is important in the development of urinary bladder cancers. COX-2 is up-regulated in invasive and noninvasive transitional cell carcinoma (TCC) in the human urinary bladder (11–13), and the level of COX-2 expression is positively associated with increasing stage and grade of TCC (14–16). Elevated COX-2 expression has also been reported in rat and canine models of bladder cancer (17, 18), and COX inhibitors reduce tumor growth and incidence in these models (19, 20). Collectively, the results from expression studies in humans and preclinical studies in animals support the hypothesis that COX-2 plays an important role in TCC development and progression (21).

Although compelling, the data on the involvement of COX-2 and prostaglandin synthesis in bladder cancer development has been primarily correlative in nature. To determine if COX-2 overexpression could be causally related to cancer development in these tissues, we utilized a transgenic mouse line (K5.COX-2 mice) that expresses a COX-2 transgene under control of a keratin 5 promoter (22). The COX-2 transgene of K5.COX-2 mice is expressed in the basal epithelial cells of skin and in other tissues with simple and stratified epithelium, including the urinary bladder. We analyzed urinary bladder tissues from two high expressing K5.COX-2 lines for evidence of spontaneous pathologic changes. We report that COX-2 overexpression in basal epithelial cells of K5.COX-2 mice is sufficient to cause transitional cell hyperplasia (TCH) and TCC of the urinary bladder. Furthermore, we provide evidence that COX-2 expression in the urinary bladder is proinflammatory and enhances expression of vascular endothelial growth factor (VEGF) and vascular proliferation.

Materials and Methods

Materials. Purified rat-monoclonal anti-CD3, anti-CD45R, and anti-mouse F4/80 were from Serotec (Raleigh, NC); biotinylated rabbit-anti-rat IgG purified rat-monoclonal anti-mouse Ki-67 were from DakoCytomation (Carpinteria, CA); rabbit polyclonal anti-cytokeratin-5 was from Covance/BabCo (Princeton, NJ); goat polyclonal anti-VEGF was from Santa Cruz Biotechnology; mouse anti-Factor VIII was from DakoCytomation; mouse anti-COX-2 and the prostaglandin E_2 (PGE_2) immunoassay kit were from

Requests for reprints: Karin Müller-Decker, Section Eicosanoids and Tumor Development, A140, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. Phone: 49-6221-424531; Fax: 49-6221-424406; E-mail: K.Mueller-Decker@DKFZ.de.

Cayman Chemical (Ann Arbor, MI). Streptavidin-horseradish peroxidase was from Biogenex (San Ramon, CA); alkaline phosphatase–conjugated anti-rabbit IgG was from Dianova (Hamburg, Germany); and biotinylated goat-anti-rabbit IgG, biotinylated rabbit-anti-rat IgG, and avidin-biotin complex–horseradish peroxidase were from Vector (Burlingame, CA).

Transgenic Mouse Lines. Wild-type NMRI mice (outbred strain from RCC, Füllinsdorf, Switzerland) and K5.COX-2 transgenic lines 667+/− and 675+/+, generated as described (22), were kept in Deutsches Krebsforschungszentrum (Heidelberg, Germany) under an artificial day/night rhythm and fed Altromin standard food pellets and water ad libitum. All animal experiments were approved by the Governmental Committee for Animal Experimentation (License 053/00).

Tissue Preparation and Histologic Analysis. The urogenital tracts, including the bladder and all prostatic lobes, were excised and fixed in PBS-buffered 10% paraformaldehyde. The fixed urogenital tracts were then bisected longitudinally through the bladder and urethra and positioned with the cut faces oriented in the same direction for paraffin embedding. Five-micrometer sections were stained with H&E by standard protocols. These sections included a cross section of the entire bladder and at least one section per mouse was analyzed for histopathology by a veterinary pathologist. Immunohistochemical staining was done using modified avidin-biotin complex techniques. Deparaffinized and hydrated sections were blocked with 3% hydrogen peroxide and, with the exception of the sections to be stained for VEGF and Factor VIII, were subjected to microwave antigen retrieval in 10 mmol/L citrate buffer (pH 6.0) for 10 to 15 minutes. Sections were blocked using 10% species-appropriate serum and incubated with primary antibody. Primary antibody dilutions and treatments were as follows: Ki-67, 1:200, overnight at 4°C; keratin 5, 1:500, 1 hour at room temperature (RT); CD3, 1:100, 2 hours at RT; CD45R, 1:2,000, 1 hour at RT; F4/80, 1:25, 2 hours at RT; COX-2, 1:2,000 overnight at 4°C; VEGF, 1:75, incubated 30 minutes at RT and then overnight at 4°C; Factor VIII, 1:400, incubated 30 minutes at RT. Species-appropriate biotinylated secondary antibodies were used at 1:200 for 2 hours at RT followed by avidin-biotin complex-horseradish peroxidase or streptavidin-horseradish peroxidase treatment for 30 minutes at RT. Slides were developed with 3,3′-diaminobenzidine for ~5 minutes. Ki-67-positive cell counts were divided by bladder epithelium circumference measured using calibrated Spot V4.0.5 imaging software (Diagnostic Instruments, Sterling Heights, MI).

Immunoblot Analysis. Immunoprecipitation of COX-2 and COX-1 and subsequent immunoblot analysis were carried out by using COX isozyme–specific antisera as described (10).

Prostaglandin Measurements. Endogenous PGE_2 levels in bladder tissue were determined by enzyme immunoassay as previously described (10). Briefly, bladder samples were pulverized and suspended in 2 mL of ice-cold ethanol. After centrifugation, the supernatant was collected and diluted to 15% ethanol with 0.1 mol/L sodium formate buffer. Prostaglandins were enriched by solid-phase extraction on C18 silica cartridges eluted with ethyl acetate. The dried lipids were then analyzed according to manufacturer's instructions. The protein pellets were dissolved in 8 mol/L urea and used for measurement of protein concentrations by means of Bio-Rad (Munich, Germany) protein detection kit. Concentrations (pg/mg of protein) represent mean ± SE for $n = 4$ to 5 samples.

Results

Transgenic Expression of COX-2 in the Urinary Bladders of K5.COX-2 Mice. The expression of COX-2 in K5.COX-2 mice is driven by the bovine cytokeratin 5 promoter in the basal cells of simple and stratified epithelia in a variety of tissues including the urinary bladder (9, 10). COX-2 expression in urogenital tissues of K5.COX-2 transgenic mice was confirmed by immunohistochemistry on tissues from homozygous line 675 and hemizygous line 667. As can be seen in Fig. 1*A*, little or no COX-2 protein is expressed in bladder tissue of wild-type NMRI mice. However, intense staining of COX-2 protein was observed in bladder epithelium from K5.COX-2 mice of both transgenic lines (Fig. 1*B* and data not shown). In hyperplastic regions, the expression of COX-2 extends into the suprabasal cell layers, similar to the expression of keratin 5 (data not shown). Analysis of bladder tissue for COX-2 expression by immunoblot analysis confirmed that little or no COX-2 protein is present in wild-type bladder tissue, but K5.COX-2 bladders express high levels of COX-2 protein (Fig. 1*C*). COX-1 steady state levels were similar in wild-type and transgenic bladders (Fig. 1*C*). The elevated expression of COX-2 resulted in an ~5-fold increase in PGE_2 contents in the bladders of K5.COX-2 transgenics compared with wild-type mice (Fig. 1*D*).

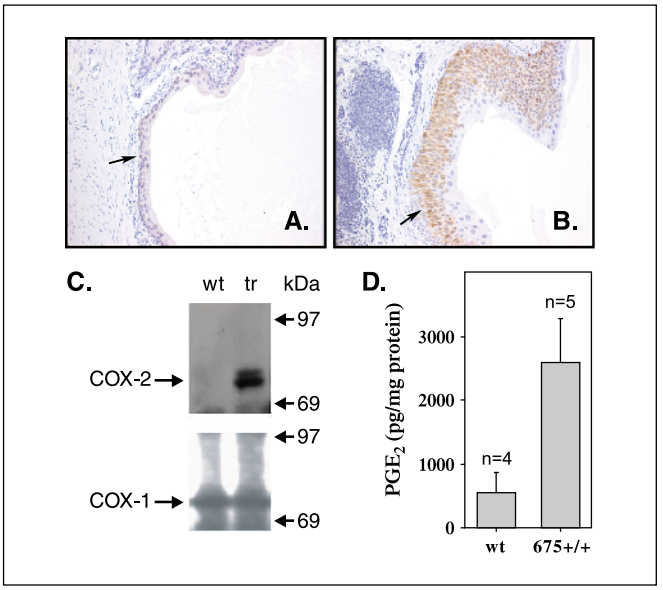

Figure 1. COX-2 is highly expressed and PGE_2 levels are elevated in the urinary bladders of K5.COX-2 transgenic mice. Bladders from wild-type and K5.COX-2 transgenic mice were stained with anti-COX-2. No staining to weak staining is observed in wild-type bladder epithelium (*A*), whereas intense staining is present in the basal epithelium of line 675 homozygous transgenic bladders (*B*). Cell lysates from transgenic and wild-type mouse bladders were analyzed for COX-2 and COX-1 protein expression by immunoblotting (*C*) and for endogenous levels of PGE_2 by an enzyme immunoassay (*D*); columns represent means ± SE.

Inflammation, Hyperplasia, and Carcinoma in K5.COX-2 Urinary Bladders. A histopathologic analysis was conducted on urinary bladders from the two K5.COX-2 transgenic lines and age-matched wild-type controls. H&E-stained sections from a total of 67 urinary bladders from 3- to 16-month-old male mice were examined. From the results of this analysis, it is clear that overexpression of COX-2 in the epithelial cells of the urinary bladder is associated with the development of inflammation, TCH, and TCC (Fig. 2). Interestingly, the initial lesion in K5.COX-2 urinary bladders seemed to be inflammation consisting of perivascular infiltrates of lymphocytes and plasma cells. By 6 months of age, there were nodules of lymphoid proliferation that were more pronounced between 10 and 16 months of age (Fig. 2*B* and *C*). The incidence and severity of the perivascular infiltrates and lymphoid nodular proliferation were increased in the homozygous founder line 675 compared with hemizygous line 667. Characterization of these lymphoid nodules by immunohistochemistry revealed that they are primarily composed of B lymphocytes. Most of the cells within the nodules are positive for the B-cell marker, CD45R (Fig. 3*A*). Few cells within these nodules stained for the T-cell marker CD-3 (Fig. 3*B*), whereas F4 staining

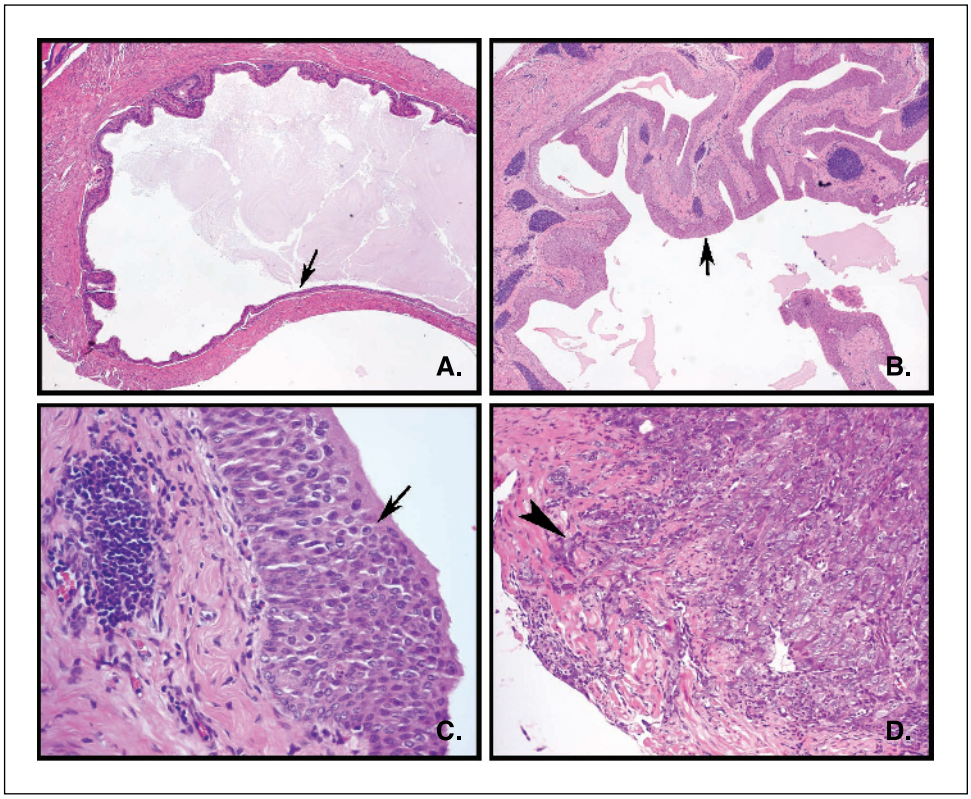

Figure 2. Expression of the COX-2 transgene leads to TCH and TCC in the bladders of K5.COX-2 transgenic mice. Bladders from wild-type (*A*) and K5.COX-2 (*B-D*) mice were stained with H&E. Note the thin epithelium (*black arrow*) in wild-type bladders in *A* (×40). Papillary TCH denoted by extensive papillary extensions into the bladder lumen (*B*, ×40) and thickened hyperplastic epithelium (*black arrows*, *B* and *C*, ×200) in a K5.COX-2 bladder. Note also the nests of darkly stained inflammatory cells (*white arrowheads*, *B*). *D*, invasive TCC in a K5.COX-2 bladder (×200); darkly stained tumor cells can be seen invading through the muscle (*black arrowhead*).

indicated a few areas of macrophage infiltration outside of the lymphoid nodules (Fig. 3*C*).

The earliest TCH observed was at 6 months, but incidence and severity of these lesions increased with age. TCH occurred in 17% and 75% of the heterozygous and homozygous K5.COX-2 mice, respectively, in animals 10 months of age or older (Table 1). Both papillary and nodular TCH was observed often within the same section. TCC occurred with an incidence of 8% and 10% in heterozygous and homozygous K5.COX-2 transgenic founder lines, respectively. The time to tumor formation seemed to be ~12 to 16 months as no animals younger than 12 months presented with TCC. The TCC tumors in K5.COX-2 mice were anaplastic, had numerous mitotic cells, and had invaded the muscle wall of the urinary bladder (Fig. 2*D*). No TCH or TCC lesions were observed in any of the urinary bladders from wild-type NMRI mice.

Increased Proliferation Associated with TCH of the Urinary Bladder. Tissue hyperplasia can arise from an increase in cellular proliferation and a decrease in apoptosis. In normal bladders from wild-type mice, we observed very few apoptotic cells as determined by terminal deoxynucleotidyl transferase–mediated nick end labeling. There was no apparent reduction in apoptosis in hyperplastic K5.COX-2 urinary bladder tissue (data not shown). To determine if there was an increase in proliferating epithelial

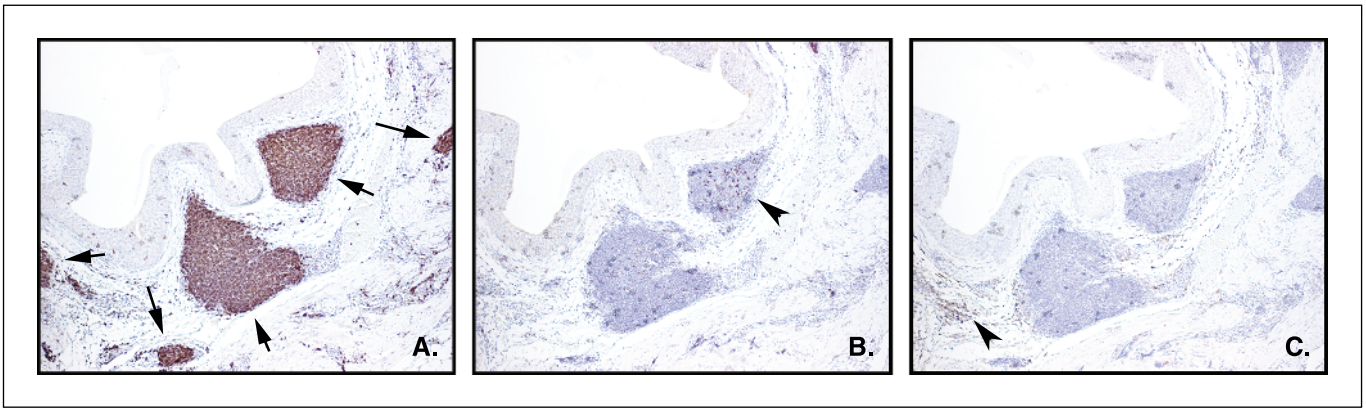

Figure 3. Inflammatory cells in K5.COX-2 bladders are predominately B-cell lymphocytes. *A,* bladders from K5.COX-2 mice were stained with anti-CD45R to test for the presence of B lymphocytes. Most of the cells in the large nests of inflammatory cells stained positive for CD45R antigen (*arrows*), indicating the presence of large numbers of B lymphocytes. *B,* staining with anti-CD3 to detect the presence of T-lymphocytes revealed the presence of small numbers of these cells within the inflammatory cell nests (*arrow*). *C,* bladders were also stained with anti-F4 to detect the presence of macrophages. Macrophages seemed sporadically in small numbers throughout the stroma and epithelium, and less frequently as patches of multiple cells (*arrow*).

Table 1. Phenotype of bladders from two lines of K5.COX-2 transgenic mice and wild-type controls 10-16 months of age

Genotype	TCH*	TCC*
Wild type	0/11 (0)	0/11 (0)
K5.COX-2 line 667	2/12 (17)	1/12 (8)
K5.COX-2 line 675	15/20 (75)	2/20 (10)

*n positive/n total (%).

cells in hyperplastic urinary bladders from K5.COX-2 transgenic mice compared with wild-type controls, a subset of tissue sections were analyzed for expression of the proliferation marker Ki-67 (Fig. 4). Sections of bladders from six wild-type mice, six K5.COX-2 mice with nonhyperplastic urinary bladder tissue, and six K5.COX-2 mice with bladders exhibiting TCH were analyzed. In nonhyperplastic urinary bladder tissues (Fig. 4*A*), very few Ki-67 positively stained epithelial cells were observed (mean, 0.24 cells/mm; 95% confidence interval, 0.08-0.40). There was no increase in Ki-67-positive epithelial cells in nonhyperplastic K5.COX-2 urinary bladders compared with wild-type bladders. In urinary bladder tissues with TCH (Fig. 4*B*), there was an ~40-fold increase in the number of Ki-67-positive staining epithelial cells (mean, 8.0 cells/mm; 95% confidence interval, 3.8-12.2) compared with nonhyperplastic tissues. These results indicate that increased proliferation is the primary mechanism by which COX-2 expression is inducing hyperplasia in the mouse urinary bladder.

Vascular Proliferation and VEGF Expression in K5.COX-2 Urinary Bladders. In mice 10 to 16 months of age, vascular proliferation within the lamina propria (stroma) was observed in ~50% of urinary bladders from K5.COX-2 line 667 and 75% of urinary bladders from line 675. In contrast, only one urinary bladder section from a wild-type mouse (9% of total) had any evidence of vascular proliferation. The presence of vascular proliferation was strongly associated with TCH as 16 of 17 urinary bladders with TCH also had vascular proliferation. Only 6 of 25 bladders without TCH had evidence of vascular proliferation. Immunohistochemical staining of urinary bladder tissue for Factor VIII showed the extent of vascular proliferation in hyperplastic bladders (Fig. 5*B*) compared with nonhyperplastic bladder tissue (Fig. 5*A*). The number of blood vessels present in the lamina propria underlying areas of hyperplastic epithelium was greatly increased, and numerous abnormally large blood vessels were found to be present.

Because PGE_2 has been reported to induce VEGF expression (23), we stained adjacent sections for VEGF. Very little expression of VEGF was seen in nonhyperplastic bladder epithelium of wild-type mice (Fig. 5*C*), whereas strong staining for VEGF was found throughout the hyperplastic epithelium of K5.COX-2 urinary bladders (Fig. 5*D*).

Discussion

There is substantial correlative evidence implicating COX-2 activity in the development of bladder cancer. Whereas COX-1 expression is not elevated in TCC and is confined to the stroma in benign and malignant bladders (11), COX-2 is elevated in dysplasia, carcinoma *in situ*, and invasive urinary bladder cancers (21). Expression of COX-2 correlates with tumor stage and grade in human bladder TCC (12, 15). COX-2 activity in urothelial mucosal tissues was reported to be elevated in smokers compared with nonsmokers and to correlate with the number of cigarettes smoked by bladder cancer patients (24). In one case-control study, nonsteroidal anti-inflammatory drug users were found to have a decreased risk for urinary bladder cancer (25).

Evidence for a functional role of COX-2 in bladder cancer is primarily from preclinical models. In a rat bladder cancer model, COX-2-selective inhibitors effectively inhibited tumor growth and incidence (19). The COX inhibitor piroxicam reduced tumor volumes and enhanced remission of spontaneous urinary bladders tumors in dogs (20). Although supportive of a functional role of COX-2 in the etiology of bladder cancer, these inhibitors also have COX-independent effects.

Transgenic mice offer a unique opportunity to study tissue-specific effects by expressing putative cancer causing genes in an *in vivo* system. Transgenic expression of COX-2 using a mouse mammary tumor virus promoter is sufficient to induce focal mammary gland hyperplasia, dysplasia, and metastatic tumors in multiparous female mice (8). The primary mechanisms by which COX-2 causes tumorigenesis in this model seem to be through regulation of genes involved in apoptosis and angiogenesis (23). We have reported on the ability of keratin 5 promoter–driven expression of COX-2 in K5.COX-2 transgenic mice to enhance skin tumorigenesis following treatment with dimethlybenz(*a*)anthracene. Interestingly, COX-2 was not sufficient to initiate skin carcinogenesis in this model but was sufficient to "autopromote" tumorigenesis in skin initiated by a subcarcinogenic dose of a genotoxic carcinogen (10). COX-2 expression is elevated in basal keratinocytes in K5.COX-2 mice and results in abnormal epidermal differentiation leading to hyperplasia, dysplasia, and increased vascularization (22). Because basal epithelial cells in the urinary bladder also express keratin 5 (26), we used K5.COX-2 mice to determine the effect of COX-2 expression on histopathology in these tissues.

Strong expression of COX-2 and an increase in PGE_2 levels were observed in the urinary bladders of both of the K5.COX-2 lines

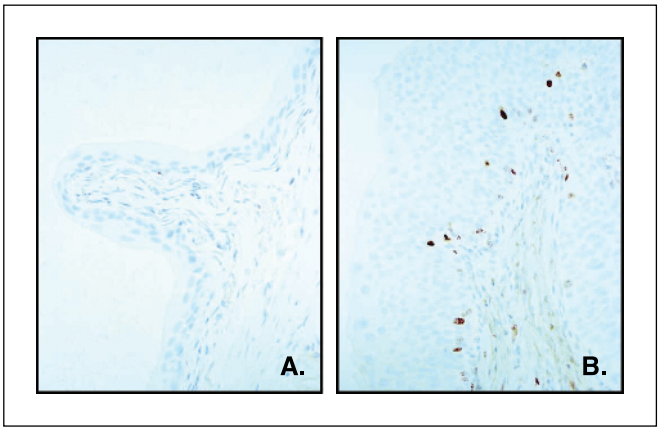

Figure 4. The number of epithelial cells positive for the proliferation marker Ki67 is increased in bladders from K5.COX-2 mice. Bladders from wild-type and K5.COX-2 mice were stained with anti-Ki67 (*A* and *B*, ×200). Very few cells stained positive in the wild-type bladder epithelial cells (*A*), whereas K5.COX-2 bladder epithelium (*B*) had areas with multiple Ki67-positive cells (*brown stain*). Most of the positive cells were found toward the basement membrane; however, some positive cells were observed within the upper layers of the hyperplastic epithelium.

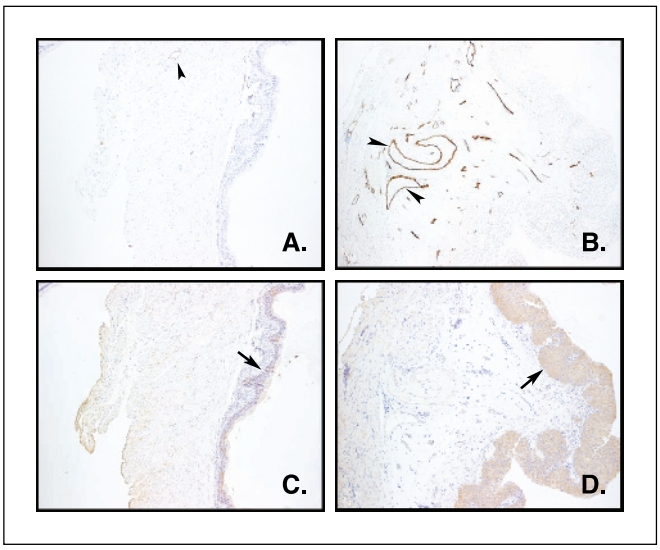

Figure 5. The expression of VEGF is enhanced in epithelial cells of K5.COX-2 bladders, and the number of blood vessels in the bladder stroma is greatly increased. Wild-type (*A* and *C*) and K5.COX-2 (*B* and *D*) bladders were stained with anti-Factor VIII (*A* and *B*) or anti-VEGF (*C* and *D*). Factor VIII staining of the wild-type bladders (*A*) showed relatively few blood vessels in the bladder stroma (*arrowhead*). In striking contrast, many blood vessels are revealed in K5.COX-2 bladder stroma (*B*, *brown stain*), including some relatively large vessels (*arrowheads*). Whereas staining of the epithelium of the wild-type bladders (*C*) for VEGF protein was either absent or very weak (*arrow*), the K5.COX-2 bladders (*D*) were stained very strongly throughout the hyperplastic epithelium (*arrow*).

examined whereas COX-1 levels were unchanged. Inflammation was strongly associated with the presence of TCH in bladders from K5.COX-2 mice. The highest incidence of inflammation and TCH occurred in line 675, which is maintained as a homozygous line. Mice from the hemizygous line 667 exhibited similar lesions, although with a lower incidence, indicating that the bladder phenotype was not due to an insertional effect of the transgene. Because prostaglandins have been reported to be proinflammatory, it is possible that COX-2-induced inflammation is a causative factor in the development of the TCH in these mice. The significance of the finding that B lymphocytes are the predominant cells in the lymphoid nodules is not clear. PGE_2 has been reported to synergize with interleukin (IL)-4 to cause differentiation, including antibody isotype switching (from immunoglobulin M to immunoglobulin E), and to mildly inhibit proliferation of B lymphocytes *in vitro* (27). PGE_2 can also induce a switch from T-helper 1–type cytokines (e.g., IL-2, IFN-γ, and IL-12) to the production of T-helper 2–type cytokines (e.g., IL-4, IL-5, and IL-10; ref.28). However, these activities of PGE_2 seem to be dependent on the state and mode of activation of the lymphocytes (29). Further analysis of the levels of individual prostaglandins and cytokines present in K5.COX-2 bladder tissue will be necessary to better understand the mechanisms behind the observed inflammatory response.

The development of TCH in the bladder of K5.COX-2 mice was associated with a dramatic increase in proliferating cells in the basal cell layers of the urothelium. Prostaglandin synthesis via COX-2 is necessary for proliferation induced by growth factors and tumor promoters in a number of tissues and cell types (30–33); however, it is unclear if the hyperplasia observed in K5.COX-2 bladders was due solely to prostaglandins or if inflammatory cytokines also were contributory. No decrease in apoptosis was observed by terminal deoxynucleotidyl transferase–mediated nick end labeling analysis in the urothelium of K5.COX-2 mice. In contrast, mouse mammary tumor virus–human COX-2 transgenic mice exhibited decreased apoptosis in involuting mammary epithelium compared with wild-type littermates (8). The low number of apoptotic cells in the wild-type bladders may preclude finding any reduction in transgenic mice by the methods utilized in this study. In concordance with the findings in the mouse mammary tumor virus–human COX-2 mammary model and the skin phenotype of K5.COX-2 mice, a dramatic increase in vascularization was observed in the lamina propria of the urinary bladder. Neovascularization depends on an increase in proangiogenic factors relative to antiangiogenic factors. The proangiogenic factor VEGF was clearly elevated in the urothelium of K5.COX-2 mice and likely contributed to the observed increase in vascularization.

Although bladder TCH developed in mice as young as 6 months and in 75% of line 675 mice, TCC developed only in mice aged 10 to 16 months and in just 10% of line 675 and 8% of line 667 mice. The low incidence of TCC and the relatively late occurrence implies that additional mutations are required for progression from TCH to TCC. One possible mechanism by which elevated COX enzyme activity could lead to genetic mutations is through increased formation of DNA adducts resulting from increased lipid peroxidation (34). Malondialdehyde is generated from the peroxidation of membrane phospholipids (35) and is also produced as a by-product of prostaglandin synthesis (36). Malondialdehyde has been shown to react with DNA to form adducts (37) and to be mutagenic (38). Another DNA lesion that occurs as a result of oxidative stress is 8-hydroxydeoxyguanosine. The level of 8-hydroxydeoxyguanosine in DNA is increased in inflammation (39). COX-2-selective inhibitors have been shown to decrease the formation of 8-hydroxydeoxyguanosine in a rat model of liver carcinogenesis (39) and a rat model of colonic inflammation (40), suggesting that the reactive oxygen species may come from either COX-mediated oxidative metabolism of arachidonic acid or from inflammatory cells activated by prostaglandin products of COX. Further studies will be needed to determine if the elevated COX-2 expression in K5.COX-2 mice is sufficient to cause an increase in DNA adduct formation.

It is clear from our data that transgenic expression of COX-2 is sufficient to cause TCH and ultimately TCC in the mouse bladder. Although further studies are necessary to fully elucidate the mechanisms involved, COX-2 expression resulted in inflammation, increased proliferation of the basal urothelium, and enhanced vascularization of the lamina propria. These findings support the hypothesis that COX-2 can play a functional role in urinary bladder carcinogenesis. Indeed, they further validate the rationale for conducting the current phase III trial testing the ability of the COX-2-selective inhibitor celecoxib to prevent bladder cancer recurrence in high-risk patients having undergone transurethral resection (21).

Acknowledgments

Received 10/6/2004; revised 11/30/2004; accepted 12/15/2004.

Grant support: National Cancer Institute grants CA100140 and CA091865, National Institutes of Environmental Health Sciences grant ES07784 (R.D. Klein and S.M. Fischer), and Deutsche Krebshilfe eV, Bonn, Germany (K. Müller-Decker and G. Fürstenberger).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank B. Steinbauer and D. Kucher (Deutsches Krebsforschungszentrum, Heidelberg, Germany), for their excellent assistance in mouse breeding.

References

1. Kam PC, See AU. Cyclo-oxygenase isoenzymes: physiological and pharmacological role. Anaesthesia 2000;55:442–9.
2. Gasparini G, Longo R, Sarmiento R, Morabito A. Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents? Lancet Oncol 2003;4:605–15.
3. Marks F, Furstenberger G. Cancer chemoprevention through interruption of multistage carcinogenesis. The lessons learnt by comparing mouse skin carcinogenesis and human large bowel cancer. Eur J Cancer 2000;36: 314–29.
4. Tiano HF, Loftin CD, Akunda J, et al. Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. Cancer Res 2002;62:3395–401.
5. Oshima M, Dinchuk JE, Kargman SL, et al. Suppression of intestinal polyposis in Apc δ716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 1996;87: 803–9.
6. Fischer SM, Lo HH, Gordon GB, et al. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. Mol Carcinog 1999; 25:231–40.
7. Lanza-Jacoby S, Miller S, Flynn J, et al. The cyclooxygenase-2 inhibitor, celecoxib, prevents the development of mammary tumors in Her-2/neu mice. Cancer Epidemiol Biomarkers Prev 2003;12:1486–91.
8. Liu CH, Chang SH, Narko K, et al. Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J Biol Chem 2001;276:18563–9.
9. Müller-Decker K, Berger I, Ackermann K, et al. Cystic duct dilatations and epithelial proliferations in mouse mammary glands upon keratin 5 promoter-driven overexpression of cyclooxygenase-2. Am J Pathol. In press 2005.
10. Muller-Decker K, Neufang G, Berger I, Neumann M, Marks F, Furstenberger G. Transgenic cyclooxygenase-2 overexpression sensitizes mouse skin for carcinogenesis. Proc Natl Acad Sci U S A 2002;99:12483–8.
11. Komhoff M, Guan Y, Shappell HW, et al. Enhanced expression of cyclooxygenase-2 in high grade human transitional cell bladder carcinomas. Am J Pathol 2000;157:29–35.
12. Shariat SF, Kim JH, Ayala GE, Kho K, Wheeler TM, Lerner SP. Cyclooxygenase-2 is highly expressed in carcinoma *in situ* and T1 transitional cell carcinoma of the bladder. J Urol 2003;169:938–42.
13. Shirahama T. Cyclooxygenase-2 expression is upregulated in transitional cell carcinoma and its preneoplastic lesions in the human urinary bladder. Clin Cancer Res 2000;6:2424–30.
14. Shariat SF, Matsumoto K, Kim J, et al. Correlation of cyclooxygenase-2 expression with molecular markers, pathological features and clinical outcome of transitional cell carcinoma of the bladder. J Urol 2003; 170:985–9.
15. Shirahama T, Arima J, Akiba S, Sakakura C. Relation between cyclooxygenase-2 expression and tumor invasiveness and patient survival in transitional cell carcinoma of the urinary bladder. Cancer 2001;92: 188–93.
16. Kim SI, Kwon SM, Kim YS, Hong SJ. Association of cyclooxygenase-2 expression with prognosis of stage T1 grade 3 bladder cancer. Urology 2002;60:816–21.
17. Kitayama W, Denda A, Okajima E, Tsujiuchi T, Konishi Y. Increased expression of cyclooxygenase-2 protein in rat urinary bladder tumors induced by *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine. Carcinogenesis 1999;20:2305–10.
18. Khan KN, Knapp DW, Denicola DB, Harris RK. Expression of cyclooxygenase-2 in transitional cell carcinoma of the urinary bladder in dogs. Am J Vet Res 2000;61:478–81.
19. Grubbs CJ, Lubet RA, Koki AT, et al. Celecoxib inhibits *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancers in male B6D2F1 mice and female Fischer-344 rats. Cancer Res 2000;60: 5599–602.
20. Mohammed SI, Bennett PF, Craig BA, et al. Effects of the cyclooxygenase inhibitor, piroxicam, on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. Cancer Res 2002;62:356–8.
21. Sabichi AL, Lippman SM. COX-2 inhibitors and other nonsteroidal anti-inflammatory drugs in genitourinary cancer. Semin Oncol 2004;31:36–44.
22. Neufang G, Furstenberger G, Heidt M, Marks F, Muller-Decker K. Abnormal differentiation of epidermis in transgenic mice constitutively expressing cyclooxygenase-2 in skin. Proc Natl Acad Sci U S A 2001; 98:7629–34.
23. Chang SH, Liu CH, Conway R, et al. Role of prostaglandin E_2-dependent angiogenic switch in cyclooxygenase 2-induced breast cancer progression. Proc Natl Acad Sci U S A 2004;101:591–6.
24. Badawi AF, Habib SL, Mohammed MA, Abadi AA, Michael MS. Influence of cigarette smoking on prostaglandin synthesis and *cyclooxygenase-2* gene expression in human urinary bladder cancer. Cancer Invest 2002; 20:651–6.
25. Castelao JE, Yuan JM, Gago-Dominguez M, Yu MC, Ross RK. Non-steroidal anti-inflammatory drugs and bladder cancer prevention. Br J Cancer 2000;82: 1364–9.
26. Moll R, Achtstatter T, Becht E, Balcarova-Stander J, Ittensohn M, Franke WW. Cytokeratins in normal and malignant transitional epithelium. Maintenance of expression of urothelial differentiation features in transitional cell carcinomas and bladder carcinoma cell culture lines. Am J Pathol 1988;132:123–44.
27. Roper RL, Brown DM, Phipps RP. Prostaglandin E2 promotes B lymphocyte Ig isotype switching to IgE. J Immunol 1995;154:162–70.
28. Fedyk ER, Adawi A, Looney RJ, Phipps RP. Regulation of IgE and cytokine production by cAMP: implications for extrinsic asthma. Clin Immunol Immunopathol 1996;81:101–13.
29. Watanabe S, Yssel H, Harada Y, Arai K. Effects of prostaglandin E_2 on Th_0-type human T cell clones: modulation of functions of nuclear proteins involved in cytokine production. Int Immunol 1994;6:523–32.
30. Mohseni H, Zaslau S, McFadden D, Riggs DR, Jackson BJ, Kandzari S. COX-2 inhibition demonstrates potent anti-proliferative effects on bladder cancer *in vitro*. J Surg Res 2004;119:138–42.
31. Furstenberger G, Marks F. Indomethacin inhibition of cell proliferation induced by the phorbolester TPA is reversed by prostaglandin E_2 in mouse epidermis *in vivo*. Biochem Biophys Res Commun 1978;84:1103–11.
32. Pentland AP, Needleman P. Modulation of keratinocyte proliferation *in vitro* by endogenous prostaglandin synthesis. J Clin Invest 1986;77:246–51.
33. Badawi AF. The role of prostaglandin synthesis in prostate cancer. BJU Int 2000;85:451–62.
34. Bartsch H, Nair J. Ultrasensitive and specific detection methods for exocylic DNA adducts: markers for lipid peroxidation and oxidative stress. Toxicology 2000;153:105–14.
35. Vaca CE, Wilhelm J, Harms-Ringdahl M. Interaction of lipid peroxidation products with DNA. A review. Mutat Res 1988;195:137–49.
36. Hayaishi O, Shumizu T. Metabolic and functional significance of prostaglandins in lipid peroxide research. In: Yagi K, editor. Lipid peroxides in biology and medicine. New York: Academic Press; 1982. p. 41–53.
37. Basu AK, O'Hara SM, Valladier P, Stone K, Mols O, Marnett LJ. Identification of adducts formed by reaction of guanine nucleosides with malondialdehyde and structurally related aldehydes. Chem Res Toxicol 1988; 1:53–9.
38. Basu AK, Marnett LJ. Unequivocal demonstration that malondialdehyde is a mutagen. Carcinogenesis 1983;4:331–3.
39. Denda A, Endoh T, Tang Q, Tsujiuchi T, Nakae D, Konishi Y. Prevention by inhibitors of arachidonic acid cascade of liver carcinogenesis, cirrhosis and oxidative DNA damage caused by a choline-deficient, L-amino acid-defined diet in rats. Mutat Res 1998;402:279–88.
40. Tardieu D, Jaeg JP, Deloly A, Corpet DE, Cadet J, Petit CR. The COX-2 inhibitor nimesulide suppresses superoxide and 8-hydroxy-deoxyguanosine formation, and stimulates apoptosis in mucosa during early colonic inflammation in rats. Carcinogenesis 2000; 21:973–6.