This study investigates the apoptotic activity of the cyclooxygenase-2 (COX-2) inhibitor celecoxib in prostate carcinoma cells. COX-2 is constitutively expressed in androgen-responsive LNCaP and androgen-nonresponsive PC-3 cells. Exposure of these cells to celecoxib induces characteristic features of apoptosis, including morphological changes, DNA laddering, and caspase-3 activation, whereas piroxicam, a COX-1-specific inhibitor, displays no appreciable effect on either cancer cell line even after prolonged exposure. Moreover, the potency of celecoxib in apoptosis induction is significantly higher than that of other COX-2 inhibitors examined despite the observation that these inhibitors exhibit similar IC50 in COX-2 inhibition. It is noteworthy that normal human prostate epithelial cells, expressing a marginally detectable level of COX-2, are insensitive to the induction of apoptosis by celecoxib. These data suggest a correlation between COX-2 expression and sensitivity to the apoptotic effect of the COX-2 inhibitor. In an effort to delineate the underlying mechanism, we examined the effect of celecoxib on the expression of Bel-2 as well as the activation of the key anti-apoptotic kinase Akt. In contrast to an earlier report that attributed the apoptotic activity of NS398 in LNCaP cells to Bel-2 down-regulation, we provide evidence that the induction of apoptosis by celecoxib in LNCaP and PC-3 cells is independent of Bel-2. First, treatment with celecoxib does not alter the cellular Bel-2 level in both cell lines. Second, enforced Bel-2 expression in PC-3 cells does not confer protection against the induction of apoptosis by celecoxib. Our data show that celecoxib treatment blocks the phosphorylation of Akt. This correlation is supported by studies showing that overexpression of constitutively active Akt protects PC-3 cells from celecoxib-induced apoptosis. Nevertheless, how celecoxib down-regulates Akt is not clear because the drug does not adversely affect phosphoinositide 3-kinase activity in vivo and okadaic acid, a protein phosphatase 2A inhibitor, cannot rescue the inhibition. In summary, our data demonstrate that inhibition of Akt activation may play a crucial role in the induction of apoptosis by celecoxib.

An expanding body of information has suggested the potential application of nonsteroidal anti-inflammatory drugs (NSAIDs)1 in cancer chemoprevention (1–3). Epidemiological studies indicate that the use of aspirin and other NSAIDs reduces the risk of colorectal cancer by 40–50% (4–7) and, to a lesser extent, the risk of breast cancer (6, 8). The fact that all NSAIDs in clinical use are cyclooxygenase (COX) inhibitors provides a putative link between the inhibition of COX activity and the chemopreventive effect of NSAIDs (1, 2, 9). Two isoforms of COX have been identified, each of which displays a distinct physiological profile. COX-1, constitutively expressed in almost all tissues, is important for the maintenance of homeostatic function, whereas COX-2, an inducible isozyme, is dramatically up-regulated during pathological conditions such as inflammation and cancers (10, 11). The COX-2 gene, an immediate-early response gene, is rapidly induced in response to tumor promoters, cytokines, and growth factors (10). Moreover, COX-2 expression is regulated by oncogenes and p53, in a positive and negative manner, respectively (12–14). The involvement of p53 in the regulation COX-2 expression may help us understand why COX-2 is up-regulated in various forms of cancer that contain mutant p53 (13, 15–19). The premise that COX-2 is involved in the pathologic processes of cancer growth and progression is further supported by animal studies showing that tumorigenesis is inhibited in COX-2 knockout mice (20, 21).

Evidence suggests that increase in tumorigenic potential by COX-2 overexpression is associated with resistance to apoptosis (22). Selective inhibitors of COX-2 have been demonstrated to induce apoptosis in a variety of cancer cells, including those of colon (23–25), stomach (26), prostate (27), and breast. These observations are consistent with the COX-2 inhibitor being a chemopreventive agent that increases the susceptibility of cancer cells to apoptosis. The biochemical mechanism underlying COX-2 inhibitor-induced apoptosis, however, remains elusive. At least three distinct mechanisms have been proposed. First, a recent study shows that NSAID treatment of colon tumor cells resulted in an increase in arachidonic acid that, in turn, stimulated the production of ceramide, a known death signal, by activating neutral sphingomyelinase (28). Second, the COX-2 inhibitors SC-58125 and NS398 have been reported to sensitize colon and prostate cancer cells, respectively, to apoptosis by down-regulating the anti-apoptotic protein Bel-2 (24, 27). Third, studies using sulindac sulfone, a sulindac metabo-

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1 The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase; PI 3-kinase, phosphoinositide 3-kinase; MyrAkt, myristoylated Akt; PI (3,4,5)P3, phosphatidylinositol 3,4,5-trisphosphate; HA, hemagglutinin; mAb, monoclonal antibody; SFFV, spleen focus-forming virus; TBS, Tris-buffered saline.
lute that lacks the ability to inhibit COX-2, suggest the involvement of a COX-2-independent pathway during the apoptosis signaling (29-31). Here, we propose a unique mechanism for the apoptotic effect of celecoxib in human prostate carcinoma cells. Our data suggest that celecoxib induces apoptosis, in part, by blocking the activation of the anti-apoptotic kinase Akt (also called protein kinase B). This mechanism is noteworthy because celecoxib displays a significantly higher potency in apoptosis induction than other COX-2 inhibitors examined. Moreover, in contrast to the previous reports that correlated the apoptotic effect of COX-2 inhibitors with Bel-2 down-regulation (24, 27), we raise evidence that the induction of apoptosis by celecoxib is independent of Bel-2.

MATERIALS AND METHODS

Celecoxib and rofecoxib were prepared from commercial Celebrex® and Vioxx®, capsules, respectively, by solvent extraction followed by recrystallization. The identity and purity of both molecules were verified by NMR and mass spectrometry. DuP697, a selective COX-2 inhibitor, and rabbit anti-COX-2 antibodies were a kind gift from Professor Hsin-Hsiung Tai (University of Kentucky), and piroxicam was purchased from Sigma. NS398 was obtained from Calbiochem. Rabbit polyclonal antibodies against Akt and P.477/Ser Akt were purchased from Novus Biologicals. Mouse monoclonal antibodies (mAbs) against Bel-2 and Tyr(P) were obtained from Transduction Laboratories, mAb against the p85 subunit of PI 3-kinase a was purchased from Upstate Biotechnology, and anti-actin mAb was from Amersham Pharmacia Biotech or ICN. Goat anti-rabbit IgG-horseradish peroxidase conjugates were purchased from Jackson ImmunoResearch. Anti-mouse IgG-biotin conjugates and avidin-horseradish peroxidase conjugates were products from Pierce.

Cell Culture—Normal human prostate epithelial cells were purchased from Clonetics. To assess the potential impact of androgen sensitivity on celecoxib-induced cell death, both androgen-responsive LNCaP (p53+/+)

and androgen-nonresponsive PC-3 (p53−/−) human prostate cancer cell lines were tested. Whereas LNCaP cells exhibit androgen sensitivity, PC-3 cells do not have androgen receptors and are hormone-insensitive (32). The PC-3 transfectant PC-3/Bel-2 that overexpressed human Bel-2 from a constitutive spleen focus-forming virus (SFFV)-promoter was prepared as described previously (33). Vector SFFV-Neo and human Bel-2 expression construct pSFFV-Neo/Bel-2-Flag were gifts from Gabriel Nunez (University of Michigan, Ann Arbor, MI). The transfectants PC-3/Bel-2 cells were highly resistant to apoptosis induced by either TNF-α or Par-4 overexpression, indicating that the overexpressed Bel-2 protein was functional (33). Prostate cancer cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37 °C in a humidified 5% CO2 incubator. Cells were replenished daily with a new medium and were passaged 1:4 with fresh medium every 3 days.

Transient Transfection—The expression construct pcCMV6.Myr.Akt.HA containing a myristoylation sequence from p60src (34) was a gift from Professor Alex Toker (Harvard Medical School). For transfection, PC-3 cells were seeded into 60-mm tissue culture dishes with 25% confluence and grown for 24 h to 50–60% confluence. Each dish was washed with 4 ml of serum-free Opti-MEM (Life Technologies), and 2 ml of the same medium was added. Aliquots containing 0.2 μg of the pcCMV6.Myr.Akt.HA expression vector or a control pcCMV/Blue plasmid in 100 μl of serum-free Opti-MEM were preincubated with 8 μl of the Plus reagent from the LipofectAMINE Plus reagent kit at 25 °C for 15 min, and 8 μl of the LipofectAMINE reagent in 100 μl of Opti-MEM was added to the mixture. The mixture was incubated at 25 °C for 15 min and added to each dish with gentle mixing. After 5 h at 37 °C, the transfection media were replaced with 2 ml of 10% fetal bovine serum-supplemented RPMI 1640 medium. The transfected cells were collected at different time intervals for Western blot analysis of myristoylated Akt (MyrkAtk) expression.

Cell Viability and DNA Fragmentation Analysis—Prostate cancer cells were grown in 10% fetal bovine serum-supplemented RPMI 1640 medium for 48 h and were exposed to various concentrations of celecoxib or DuP697 dissolve in MeSO (final concentration, 0.1%) in serum-free RPMI medium for different time intervals. Controls received MeSO vehicle at a concentration equal to that in celecoxib-treated cultures. During the treatment, the percentage of cells floating in the medium increased over time. Adherent cells were harvested by trypsinization, and floating cells were recovered by centrifugation at 3,200 × g for 5 min. Cell morphology was observed with a light microscope at x200. Both adherent and floating cells were combined for the assessment of cell viability. Cell viability was determined by trypan blue exclusion. DNA was extracted by the Easy DNA kit (Invitrogen) according to the manufacturer’s instructions and electrophoresed in a 2% agarose gel, and visualized by ethidium bromide staining.

Immunoblotting—The general procedure for the Western blot analysis of COX-2, Bel-2, Akt, phospho-Akt, and actin was as follows. Cells were washed in phosphate-buffered saline, resuspended in SDS gel-loading buffer, sonicated by an ultrasonic sonicator for 5 s, and boiled for 5 min. After a brief centrifugation, equivalent protein concentrations from the soluble fractions were resolved in 10% (COX-2, Akt, P.477/Ser Akt, and actin) or 12% (Bel-2) SDS-polyacrylamide gels on a Minigel apparatus (Bio-Rad) and transferred to a nitrocellulose membrane using a semidry transfer cell (Bio-Rad). The transblotted membrane was washed twice with TBS containing 0.05% Tween 20 (TBST). After blocking with TBS containing 3% low fat milk for 30–60 min, the membrane was incubated with the primary antibody at the appropriate dilution (rabbit anti-Akt and anti-P.477/Ser Akt antibodies, 1:1000; anti-COX-2 antibodies, 1:5000; anti-Bel-2 mAb, 1:500; anti-actin mAb, 1:1000) in TBS-1% low fat milk at 4 °C for 12 h and washed twice with TBST. For Akt, phospho-Akt, and COX-2 transblots, the membrane was probed with goat anti-rabbit IgG-horseradish peroxidase conjugates (1:5000) for 1 h at room temperature and washed twice with TBST. With regard to Bel-2, and actin analyses, the membranes were first incubated with goat anti-mouse IgG-biotin conjugates for 1 h at room temperature, washed twice with TBST, incubated with avidin-horseradish peroxidase conjugates (1:500) for 30 min at room temperature, and washed twice with TBST. The immunoblots were visualized by enhanced chemiluminescence.

In Vitro PI 3-Kinase Assay—In vitro phosphorylation of phosphatidylinositol was carried out with anti-Tyr(P) or anti-p85-immunoprecipitated PI 3-kinase. Prostate cancer cells, with or without the COX-2 inhibitor treatment, were treated with 1% Nonidet P-40 in phosphate-buffered saline containing 1 mM dithiothreitol, 100 μM 4-(2-aminoethoxy)benzene-sulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin (lysis buffer), and soluble cell lysates were collected after centrifugation at 1500 × g for 5 min. Anti-p85 or anti-Tyr(P) mAb (5 μg) was added to 500 μl of the cell lysate, the protein concentration of which was adjusted to 0.8–1 μg/ml with phosphate-buffered saline. The mixture was incubated with gentle rocking at 4 °C for 12 h, 10 μg of protein A-Sepharose (Amersham Pharmacia Biotech) were added, and the incubation was continued for another 2 h. The immunoprecipitates were washed, in detergent-containing phosphate-buffered saline containing 0.1 M Tris/HCl, pH 7.5, containing 0.5 mM LiCl, and 10 μM sodium vanadate, and twice with 10 mM Tris/HCl, pH 7.5, containing 100 mM NaCl, 10 μM sodium vanadate, and 1 mM EDTA. Adequate amounts of the washed antibody conjugates, in 10 μl, were added to 80 μl of 30 mM Heps, pH 7.5, containing 125 mM ATP, 10 μCi of [γ-32P]ATP, and 6.25 mM MgCl2, and the reaction was initiated by adding 20 μg of bovine brain extract (Type I: Sigma) suspended in 10 μl of 30 mM Heps, pH 7.5. After 10 min at 37 °C, the reaction was terminated by adding 5 μl of 1 M EDTA and 25 μl of 5 M HCl followed by 160 μl of chloroform: methanol (1:1; v/v). Samples were centrifuged at 6,000 × g for 5 min, and the lower organic phase was removed, applied to 1% oxalic acid-treated TLC plates, and then developed with n-propanol:2 M acetic acid (65:35) overnight. After drying, spots were located by autoradiography and compared with standards. The autoradiograms were scanned by a Photodyne image system and quantified using the NIH Image program (version 1.59).

RESULTS

LNCaP and PC-3 Cells Constitutively Express COX-2 and Are Susceptible to the Induction of Apoptosis by the COX-2 Inhibitor Celecoxib—As evidence indicates that COX-2 is up-regulated in many cancer cell lines including prostate carcinoma (35), we compared the expression of COX-2 in androgen-responsive LNCaP and androgen-nonresponsive PC-3 cell lines to its normal human prostate epithelial cells by Western blot analysis. Fig. 1A reveals that the level of COX-2 expression in LNCaP cells and PC-3 cells was at least an order of magnitude higher than that of normal prostate epithelial cells. Between the two cancer cell lines, LNCaP cells displayed a higher COX-2 level than PC-3 cell, in a ratio of approximately 2 to 1. The effect of the COX-2 inhibitor celecoxib on the cell viability...
was examined. Fig. 1B depicts the dose- and time-dependent effect of celecoxib on the viability of LNCaP and PC-3 cells in serum-starved RPMI, indicating that celecoxib-induced cell death was independent of androgen sensitivity or p53 functional status. Virtually all cells died after exposure to 50 μM celecoxib for 3–4 h. However, other COX-2 inhibitors examined, such as DuP697, NS398, and rofecoxib, displayed a substantially reduced efficacy in apoptosis induction. For these COX-2 inhibitors, more than 36 h was required to achieve 50% cell death even at 100 μM. The mechanism underlying this discrepancy warrants investigation in view of the potential chemotherapeutic effect of COX-2 inhibitors. It is noteworthy that normal prostate epithelial cells were insensitive to celecoxib-induced apoptosis, suggesting a correlation between COX-2 expression and susceptibility to the induction of apoptosis by celecoxib.

Similar results were obtained when the incubation was carried out in serum-free Opti-MEM. Moreover, supplement of 10% fetal bovine serum to RPMI medium did not abrogate the growth inhibition effect, but it prolonged the time course required for complete cell death by almost an order of magnitude (data not shown). These data indicate that celecoxib-induced apoptosis may be mediated by growth factors. Light microscopic examination of the celecoxib-treated cells revealed pronounced morphological changes. Fig. 2A shows the morphology of untreated LNCaP cells (left panel) and LNCaP cells treated with 50 μM celecoxib for 1 h (right panel). The cells became shrunken, round, and detached from the dish. Analysis of DNA from celecoxib-treated LNCaP cells showed the dose- and time-dependent generation of nucleosomal-sized ladders of DNA fragments (Fig. 2B). In addition, because cells undergoing apoptosis execute the death program by activating caspases (36), we analyzed whether treatment with celecoxib gave rise to the activation of caspase-3, a key executioner of apoptosis (37). The Western blot analysis shown in Fig. 2C analyzed the time course of caspase-3 activation following celecoxib (50 μM) treatment, in which the enzymatically inactive 32-kDa precursor was converted to the active 17-kDa proteolytic cleavage product. As shown, exposure to celecoxib led to a precipitous disappearance of the 32-kDa precursor, accompanied by a concomitant increase in the 17-kDa fragment. The kinetics of the caspase-3 activation was consistent with that of the DNA fragmentation. Nearly all 35-kDa precursor disappeared 1 h posttreatment. These characteristic features of apoptosis were also noted in celecoxib-treated PC-3 cells. We further examined the apoptotic activity of piroxicam, an NSAID with a relative COX-1 to COX-2 specificity ratio of 250. However, piroxicam did not display appreciable apoptotic effect on either cell line over an extended period of time (data not shown). These data imply that the induction of apoptosis in prostate cancer cells by NSAIDs is a COX-2 inhibitor-specific event.

Treatment with Celecoxib Does Not Affect Bcl-2 Expression—In light of the recent reports that attributed COX-2 inhibitor-induced apoptosis to Bcl-2 down-regulation (24, 27), we investigated whether Bcl-2 expression was affected in LNCaP and PC-3 cells after celecoxib treatment. Western blot analysis reveals that the Bcl-2 level in LNCaP and PC-3 cells remained virtually unchanged (after being normalized to actin) through-

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3 C.-S. Chen, unpublished data.
To confirm the premise posttreatment. Only negligible amounts of phospho-Akt remained undetectable at 4 h. Relatively, PC-3 and PC-3/Bcl-2 cells treatment, it decreased precipitously at 2 h and became virtually no significant change within the 4-h period. In LNCaP cells, phospho-Akt in all three cell lines, whereas the control showed a dramatic impact of celecoxib treatment on the level of ceramide and the PLA2 inhibitor AACOCF3,4 suggesting that celecoxib-induced apoptosis was mediated through the inhibition of Akt activation, an activated form of Akt with the Src myristoylation signal fused in-frame to the c-Akt coding sequence (MyrAkt) (34) was introduced into PC-3 cells by transient transfection. The expression of MyrAkt in transiently transfected PC-3 cells was verified by Western analysis, which displayed a reduced mobility relative to Akt due to the HA epitope tag. Fig. 6A shows the expression of MyrAkt in the PC-3 transient transfectants in a time-dependent manner, which peaked at 48 h after transfection, whereas endogenous Akt remained unaltered throughout that period. Accordingly, the MyrAkt-overexpressing PC-3 cells were collected at 48 h and tested for susceptibility to the induction of apoptosis by 50 μM celecoxib in serum-free Opti-MEM. As shown in Fig. 6B, PC-3 cells transiently transfected with the MyrAkt vector showed a 42% reduction in celecoxib-induced cell death (curve b) as compared with the transfectants with an empty pCMV vector (curve c). Taken together, these results suggest a crucial role of Akt in celecoxib-induced apoptosis.

**Decrease in Phospho-Akt Is Not Caused by the Attenuation of PI 3-Kinase Activity in Vivo**—Because Akt represents one of the major downstream effectors of PI 3-kinase, PI 3-kinase activity, both in vitro and in vivo, was examined after celecoxib treatment. The rationale for the in vitro study was that endogenous molecules such as ceramide, cytidine diphospho-diacylglycerol, and phosphatidate are known to inhibit PI 3-kinase by interfering with substrate interaction by PI 3-kinase. Our data indicate that celecoxib did not display any appreciable inhibition on PI 3-kinase, which was immunoprecipitated with anti-p85 mAb from the lysates of LNCaP cells (data not shown). To evaluate the in vivo effect of celecoxib, LNCaP cells were exposed to 50 μM celecoxib in serum-free Opti-MEM for 0–3 h, lysed, and treated with anti-Tyr(P) mAb. PI 3-kinase activity in the immunoprecipitates was assayed using bovine brain extract as substrate, which contained a mixture of PI, phosphatidylinositol 4-phosphate, and phosphatidylglycerol 4,5-biphosphate. Again, celecoxib did not exert an appreciable effect on the PI 3-kinase activity of the immune complex prepared with anti-Tyr(P) antibody (Fig. 7A). The activity remained virtually unchanged.

![Figure 3](image3.png)

**Fig. 3.** Celecoxib does not affect Bcl-2 expression in LNCaP and PC-3 cells. Cells were treated with 50 μM celecoxib in serum-starved RPMI 1640 medium for the indicated time and lysed, and the supernatants were electrophoresed and probed by Western blot with anti-Bcl-2 mAb, followed by anti-actin antibodies. The bar graphs indicate the relative amounts of Bcl-2 after being normalized to the respective actin level.

![Figure 4](image4.png)

**Fig. 4.** Ectopic Bcl-2 expression does not protect PC-3 cells from the induction of apoptosis by celecoxib. A, cellular proteins from PC-3/vector (lane 1) and PC-3/Bcl-2 (lane 2) cells were subjected to Western blot analysis with anti-Bcl-2 mAb, followed by anti-actin antibodies. The band for ectopic Bcl-2 is expected to contain the Flag tag (eight amino acids long) from the construct, and it migrated slightly slower than endogenous Bcl-2. B, dose- and time-dependent effect of celecoxib on the viability of PC-3/Bcl-2 cells (main panel) and PC-3/vector cells (inset) in serum-starved RPMI 1640 medium. No appreciable difference in cell viability was noted between celecoxib-treated PC-3/Bcl-2 and celecoxib-treated PC-3/vector.

that celecoxib-induced apoptosis was mediated through the inhibition of Akt activation, an activated form of Akt with the Src myristoylation signal fused in-frame to the c-Akt coding sequence (MyrAkt) (34) was introduced into PC-3 cells by transient transfection. The expression of MyrAkt in transiently transfected PC-3 cells was verified by Western analysis, which displayed a reduced mobility relative to Akt due to the HA epitope tag. Fig. 6A shows the expression of MyrAkt in the PC-3 transient transfectants in a time-dependent manner, which peaked at 48 h after transfection, whereas endogenous Akt remained unaltered throughout that period. Accordingly, the MyrAkt-overexpressing PC-3 cells were collected at 48 h and tested for susceptibility to the induction of apoptosis by 50 μM celecoxib in serum-free Opti-MEM. As shown in Fig. 6B, PC-3 cells transiently transfected with the MyrAkt vector showed a 42% reduction in celecoxib-induced cell death (curve b) as compared with the transfectants with an empty pCMV vector (curve c). Taken together, these results suggest a crucial role of Akt in celecoxib-induced apoptosis.

**Decrease in Phospho-Akt Is Not Caused by the Attenuation of PI 3-Kinase Activity in Vivo**—Because Akt represents one of the major downstream effectors of PI 3-kinase, PI 3-kinase activity, both in vitro and in vivo, was examined after celecoxib treatment. The rationale for the in vitro study was that endogenous molecules such as ceramide, cytidine diphospho-diacylglycerol, and phosphatidate are known to inhibit PI 3-kinase by interfering with substrate interaction by PI 3-kinase. Our data indicate that celecoxib did not display any appreciable inhibition on PI 3-kinase, which was immunoprecipitated with anti-p85 mAb from the lysates of LNCaP cells (data not shown). To evaluate the in vivo effect of celecoxib, LNCaP cells were exposed to 50 μM celecoxib in serum-free Opti-MEM for 0–3 h, lysed, and treated with anti-Tyr(P) mAb. PI 3-kinase activity in the immunoprecipitates was assayed using bovine brain extract as substrate, which contained a mixture of PI, phosphatidylinositol 4-phosphate, and phosphatidylglycerol 4,5-biphosphate. Again, celecoxib did not exert an appreciable effect on the PI 3-kinase activity of the immune complex prepared with anti-Tyr(P) antibody (Fig. 7A). The activity remained virtually unchanged.

In cells, phospho-Akt levels are regulated by a balance be-

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4 V. M. Rangnekar and C.-S. Chen, unpublished data.
between phosphorylation by phosphoinositide-dependent kinases and dephosphorylation by protein phosphatase 2A. Our data indicate that okadaic acid (100 nM), a potent protein phosphatase 2A inhibitor, could not reverse the effect of celecoxib on Akt (data not shown). Nor could okadaic acid rescue LNCaP and PC-3 cells from celecoxib-induced apoptosis. This finding suggests that the reduction in phospho-Akt levels was not related to enhanced dephosphorylation by protein phosphatase 2A-like phosphatase.

**DISCUSSION**

The therapeutic application of NSAIDs in cancer chemoprevention has been the focus of many recent investigations. Although the signaling mechanism responsible for the apoptotic activity of NSAIDs remains unclear, this unique pathway appears to be a COX-2 inhibitor-specific event. Despite the observation that LNCaP and PC-3 cells express COX-2 at different levels, their susceptibility to the apoptotic effect of celecoxib is
similar to each other. Considering the molecular basis for COX-2 inhibitor-induced apoptosis, it has been proposed that it might due to the stimulation of ceramide production (28) or the down-regulation of Bcl-2 expression (24, 27). The present data provide a novel mechanism for celecoxib: it selectively blocks the activation of Akt, thereby attenuating the activity of a major anti-apoptotic pathway. Consequently, overexpression of constitutively active Akt protects prostate cancer cells from celecoxib-exerted apoptosis. Studies on whether this unique mechanism applies to other COX-2 inhibitors are under way because celecoxib displays an apoptotic activity at least an order of magnitude higher than that of other COX-2 inhibitors examined. Moreover, it remains unclear how celecoxib affects Akt phosphorylation because it does not adversely affect PI 3-kinase activity in vivo. The finding that okadaic acid, a potent protein phosphatase 2A inhibitor, could not reverse the effect of celecoxib on Akt suggests that the reduced phospho-Akt level might arise from decreased phosphorylation rather than enhanced dephosphorylation. Taken together, the action of celecoxib may entail intermediate regulators of Akt downstream of PI 3-kinase or involve pathways independent of PI 3 kinase. This premise is reminiscent of a recent report that the inhibitory effect of C2-ceramide on Akt activation is independent of PI 3-kinase activity (41).

The finding that apoptosis induction by celecoxib is not abrogated by enforced Bcl-2 expression in PC-3 cells connotes significant therapeutic implications. Bcl-2 overexpression has been correlated with the progression of prostate carcinoma (44–46). Up-regulation of this anti-apoptotic protein raises the apoptotic threshold, which increases apoptosis resistance in the face of cellular abnormalities. For example, a recent report indicates that ectopic expression of Bcl-2 can impair apoptosis signaling by most of the cell stresses that activate the ceramide/c-Jun NH2-terminal kinase pathway (47). Therefore, the independence of Bcl-2 function suggests the potential use of celecoxib and/or other COX-2 inhibitors in the treatment of metastatic prostate cancer.

In summary, the present study proposes a new mechanism to account for the apoptotic activity of COX-2 inhibitors. However, it remains in dispute whether the apoptotic effect of COX-2 inhibitors is related to the prostanoid-mediated function of COX-2. Our data indicate that normal prostate epithelial cells do not express significant levels of COX-2 and that they are not susceptible to the induction of apoptosis by celecoxib. This correlation argues for the dependence of the apoptotic activity on COX-2 inhibition. Nevertheless, several lines of evidence suggest that the ability of COX-2 inhibitors to promote apoptosis may be dissociated from the enzyme activity of COX-2. First, there exists a discrepancy by nearly three orders of magnitude between the concentration of celecoxib and other COX-2 inhibitors needed to inhibit COX-2 activity and that required to induce apoptosis. This discrepancy raises a possibility that celecoxib mediates apoptosis through a target other than COX-2. Second, our data indicate that different COX-2 inhibitors with similar IC50 values in COX-2 inhibition display different degree of efficacy in the induction of apoptosis, suggesting the involvement of different apoptotic mechanisms among these molecules. Moreover, addition of exogenous prostaglandin E2 does not provide appreciable protection against celecoxib-induced apoptosis in either prostate cancer cell line (data not shown). Thus, the sensitivity to the induction of apoptosis by COX-2 inhibitors may be independent of cellular levels of prostaglandins. Third, although constitutive expression of COX-2 enhances the metastatic potential of intestinal epithelial cells, ectopic expression of COX-2 in ECV endothelial cells results in growth disadvantage and increased apoptotic death (48). Taken together, it is plausible that COX-2 inhibitor-mediated apoptosis mediates through both COX-2 activity-dependent and -independent pathways. Thus, the mechanisms by which COX-2 inhibitors exert apoptotic effect in cancer cells warrant further investigation.

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The Cyclooxygenase-2 Inhibitor Celecoxib Induces Apoptosis by Blocking Akt Activation in Human Prostate Cancer Cells Independently of Bcl-2
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