ANTICANCER RESEARCH *25*: 4543-4550 (2005)

Cyclooxygenase-2 Protein Expression in Relation to Apoptotic Potential and its Prognostic Significance in Bladder Urothelial Carcinoma

K. DIAMANTOPOULOU[1], A. LAZARIS[1], E. MYLONA[1], A. ZERVAS[3], K. STRAVODIMOS[3], I. NIKOLAOU[2], S. ATHANASSIADOU[2] and L. NAKOPOULOU[2]

[1]*First Department of Pathology, Medical School, Athens;*
[2]*Second Department of Pathology, Attikon University Hospital, Athens;*
[3]*Department of Urology, "Laikon" Hospital, National and Kapodistrian University of Athens, Athens, Greece*

Abstract. *Background: Cyclooxygenase-2 (COX-2), a critical enzyme in the conversion of arachidonic acid to prostaglandin E2, influences the biological behavior of human tumors, being involved in carcinogenesis, tumor progression, reduced apoptosis and differentiation. The aim of the present study was to investigate the role of COX-2 protein expression in urothelial carcinoma (UC) of the urinary bladder in relation to clinicopathological data and indices of apoptotic potential. Materials and Methods: Immunohistochemistry was applied to 134 paraffin-embedded specimens of UC for the detection of COX-2, p53, bcl-2, caspase-3, bax protein, MLH1 and hTERT. Results: Ninety-four UCs (70.1%) had an enhanced expression of COX-2. The COX-2 semi-quantitative expression was unrelated to tumor grade and local invasion, but it was positively linked with caspase-3 (CPP32) and bax protein semi-quantitative immunoreactivity (p=0.007 and p=0.026), as well as with the quantitative expression of MLH1 (p=0.019). COX-2 was also found to be inversely correlated with the nuclear localization of the catalyst component of the telomerase complex, hTERT (p=0.009). Multivariate statistical analysis showed that COX-2 immunopositivity was independently associated with worse prognosis of patients with non muscle-invasive UCs (p=0.002). Conclusion: COX-2 overexpression, being possibly a subsequence of apoptosis activation, is associated with an unfavorable overall survival of patients with pTa-T1 UCs.*

Correspondence to: L. Nakopoulou, Professor, Department of Pathology, School of Medicine, National and Kapodistrian University of Athens, 75 Mikras Asias St., Goudi, GR-11527 Athens, Greece. Tel: +30210-7462116, Fax: +30210-7462157

Key Words: Bladder urothelial carcinoma, cyclooxygenase-2, apoptosis.

Prostaglandins (PGs), especially PGE2, appear to be important in the pathogenesis of cancer because they affect mitogenesis, cellular adhesion, invasion, apoptosis and immunosurveillance (1). The metabolism of arachidonic acid by cyclooxygenases (COXs) initiates the formation of PG. COX exists in two isoforms, COX-1 and COX-2, both of which are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) (2). Whereas COX-1 is expressed constitutively in most normal tissues and is required for their normal physiological function, COX-2 is usually not detectable, but is induced in response to various stimuli such as inflammatory cytokines, growth factors, oncogenes and tumor promoters (3-5).

Much interest has centered on the ability of COX-2 to suppress apoptosis, since diminished apoptosis is thought to favor carcinogenesis by permiting survival of cells that have acquired mutations (6). Several hypotheses have been advanced to account for the suppression of apoptosis in response to COX-2 overexpression, including the PG-mediated up-regulation of bcl-2 (7) and the decrease of arachidonic acid, an apoptosis stimulator, through its COX-2-mediated conversion to PG (8). Conversely, many NSAIDs enhance apoptotic cell death (9), although this is unlikely to be solely due to inhibition of COX activity, since NSAID-induced apoptosis has also been demonstrrated in cell lines that do not express COX-2 (10). Additionally, non-cyclooxygenase-inhibiting sulindac metabolites, such as sulindac sulphone, retain the ability to induce apoptosis (11). Furthermore, some studies have shown that various agents up-regulate COX-2 protein expression whilst inducing apoptosis (12-15). In other words, the role of COX-2 in the modulation of apoptosis is poorly characterized.

Overexpression of the inducible form of COX-2 is known to confer invasive ability on tumor cells *in vitro* and has been involved in the development of urothelial carcinoma (UC)

of the human urinary bladder (1, 16, 17). Celecoxib, a selective COX-2 inhibitor, has been shown to reduce the incidence of urinary bladder cancer induced by a nitrosamine (18). However, the prognostic significance of COX-2 in UC remains questionable (19). To investigate the role of COX-2 in patients with bladder UC, COX-2 immunohistochemical expression was analyzed in a well-documented series of 134 UCs in relation to various clinicopathological parameters and indices of apoptotic potential.

Materials and Methods

One hundred and thirty-four patients [mean age at diagnosis 68.8 years (age range, 31-89 years)] with UC were included in this study. Formalin-fixed tissue samples were obtained from the first transurethral resection of the bladder or cystectomies. According to the WHO classification, the UCs were graded 1, 2 or 3 and local invasion was classified into two groups, pTa-T1 and pT2-4 (20). The patients were observed for survival analysis; the median follow-up was 48 months (range 18-145) during which 47 patients died of UC.

Immunohistochemistry was used to detect the expression levels of COX-2. The immunohistochemical staining for COX-2 was performed on 4-μm-thick formalin-fixed paraffin sections using an avidin-biotin immunoperoxidase technique. Briefly, the sections were dewaxed, rehydrated and treated for quenching of endogenous peroxidase activity. To enhance antigen retrieval, the sections were microwave-treated in 10 mM citrate buffer, pH 6.0, at 750 W for 2 cycles of 5 min each. After blocking non-specific binding, the sections were incubated overnight at 4°C with a goat polyclonal antibody to COX-2 (c-20), raised against peptide mapping at the carboxy terminus of COX-2 of human origin (Santa Cruz Biotechnology, CA, USA) at a dilution of 1:130. The sections were incubated in biotinylated horse anti-goat secondary antibody, followed by peroxidase-conjugated avidin-biotin complex (Vectastatin Elite ABC Kit, Vector Lab, Burlingame, CA, USA). 3',3'-Diaminobenzidine tetrahydrochloride was used as a chromogen. Finally, the sections were counterstained with Harris hematoxylin and mounted.

For negative controls, the primary antibody was omitted. For statistical reasons, specimens were considered COX-2-negative when less than 10% of the cancer cells were stained and as COX-2-positive when equal to or greater than 10% of the cancer cells were stained, as previously used (21).

With regard to COX-2 immunostaining, statistical correlations were investigated with classical clinicopathological prognostic indicators, patients' overall survival and several immunohistochemical marker expressions (*i.e.* p53, bcl-2, hTERT, CPP32, bax protein, MLH-1) (22-24).

The latter data were available from our archives. With regard to MLH-1 protein, its immunohistochemical staining was evaluated quantitatively by an image analysis method (unpublished data).

Statistics. Pearson's Chi-square statistics with continuity correction were employed to assess the COX-2 expression and the categorical parameters of interest. The association of COX-2 with MLH1 was investigated by the Mann-Whitney *U*-test. Overall survival distribution curves were assessed by log-rank statistics followed by Cox's proportional hazards regression model.

Results

COX-2 was not expressed in either normal urothelia, when the latter were observable in the examined specimens, or in the tumor stroma. With regard to COX-2 expression in cancer cells, of 134 UCs, 94 (70.1%) were COX-2-positive, whereas 40 (29.9%) were COX-2-negative. In immunopositive cases, a diffuse cytoplasmic staining pattern was observed (Figures 1, 2) in tumor cells, occasionally with submembranous intensification in tumor-adjacent *in situ* UCs (Figure 3). The staining intensity was generally heterogeneous among the positive cells, ranging from moderate reactivity to a dark brown reaction product.

In the total specimens (Table I), Fisher's exact test showed that CPP32 immunoreactivity was positively correlated with COX-2 expression at a statistically significant level ($p=0.007$). In patients less than 70 years old, COX-2 reactivity was positively linked with bax immunoexpression (Fisher's exact test $p=0.026$). Regarding MLH1 quantitative expression, a positive link with COX-2 reactivity was detected in all the specimens (Mann-Whitney test, $p=0.019$), as well as in the Ta-T1 subgroup ($p=0.012$) (Figure 4A), whereas COX-2 immunodetection was inversely correlated with the nuclear localization of hTERT in all the specimens ($p=0.009$ in Fishers' exact test) and in the Ta-T1 subgroup as well ($p=0.002$) (Figure 4B). COX-2 expression was unrelated both to classic prognosticators, *i.e.,* tumor grade and pathological stage (Table I), and to any of the other immunohistochemical markers assessed (p53 and bcl-2).

Whether COX-2 expression directly influences patients' survival was investigated. A Cox proportional hazards model was constructed using established prognostic factors and COX-2 expression. The analysis showed that the patient's age ($p=0.008$) and tumor pathological T stage ($p<0.0001$), but not COX-2 expression ($p=0.243$), were independent prognostic parameters. Interestingly, when patients with superficial disease (*i.e.* Ta-T1 UCs) were separately examined, COX-2 positivity emerged as a significant independent adverse prognosticator (Cox regression model $p=0.002$), along with p53 protein accumulation ($p=0.049$) and patient's age ($=0.019$) (Table II).

Discussion

In parallel with our results, the inducible isoform of COX-2 is known to be absent in the normal urothelium (1), while it is overexpressed in UCs, possibly being involved in their pathogenesis (25). Our data indicate that COX-2 is expressed in 70% of human UCs, as detected by immunohistochemistry;

in the present study, 10% of immunoreactive cancer cells defined the COX-2 immunopositivity status. Using the same cut-off point, other investigators have reported a similar incidendence of COX-2-positive immunostaining among UCs (21), while others, using different cut-off points, reported a different incidence (16, 17). With regard to classical pathological prognosticators, in contrast to our negative findings, in other similar studies local invasion was correlated with COX-2 expression (16, 21) and enhanced immunolabelling of COX-2 has been observed in high-grade human UCs of the bladder by some researchers (2), but not by others (21, 26).

In the present study, and in contradiction to the attributed anti-apoptotic role of COX-2, COX-2 immunopositivity was found to be positively linked with the two pro-apoptotic factors, caspase-3 (CPP-32) and the bax protein.

Furthermore, COX-2 immunopositivity was positively correlated with the quantitative expression of MLH1. MLH1 is one of the two principal genes (*MSH2* and *MLH1*) of the human DNA mismatch repair (MMR) system, which is responsible for correcting DNA mismatches arising during replication (27). Inactivation of genes encoding the MMR system, and consequent defects in the proteins MLH1 and MSH2, result in a large increase in spontaneous mutability leading to the development of microsatellite instability and malignancy (28). In our cases, negative COX-2 staining was significantly more common in UCs with probably defective MMR, defined by the absence of MLH1 protein expression. MLH1-immunopositive tumors, which are not likely to be MMR-defective and thus have positive apoptotic potential, were found to overexpress COX-2, further suggesting an implication of COX-2 in apoptosis. Of course, the functional status of the rest of the MMR genes should be assessed in order for definitive conclusions to be reached. As far as gastrointestinal cancer is concerned, in parallel with our findings in UC, COX-2 appears to be significantly reduced in colorectal and gastric cancers with defective MMR (29-31).

Additionally, COX-2 immunoreactivity was found to be inversely correlated with the nuclear localization of the catalyst component of the telomerase complex, hTERT. Telomerase is a large ribonucleoprotein complex that stabilizes and extends telomeres of eukaryotic chromosomes, regulating cell replicative potential and lifespan (32). Human telomerase is composed of an RNA subunit and several protein components, including the catalyst telomerase reverse transcriptase (hTERT), which is considered to be the active component of the complex (33). Activation of telomerase has been associated with the immortalization of neoplastic cells, whereas its inhibition has been implicated in cellular senescence and apoptosis (34). hTERT, in particular, is considered to play an anti-apoptotic role, which has been shown in several cell types such as cardiac muscle cells (35), human embryonic kidney and endothelial cells (36) or vascular smooth muscle cells (37) and which is, at least partly, attributed to the interaction of nuclear hTERT with the DNA damage signal protein PARP and its participation in the maintenance of genome integrity (32). In other words, in our study and in accordance with the aforementioned positive association of COX-2 with indices of apoptotis potential (CPP32, bax protein and MLH1), COX-2 was inversely correlated with the anti-apoptotic nuclear hTERT, further supporting its positive relationship to apoptosis.

Given the anti-apoptotic role which is attributed to COX-2 overexpression (7, 8) and the enhancement of apoptosis that has been shown to be induced by NSAIDs (9), the prementioned relationship between COX-2 immuno-expression and indices of apoptotic potential seems controversial. However, studies have shown that agents such as NSAIDs (12), TGFβ (13), butyrate (14) and ceramide (15), at doses causing apoptosis, cause an increase in COX-2 protein expression, supporting that the induced expression of COX-2 is likely to be a response to an apoptotic stimulus. It is thus possible that, by up-regulating COX-2, cancer cells are resisting apoptosis. In support of that notion, DNA damage in colonic cells, with the subsequent induction of the caspases' apoptotic pathway, has been shown to enhance COX-2 promoter activity (38).

Another interesting finding of our study was the independent adverse influence of COX-2 positivity on overall survival of patients with Ta-T1 UCs, most of which were grades II and III. To date, the prognostic significance of COX-2 expression in cancer has been most thoroughly studied in colon cancer, where increased COX-2 expression has been correlated with reduced patient survival (39). In UC, COX-2 was generally not found to predict recurrence in pTa-T1 UCs (19) but, with Kaplan-Meier analysis, COX-2 expression was a significant predictor of recurrence (and disease progression) in patients with stage T1 grade 3 UCs (17), being partially in agreement with our finding that COX-2 expression may have an adverse prognostic value in patients with Ta-T1 UC, grades II and III, in terms of overall survival. This finding may, in part, be justified by the fact that COX-2 immunoreactivity preserves its positive correlation with both MLH1 and nuclear hTERT expressions in the Ta-T1 subgroup. Indeed, COX-2 overexpression, being a possible indication of successful cellular resistance to apoptotic stimuli, could exert an unfavorable influence on the survival of patients with pTa-pT1 UCs.

In conclusion, COX-2 overexpression in our series of UCs may be secondary to mutagenesis and the subsequent activation of an apoptotic process, offering a successful resistance to the latter. Furthermore, COX-2 protein expression seems to exert an adverse prognostic impact on the overall survival of patients with Ta-T1 UCs.

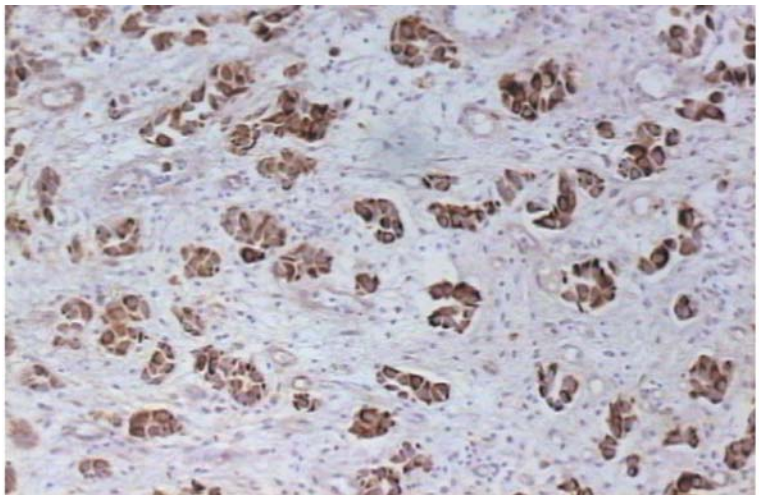

Figure 1. *Intense immunoexpression of COX-2 protein in the cytoplasm of the malignant cells of an invasive bladder urothelial carcinoma (ABC/HRPx200).*

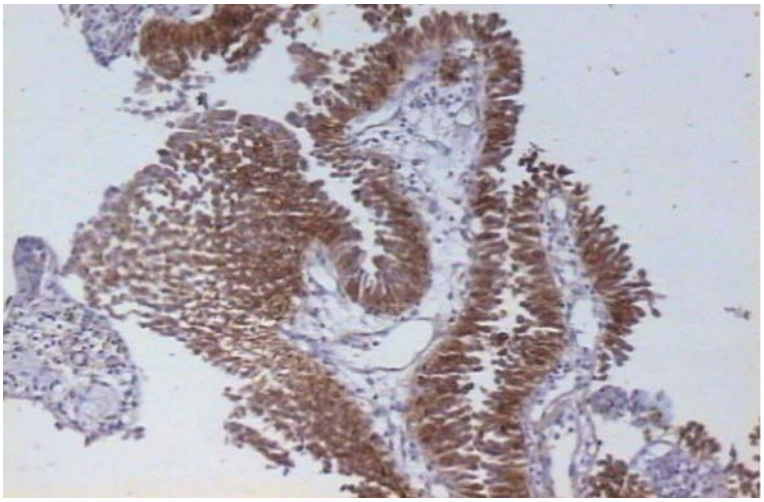

Figure 2. *COX-2 immunopositivity in a papillary urothelial carcinoma of the bladder (ABC/HRPx100).*

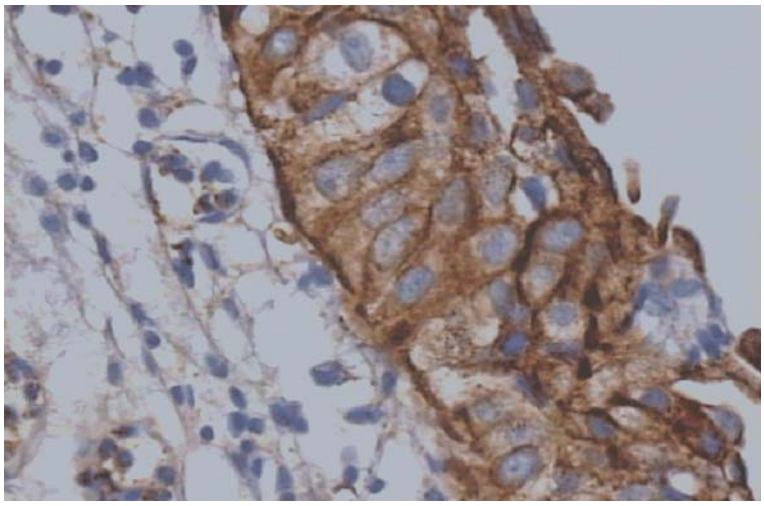

Figure 3. *In situ bladder UC: cytoplasmic COX-2 protein expression with submembranous intensification in some malignant cells (ABC/HRP x400).*

Table I. *Relation between COX-2 expression and clinicopathological characteristics in 134 patients with UCs of the urinary bladder.*

Characteristic	Positive expression of COX-2			
	No. of patients	no.	%	*P*
Total no.	134	94	70.1	
Gender				0.552
Male	119	82	68.9	
Female	15	12	80.0	
Histological grade				0.164
1	29	18	62.1	
2	42	34	81.0	
3	63	42	66.7	
Local invasion				0.975
pTa-T1	79	56	70.9	
pT2-T4	55	38	69.1	
p53 accumulation				0.578
Negative (<10% of cancer cells)	59	44	74.6	
Positive (≥10% of cancer cells)	70	48	68.6	
CPP32 expression				**0.007**
Negative (<20% of cancer cells)	57	35	61.4	
Positive (≥20% of cancer cells)	24	22	91.7	
bax expression				0.775
Negative (<20% of cancer cells)	40	28	70.0	
Positive (≥20% of cancer cells)	33	25	75.8	
bcl-2 expression				
Negative (≤10% of cancer-cells)	69	52	75.4	0.466
Positive (>10% of cancer-cells)	56	38	67.9	
Nuclear hTERT expression				**0.009**
Negative (≤10% of cancer cells)	48	42	87.5	
Positive (>10% of cancer cells)	67	44	65.7	
Cytoplasmic hTERT expression				0.347
Negative (≤10% of cancer cells)	60	43	71.7	
Positive (>10% of cancer cells)	55	43	78.2	

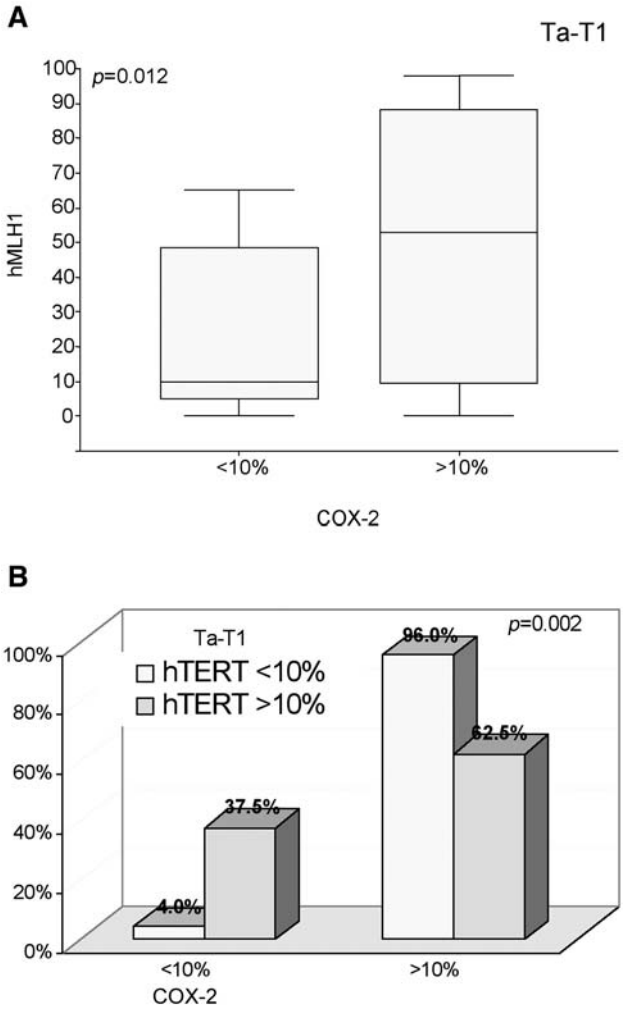

Figure 4. *Schematic representation of COX-2 immunopositivity in relation to MLH1 (A) and nuclear hTERT (B) expression in the Ta-T1 subgroup of patients.*

Table II. *Contribution of parameters of statistical significance to patients' overall survival through stepwise forward Cox's proportional hazards regression model adjusted for the following parameters: age, grade, stage, p53, bcl-2, CPP32, MLH1 and nuclear hTERT.*

							95% CI for HR	
		B	SE	df	*p*	HR	Lower	Upper
Ta-T1	COX-2	0.060	0.019	1	0.002	1.062	1.023	1.102
	p53	1.610	0.817	1	0.049	5.001	1.009	24.782
	age	0.207	0.088	1	0.019	1.229	1.035	1.460
T2-T3-T4	age	0.053	0.023	1	0.023	1.055	1.008	1.104

B, regression coefficient; SE, standard error; df, degree of freedom;HR, hazard ratio; CI, confidence interval.

References

1 Eschwege P, Ferlicot S, Droupy S, Ba N, Conti M, Loric S, Coindard G, Denis I, Ferretti L, Cornelius A, Legrand A, Bedossa P, Benoit G, Jardin A and Scardino P: A histopathologic investigation of PGE2 pathways as predictors of proliferation and invasion in urothelial carcinomas of the bladder. Eur Urol *44*: 435-441, 2003.

2 Kömhoff M, Guan Y, Shappell HW, Davis L, Jack G, Shyr Y, Koch MO, Shappell SB and Breyer MD: Enhanced expression of cyclooxygenase-2 in high grade human transitional cell bladder carcinomas. Am J Pathol *157*: 29-35.

3 Shirahama T and Sakakura C: Overexpression of cyclooxygenase-2 in squamous cell carcinoma of the urinary bladder. Clin Cancer Res *7*: 558-561, 2001.

4 Borzacchiello G, Ambrosio V, Galati P, Perillo A and Roperto F: Cyclooxygenase-1 and -2 expression in urothelial carcinomas of the urinary bladder in cows. Vet Pathol *40*: 455-459, 2003.

5 Subbaramaiah K and Dannenberg AJ: Cyclooxygenase 2: a molecular target for cancer prevention and treatment. Trends Pharmacol Sci *24*: 96-102, 2003.

6 Brown JM and Attardi LD: The role of apoptosis in cancer development and treatment response. Nat Rev Cancer *5(3)*: 231-237, 2005.

7 Sheng H, Shao J, Morrow JD, Beauchamp RD and DuBois RN: Modulation of apoptosis and bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res *58*: 362-366, 1998a.

8 Cao Y, Pearman AT, Zimmerman GA, McIntyre TM and Prescott SM: Intracellular unesterified arachidonic acid signals apoptosis. PNAS *97*: 11280-11285, 2000.

9 Husain SS, Szabo IL and Tarnawski AS: NSAID inhibition of GI cancer growth: clinical implications and molecular mechanisms of action. Am J Gastroenterol *97*: 542-553, 2002.

10 Zhang X, Morham SG, Langenbach R and Young DA: Malignant transformation and antineoplastic actions of nonsteroidal antiflammatory drugs (NSAIDs) on cyclooxygenase-hull embryo fibroblasts. J Exp Med *190(3)*: 451-459, 1999.

11 Lim JT, Piazza GA, Han EK, Delohery TM *et al*: Sulindac derivatives inhibit growth and induce apoptosis in human prostate cancer cell lines. Biochem Pharmacol *58*: 1097-1107, 1999b.

12 Elder D, Halton DE, Playle L and Paraskeva Ch: The MEK/ERK pathway mediates COX-2 selective NSAID-induced apoptosis and induced COX-2 protein expression in colorectal carcinoma cells. Int J Cancer *99*: 323-327, 2002.

13 Sheng H, Shao J, O'Mahony CA, Lamps L *et al*: Transformation of intestinal epithelial cells by chronic TGF-β1 treatment results in downregulation of the type II TGF-β receptor and induction of cyclooxygenase-2. Oncogene *18*: 855-867, 1999.

14 Crew TE, Elder DJE and Paraskeva Ch: A cyclooxygenase-2 (COX-2) selective non-steroidal anti-inflammatory drug enhances the growth inhibitory effect of butyrate in colorectal carcinoma cells expressing COX-2 protein: regulation of COX-2 by butyrate. Carcinogenesis *21*: 69-77, 2000.

15 Subbaramaiah K, Chung WJ and Dannenberg AJ: Ceramide regulates the transcription of cyclooxygenase-2. J Biol Chem *273*: 32943-32949, 1998.

16 Shirahama T, Arima J-I, Akiba S and Sakakura C: Relation between cyclooxygenase-2 expression and tumor invasiveness and patient survival in transitional cell carcinoma of the urinary bladder. Cancer *92*: 188-193, 2001.

17 Sun Il Kim, Soo Mee Kwon, Young Sig Kim and Sung Joon Hong: Association of cyclooxygenase-2 expression with prognosis of stage T1 grade 3 bladder cancer. Urology *60*: 816-821, 2002.

18 Garavito RM, Malkowski MG and DeWitt DL: The structures of prostaglandin endoperoxide H synthases-1 and -2. Prostaglandins Other Lipid Mediat *68-69*: 129-152, 2002.

19 Frieddrich MG, Toma MI, Petri S and Huland H: Cyclooxygenase-2 promotes angiogenesis in pTa/T1 urothelial bladder carcinoma but does not predict recurrence. BJU Int *92*: 389-392, 2003.

20 Eble JN, Sauter G, Epstein JI and Sesterhenn IA (eds.): World Health Organization. Classification of Tumors. Pathology and Genetics. Tumors of the Urinary System and Male Genital Organs. IARC Press, Lyon, 2004.

21 Ristimäki A, Nieminen O, Saukkonen K, Hotakainen K, Nordling S and Haglund C: Expression of cyclooxygenase-2 in human transitional cell carcinoma of the urinary bladder. Am J Pathol *158*: 849-853, 2001.

22 Giannopoulou I, Nakopoulou L, Zervas A, Lazaris AC, Stravodimos K, Giannopoulos A and Davaris P: Immunohistochemical study of pro-apoptotic factors Bax, Fas and CPP32 in urinary bladder cancer: prognostic implications. Urol Res *30*: 342-345, 2002.

23 Nakopoulou L, Vourlakou C, Zervas A, Tzonou A, Gakiopoulou H and Dimopoulos M-A: The prevalence of bcl-2, p53 and Ki67 immunoreactivity in transitional cell bladder carcinomas and their clinicopathologic correlates. Hum Pathol *29*: 146-154, 1998.

24 Mavrommatis J, Mylona E, Gakiopoulou H, Stravodimos C, Zervas A, Giannopoulos A and Nakopoulou L: Nuclear hTERT immunohistochemical expression is associated with survival of patients with urothelial bladder cancer. Anticancer Res *25*: 3109-3111, 2005.

25 Mohseni H, Zaslau S, McFadden D, Riggs DR, Jackson BJ and Kandzari S: COX-2 inhibition demonstrates potent anti-proliferative effects on bladder cancer *in vitro*. J Surg Res *119*: 138-142, 2004.

26 Mohammed SI, Knapp DW, Bostwick DG, Foster RS *et al*: Expression of cyclooxygenase-2 (COX-2) in human invasive transitional cell carcinoma (TCC) of the urinary bladder. Cancer Res *59*: 5647-5650, 1999.

27 Stone JG, Robertson D and Huolston RS: Immunohistochemistry for MSH2 and MLH1: a method for identifying mismatch repair deficient colorectal cancer. J Clin Pathol *54*: 484-48, 2001.

28 Bonnal Ch, Ravery V, Toublanc M, Bertrand G, Baccon-Gibon L, Henin D and Grandchamp B: Absence of microsatellite instability in transitional cell carcinoma of the bladder. Urology *55(2)*: 287-290, 2000.

29 Karnes WE Jr: Implications of low COX-2 expression in colorectal neoplasm with defective DNA mismatch repair. J Cell Biochem Suppl *34*: 23-27, 2000.

30 Yamamoto H, Itoh F, Fukushima H, Hinoda Y and Imai K: Overexpression of cyclooxygenase-2 protein is less frequent in gastric cancers with microsatellite instability. Int J Cancer *84(4)*: 400-403, 1999.

31 Karnes WE Jr, Shattuck-Brandt R, Burgart LJ, DuBois RN, Tester I, Cunningham JM, Kim CY, McDonnell SK, Schaid DJ and Thibodeau SM: Reduced COX-2 protein in colorectal cancer with defective mismatch repair. Cancer Res *58(23)*: 5473-5477, 1998.

32 Cao Y, Li H, Deb S and Liu J-P: TERT regulates cell survival independent of telomerase enzymatic activity. Oncogene *21*: 3130-3138, 2002.
33 Harrington L, McPahil T, Marc V, Zhou W *et al*: A mammalian telomerase-associated protein. Science (Washington DC) *275*: 973-977, 1997.
34 Kim NW, Piatyszek MA, Prowse KP, Harley CB *et al*: Specific association of human telomerase activity with immortal cells and cancer. Science *266*: 2011-2019, 1994.
35 Oh H, Taffet GE, Vouker KA, Weytman HL, Overbeck PA *et al*: Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy and survival. Proc Natl Acad Sci *98*: 10308-10313, 2001.
36 Haendeler J, Hoffmann J, Rahman S, Zeiher A and Dimmeler S: Regulation of telomerase activity and anti-apoptotic function by protein-protein interaction and phosphorylation. FEBS Lett *536(1-3)*: 180-186, 2003.
37 Cao Y, Li H, Mu F-T, Ebisui O, Funder JW and Liu JP: Telomerase activation causes vascular smooth muscle cell proliferation in genetic hypertension. FASEB J *16*: 96-98, 2002.
38 Glinghammar B, Inoue H and Rafter JJ: Deoxycholic acid causes DNA damage in colonic cells with subsequent induction of caspases, COX-2 promoter activity and the transcription factors NF-kB and AP-1. Carcinogenesis *23(5)*: 839-845, 2002.
39 Sheehan KM, Sheahan K, O'Donoghue DP *et al*: The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA *282*: 1254-1257.

Received June 22, 2005
Accepted August 26, 2005