RNA interference–mediated cyclooxygenase-2 inhibition prevents prostate cancer cell growth and induces differentiation: modulation of neuronal protein synaptophysin, cyclin D1, and androgen receptor

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Abstract
Cyclooxygenase-2 (COX-2) plays an important role in tumor development and progression. Inconsistent reports on the expression of COX-2 in early versus advanced prostate cancer raised the question on whether COX-2 inhibition affects prostate carcinogenesis. Evidence from recent studies indicates that prostate carcinogenesis depends on the altered expression of several factors including androgen receptor signaling, proinflammatory, and cell cycle regulatory genes. Very often, the outcome of androgen ablation treatment is not effective and, eventually, the cancer becomes androgen independent followed by activation of several survival genes and transcription factors. Most importantly, the extent of the influence of COX-2 on the regulation of the androgen receptor, cyclin D1, and other factors involved in cancer growth is not known. Using RNA interference–mediated COX-2 inhibition in metastatic prostate cancer cells, this study has shown that the silencing of COX-2 at the mRNA level can induce cell growth arrest and down-regulate androgen receptor and cyclin D1. We have further shown for the first time that COX-2 knockdown prostate cancer cells depict morphologic changes associated with enhanced expression of differentiation markers, particularly the neuronal protein synaptophysin along with activation of p21\(^{wt/1/Cip1}\) and p27\(^{Kip1}\). In summary, our findings determined the role of COX-2 in prostate carcinogenesis and its control on COX-2-independent targets. Second, abrogation of COX-2 and activation of synaptophysin provide evidence for the control of COX-2 on the expression of a neuronal protein. Finally, our findings provide evidence of COX-2-independent targets promoting cell growth arrest and differentiation in cells lacking COX-2 expression at the mRNA level. [Mol Cancer Ther 2006; 5(5):1117–25]

Introduction
Epidemiologic studies suggest that regular use of nonsteroidal anti-inflammatory drugs targeting cyclooxygenase-2 (COX-2) is protective against human cancer (1–4). In addition to several reports (5, 6), in our earlier studies using cell culture and transgenic animal models, we have shown that COX-2 inhibitors reduce prostate tumor growth by modulating both COX-2-dependent and -independent targets (7, 8). However, recent reports on issues related to the side effects of COX-2 inhibitors are raising concerns about their nonselectivity and their mode of action against human cancer (9, 10). The discovery of small interfering RNA (siRNA) has revolutionized approaches to decoding specific gene function and pathways (11–14). Small RNAs with perfect homology to their target gene can cause specific mRNA cleavage called RNA interference (RNAi; ref. 15). Most importantly, siRNA-directed transcriptional silencing is conserved in mammals and thus provides a means to inhibit specific mammalian gene function (13).

To assess the molecular and cellular effects on COX-2 gene silencing, in the present study, we knock down the gene COX-2 using RNAi for the first time in cells derived from the adenocarcinoma of the mouse prostate (TRAMP) in comparison with the human prostate cancer cell PC-3. These cells represent metastatic prostate cancer with a higher level of COX-2 at the mRNA level. After examining the base level expression of COX-2, we assessed the effects of COX-2 siRNA on the androgen receptor and cyclin D1 expression. Findings from the present study indicated that RNAi-mediated COX-2 inhibition resulted in overall cancer cell growth and cell cycle arrest. However, unlike the selective COX-2 inhibitor celecoxib (7), siRNA-mediated COX-2 inhibition was less effective in inducing apoptosis. Interestingly, and most importantly, our findings indicated negative regulation of the androgen receptor and cyclin D1 while activating the cyclin-dependent kinase (cdk) inhibitors p21\(^{wt/1/Cip1}\) and p27\(^{Kip1}\) and synaptophysin (a neuron specific protein) involved in differentiation in COX-2 knockdown cells. Although RNAi-mediated COX-2 inhibition shares few functional aspects to that of selective
COX-2 inhibitors, we concluded that RNAi-mediated COX-2 knockdown at the transcriptional level is more effective in inducing irreversible cell growth arrest and differentiation.

In summary, our findings determined the role of COX-2 in prostate carcinogenesis and its control on COX-2-independent targets. Second, abrogation of COX-2 and activation of synaptophysin provide evidence for the control of COX-2 on the expression of a neuronal protein. Finally, our findings provide indirect evidence for the role of drugs or chemopreventive agents that modulate COX-2 on the expression of a neuronal protein.

**Materials and Methods**

**Cell Culture**

The cells used in this study included mouse prostate cancer cells, TR-75, derived from transgenic adenocarcinoma of the mouse prostate (TRAMP) and human cancer prostate cancer cells, PC-3. Human prostate cancer cells were grown in RPMI (Gibco Life Technologies, Inc., Gaithersburg, MD) with 5% fetal bovine serum in addition to adopting the standard cell culture conditions recommended by the American Type Culture Collection (Manassas, VA) with appropriate modifications (8, 16). The TR-75 cell line from TRAMP was established in our laboratory for ongoing studies. The primary culture of TR-75, epithelial cells from prostatic adenocarcinoma removed from a 26-week-old TRAMP (C57BL/6-TgN [TRAMP] 8247Ng) mouse, was initiated by mechanical disruption of the prostate tissue followed by enzymatic digestion with dispase for 1 hour at 37°C as described earlier (17). Briefly, cell suspensions plated on T-25 cell culture flasks were allowed to attach and proliferate in DMEM high-glucose media to adopting the standard cell culture conditions recommended by the manufacturer. The TR-75 culture medium was changed every 3 days. The cells were split when they reached confluency by trypsinizing with 0.25% trypsin/EDTA (Life Technologies, Inc., Rockville, MD); simultaneously, cells from the wild-type control (C57BL/6) mouse prostate were also grown in a similar condition to get normal mouse prostate cells. For treatments, celecoxib was provided by Searle Research and Development, Pharmacia (St. Louis, MO) and NS-398 was purchased from Cayman Chemicals (Ann Arbor, MI). Celecoxib and NS-398 were dissolved in DMSO for treatments.

**RNAi Experiments for Silencing COX-2 Expression: Transient Transfection Assays**

The COX-2 gene–specific siRNA duplexes along with HiPerFect transfection reagent were purchased from Qiagen Inc. (Valencia, CA). In this study, we used two regions (siRNA-1 and siRNA-2) for the target siRNA sequences of the human COX-2 gene (NM_000963) covering the bases 299-319 (5'-AACACCGGAATTTTGGACAAG-3') and 575-595 (5'-TCCCTTGGTGTTCAAGTAAA-3'), respectively. Similarly, we also used two regions (siRNA-1 and siRNA-2) for the target siRNA sequences of the mouse COX-2 gene (NM_011198) covering the bases 563-583 (5'-AAGGAGCCTTCTAGTTCAAAA-3') and 1,183-1,203 (5'-CCGCATTGCCTCTGAAAAAT-3'), respectively. For the transfection assay with siRNA, ~10^6 cells were grown to reach 70% confluence. The original stock of the siRNA was resuspended in siRNA suspension buffer provided by the manufacturer. The resulting suspension was aliquoted in required amounts for each experiment and stored at −20°C until it was ready to use. On the day of transfection, aliquots of siRNA suspension were heated to 90°C for 1 minute followed by incubation at 37°C for 60 minutes. The siRNA was then gently introduced into the cells by mixing with the required amount of HiPerFect transfection reagent (as recommended by the manufacturer). Parallel experiments with HiPerFect transfection agent served as the control. To get a 100% COX-2 knockdown, the cells were subjected

<table>
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Table 1. Primer sequences

Mol Cancer Ther 2006;5(5). May 2006
Figure 1. COX-2 expression in TR-75 and PC-3 cells. A, RT-PCR analysis of total RNA extracted from TR-75 cells derived from transgenic adenocarcinoma of the mouse prostate and human prostate cancer cells PC-3. The PCR products were amplified as described in Materials and Methods using COX-2 gene–specific primers for mouse and human separately as listed in Table 1. Amplification of GAPDH was used as the internal control. Columns, relative expression level of COX-2 mRNA. B, immunofluorescence detection of COX-2 inhibition in TR-75 and PC-3 cells transfected with 30 nmol COX-2 siRNA for 48 and 72 h. Transfected cells were incubated for 2 h with mouse monoclonal antibody for COX-2, followed by an incubation with FITC-conjugated secondary antibody to detect green fluorescence signaling, indicating the level of COX-2 expression. C, RT-PCR analysis of total RNA extracted from control and siRNA-transfected cells after 48 and 72 h showing COX-2 down-regulation at the mRNA level. Amplification of GAPDH was used as the internal control. Columns, relative expression level of COX-2 mRNA in cells transfected with COX-2 siRNA after 48 and 72 hours. D, RT-PCR analysis of total RNA extracted from cells transfected with control and two regions of the COX-2 siRNA (30 nmol each of siRNA-1 and siRNA-2) after 48 h, in addition to RNA extracted from cells treated with COX-2 inhibitors NS-398 and celecoxib, showing a comparative effect of COX-2 at the mRNA level.
to redosing with same amount of 30 nmol siRNA for up to 72 hours. For all the biochemical and molecular target analyses, we used protein and/or RNA extracted from cells exposed to 30 nmol siRNA. A final concentration of 30 nmol of siRNA for transfection used in this study was derived from a dose-dependent assay. In addition, the cells transfected with 30 nmol siRNA for 48 to 72 hours resulted in effective reduction in the COX-2 expression as determined by reverse transcription-PCR (RT-PCR) and Western blot. Similar experiments were repeated using a second set of COX-2 siRNA in both cell types. Molecular changes observed in COX-2 knockdown cells mediated by siRNAs were compared with the effects induced by 10 μmol/L each of COX-2 inhibitors celecoxib and NS-398. All the experiments were conducted in triplicate.

**Immunofluorescence Detection of COX-2**

To assess the effect of RNAi-mediated COX-2 inhibition, we first used immunofluorescence detection of COX-2, as described earlier (7), in both TR-75 and PC-3 cells transfected with or without siRNA. Cells grown in dishes transfected with siRNA for 48 and 72 hours were trypsinized, washed in PBS, fixed in 10% formalin, and pretreated with 0.1% Triton X-100 for 15 minutes each. COX-2 expression was detected after incubating the cells with COX-2-specific antibody conjugated to FITC (Cayman Chemicals) for 1 hour at room temperature. Green fluorescence signaling of COX-2 expression was viewed under an epifluorescence microscope (Olympus AX-70) as described earlier (8). Quantification of the green fluorescence signaling in siRNA-transfected cells compared with the control is presented as a bar graph.

**RNA Isolation and Quantitative Real-time PCR**

A two-step RT-PCR was carried out with total RNA extracted from TR-75 and PC-3 cells transfected with or without 30 nmol of siRNAs (siRNA-1 and siRNA-2) and from cells treated with 10 μmol/L each of celecoxib and NS-398 for 48 hours using Trizol reagent and Qiagen columns (Life Technologies and Qiagen). We used an initial denaturing for 2 minutes at 95°C and continued the amplification with an extension at 72°C, 7 minutes for 33 cycles using COX-2 gene–specific primer sequences (Table 1) along with the amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal control. Real-time RT-PCR was carried out to evaluate the expression of mouse and human COX-2, androgen receptor, and cyclin D1 using Cepheid Smart Cycler II (Cepheid, Sunnyvale, CA).

**Clonogenic Survival Assay**

To determine the RNAi effect mediated by the COX-2 siRNA on prostate cancer cell growth, a soft-agar (Gibco Life Technologies) clonogenic assay was done as described earlier (7) using cells transfected with siRNA for 48 hours. Briefly, prostate cancer cells were grown on soft-agar plates (~50,000 per plate) made with cell culture medium containing 1.5% agar. Triplicate plates were used for each experiment along with the control. The plates were incubated for 10 days at 37°C in 5% CO2. Colonies identified by crystal violet staining of >25 cells per colony were precisely counted to determine the efficacy. The results are presented as a percentage of the control.

**Cell Cycle Analysis**

To determine the RNAi effect on cell cycle regulation, control and COX-2 siRNA–treated cells were harvested by trypsinization and fixed in 10% neutral buffer formalin followed by fixing in 80% ethanol for 24 hours. The cells were washed in PBS and resuspended in 1 mL of 5 μg/mL of propidium iodide with 0.1% RNase A (Sigma-Aldrich, St. Louis, MO) in PBS. After 30-minute incubation at room temperature in the dark, the cells were analyzed by flow cytometry with Coulter Epic Elite ESP. Cell cycle analysis was done as described by Narayanan et al. (7) with modifications of the original protocol described by Darzynkiewicz et al. (18) and Gong et al. (19).

To determine the significant difference between control and treatments, the analysis was repeated with triplicate samples for each treatment.

**Western Blot Analysis**

To identify the specific proteins altered in COX-2 knockdown cells, total protein lysate was extracted from cells treated with 30 nmol siRNA-1 and siRNA-2 for 48 hours. Protein lysates from cells treated with 10 μmol/L each of NS-398 and celecoxib were also used for Western blot analysis. Briefly, cells were harvested by trypsinization using lysate buffer mixed with a cocktail of protease inhibitors as described earlier (7). Equal amounts of protein (10 μg/lane) were fractionated on a 10% SDS-PAGE gel for each sample and transferred to polyvinylidene difluoride membranes. Western blot procedure was carried out using specific mouse monoclonal antibodies for COX-2, androgen receptor, cyclin D1, p21(Waf1/Cip1), and p27(Kip1) (Santa Cruz Biotechnology, Santa Cruz, CA) and synaptophysin (Sigma). β-Actin was used as the internal control. Reactive protein bands were developed using enhanced chemiluminescence detection (GE Healthcare Bio-Sciences Corp., Piscataway, NJ).

**Statistical Analysis**

The significant differences in the cellular effects and in the expression of COX-2 between control and siRNA-treated cells showing the effect on G1 phase of cells were compared using one-way ANOVA followed by Tukey’s multiple comparisons procedure (20).

**Results**

**Inhibition of COX-2 by siRNA**

The metastatic prostate cancer cells TR-75 and PC-3 used in this study showed a higher level of COX-2 expression at the transcription (mRNA) level (Fig. 1A). Immunofluorescence detection of COX-2 after transfection with 30 nmol of siRNA for 48 hours indicated a sharp decline in the COX-2 expression compared with that in the control. A further decrease in the expression of COX-2 was evident after 72 hours (Fig. 1B). Immunofluorescence detection of COX-2, indicating a reduced expression at the cellular level, was consistent with the findings from RT-PCR analysis (Fig. 1C). Down-regulation of COX-2 expression mediated by siRNA was consistent with the effect induced by known COX-2 inhibitors NS-398 and celecoxib in TR-75 and PC-3 cells (Fig. 1D).
RNAi-Mediated COX-2 Knockdown Induces Cell Growth Inhibition and Cell Cycle Arrest

RNAi-mediated COX-2 inhibition was associated with cell growth inhibition and cell cycle arrest. Clonogenic survival assay with TR-75 and PC-3 cells transfected with 30 nmol siRNA for 48 and 72 hours indicated cell growth inhibition (indirectly determined by the rate of cell survival) by 80% compared with the control (Fig. 2A; \( P < 0.001 \)); however, there was no significant difference in the rate of cell growth inhibition between 48 and 72 hours. Flow cytometry analysis of cells transfected with siRNA indicated cell cycle arrest at the G1-S phase in both TR-75 and PC-3 cells with a G1 peak showing 82.53% and 75.84%, respectively, compared with the control (\( P < 0.05 \)), with much less number of cells at the S and G2-M phase (Fig. 2B).

RNAi-Mediated COX-2 Inhibition Affects Cyclin D1 and Androgen Receptor Expression

It is evident from our current and earlier studies that TR-75 cells derived from the 26- to 28-week-old TRAMP continue to express a high level of androgen receptor. In this study, the nuclear oncogene cyclin D1 involved in cell cycle regulation was down-regulated in cells transfected with COX-2-specific siRNA. Interestingly, COX-2 inhibition was also associated with androgen receptor inhibition in TR-75 cells. However, androgen receptor expression was not detected in siRNA-transfected PC-3 cells (Fig. 3A). Findings from Western blot analysis indicated down-regulation of cyclin D1 in both TR-75 and PC-3 cells. Down-regulation of androgen receptor determined by Western blot in siRNA-transfected TR-75 cells was consistent with mRNA level determined by RT-PCR (Fig. 3B and C). Bar graph represents the quantification of the expressed proteins.

Morphologic Changes Associated with Activation of Synaptophysin and Cdk Inhibitors p21\(^{Waf1/Cip1}\) and p27\(^{Kip1}\)

To determine the fate of growth-arrested cells, morphologic changes of TR-75 cells transfected with COX-2 siRNA was monitored constantly for more than 96 hours. After 96 hours of transfection, TR-75 cells stopped dividing and indicated a gradual change in the morphology, indicating elongation and differentiation similar to neuronal type of cells. The reduced cell growth was associated with appearance of a significant number of elongated neuronal types of cells as shown in Fig. 4. Western blot analysis of the total protein from both TR-75 and PC-3 cells transfected with siRNA after 48 hours showed an elevated level of known markers of differentiation including cdk inhibitor proteins p21\(^{Waf1/Cip1}\) and p27\(^{Kip1}\) and the protein synaptophysin compared with that of the control. Activation of cdk inhibitor proteins p21\(^{Waf1/Cip1}\) and p27\(^{Kip1}\) and synaptophysin in siRNA knockdown cells was consistent with effect induced by COX-2 inhibitors NS-398 and celecoxib, although in PC-3 cells, much variations were not detected in the expression level of p27 by COX-2 inhibitors NS398 and celecoxib (Fig. 5A and B). Bar graph represents the quantification of the expressed proteins.

Discussion

Cyclooxygenase is a rate-limiting enzyme that catalyzes the biosynthesis of prostaglandins and thromboxanes from arachidonic acid. COX-2 is an immediate-early gene and is induced by oncogenes, growth factors, cytokines, endotoxins, and phorbol esters (21–23). COX-2 plays an important role in tumor development and progression (24, 25). Our finding from this study using RNAi-mediated COX-2 inhibition is advantageous because siRNAs with perfect homology to their target gene can cause specific mRNA cleavage (15). Most importantly, siRNA-directed transcriptional silencing is conserved in mammals and thus provides a means to inhibit specific mammalian gene function (13). Although pharmacologic intervention of
cancer is primarily focused on the inhibition of COX-2, findings from this study using RNAi-mediated COX-2 depletion for the first time indicate its transcriptional control on several non-COX-2 targets involved in cancer cell growth arrest and differentiation mechanisms.

Our findings from this study indicate that COX-2 inhibition by siRNA in both mouse and human prostate cancer cells modulate similar targets of selective COX-2 inhibitor celecoxib (e.g., down-regulation of cyclin D1 and activation of cdk inhibitor p21^(waf1/Cip1) by nonsteroidal anti-inflammatory drugs; ref. 7). However, in addressing the question of whether COX-2 inhibition in general plays an important role in mediating apoptosis, our findings from RNAi-mediated COX-2 inhibition indicated a very weak apoptotic effect compared with that of COX-2 inhibitors (7). Clonogenic survival assay and flow cytometry analysis with TR-75 and PC-3 cells transfected with 30 nmol siRNA for 48 hours induced cell growth inhibition and cell cycle arrest, but not apoptosis. These observations suggest that COX-2 inhibition at the mRNA level in prostate cancer cells is not an obligatory function to induce apoptosis. Although RNAi-mediated COX-2 depletion and COX-2 inhibition by specific inhibitors share a few common targets, these two treatments may have very different cellular effects, as has been reported earlier by Song et al. (26) with Tet-On antisense COX-2 clones in prostate cancer cells. However, an earlier

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**Figure 3.** Down-regulation of androgen receptor and cyclin D1.

A, RT-PCR analysis. Agarose gel electrophoresis (2%) showing the siRNA effect on cyclin D1 and androgen receptor expression. RT-PCR analysis was done with total RNA extracted from control, TR-75, and PC-3 cells transfected with 30 nmol siRNA for 48 h. Amplification was done as described in Materials and Methods using specific primers listed in Table 1. B and C, Western blot analysis of total protein extracted from COX-2 knockdown TR-75 and PC-3 cells showing inhibition of COX-2, cyclin D1, and androgen receptor. Total protein lysates from TR-75 and PC-3 cells transfected with siRNA were used for Western blot analysis as described in Materials and Methods. Columns, relative expression levels of COX-2, androgen receptor, and cyclin D1.
study with RNAi to dissociate the effects of nonsteroidal anti-inflammatory drugs against ovarian cancer indicated that neither COX-1-specific nor COX-2-specific siRNAs had an effect on ovarian cancer cell growth (27).

In this study, we observed the coupling of cell cycle exit with cellular differentiation in prostate cancer cells exposed to COX-2 siRNA, which was consistent with our earlier findings on COX-2 inhibition mediated by cell cycle arrest with celecoxib (7). Although the above events involve synchronization and modulation of several molecular targets, in this study, the COX-2 inhibition by siRNA as well as by known COX-2 inhibitors celecoxib and NS-398 resulted in the activation of p21^{Waf1/Cip1} and p27^{Kip1} and down-regulation of oncoprotein cyclin D1 and androgen receptor in TR-75 cells from adenocarcinoma of the mouse prostate. It is evident that androgen receptor activity is required for prostate growth, differentiation, and secretion, and studies have also shown that cyclin D1 is a potent inhibitor of androgen receptor activity (28). Because cyclin D1 has been shown to harbor multiple transcriptional functions independent of cell cycle (29, 30), its role in regulating the androgen receptor expression in COX-2 knockdown cells is yet to be investigated. We have further shown for the first time that COX-2 knockdown prostate cancer cells depict morphologic changes associated with enhanced expression of differentiation markers, particularly the neuronal protein synaptophysin, along with the activation of p21^{Waf1/Cip1} and p27^{Kip1}. Reports on the differential expression of p27 and cyclin D1 leading to neuronal differentiation present a very critical event to optimize their regulation against tumors with a higher level of COX-2 expression (31, 32). Recent studies using COX-2-specific siRNA indicated a direct control of COX-2 in modulating more than one molecular variable. Contrary to our observation from this study, adenovirus-mediated COX-2 siRNA effectively inhibited COX-2 protein expression and enhanced apoptosis without inhibiting extracellular signal–regulated kinase 1/2 phosphorylation (33). Moreover, COX-2-specific siRNA markedly abolished human umbilical vascular endothelial cell tube formation (34) and thus provided evidence for antiangiogenic effect in cells lacking COX-2. In addition, COX-2-overexpressing non–small-cell lung cancer cells exposed to survivin siRNA decreases apoptosis resistance (35). A more in-depth analysis on the mechanistic aspect of siRNA-mediated COX-2 inhibition in prostate cancer cells indicated a major shift from COX-2-dependent to COX-2-independent molecular variables involved in prostate cancer. It is also evident from our findings that siRNA-mediated COX-2 inhibition resulted in cell cycle arrest (G_1) associated with activation of cdk inhibitors p21^{Waf1/Cip1} and p27^{Kip1}. Although the question remains on whether siRNA-mediated COX-2 inhibition can directly regulate the biomarkers p21^{Waf1/Cip1}, p27^{Kip1}, and cyclin D1 or androgen receptor, it has been reported earlier by us and also by others that in cancer cells, treatment with nonsteroidal anti-inflammatory drugs resulted in down-regulation of cyclin D1 and androgen receptor. Most importantly, we have also shown earlier that selective COX-2 inhibitors induce cell cycle arrest and apoptosis and up-regulate cdk inhibitors and, thus, cause accumulation of cells at the G_0-G_1 phase (7, 36–39).

It is also evident from our in vivo and in vitro studies that TR-75 cells derived from 26-week-old TRAMP continue to express a higher level of androgen receptor. Further studies...
are in progress using siRNA for androgen receptor to determine whether COX-2 siRNA–mediated cell growth inhibition is a consequence of androgen receptor suppression or due to unknown secondary effect on androgen receptor transcription. However, PC-3 cells used in this study did not show androgen receptor expression. Although synaptophysin is known to be a differentiation marker in neuronal and/or neuroendocrine cells with poor prognosis, in this study, its expression is associated with cell growth arrest mediated by COX-2 inhibition in prostate cancer cells.

The results presented in our study strongly support the view on molecular targets that are blocked by the COX-2 gene. Overall, our findings determine the role of COX-2 in prostate carcinogenesis and its control on COX-2-independent targets. Second, abrogation of COX-2 and activation of synaptophysin provide evidence for the control of COX-2 on the expression of a neuronal protein involved in cellular differentiation. Currently, we are examining similar effects in cancer cells with stable transfection using lentivirus-mediated (U6 promoter) COX-2 inhibition. Findings from this study revealed the critical role of siRNA and/or COX-2 inhibitors in regulating several non-COX-2 targets such as the androgen receptor and cdk inhibitors in prostate cancer. Based on our current findings, further studies are in progress to test the approach of silencing the expression of inducible form of several other proinflammatory genes in other cell types and in preclinical models to better understand the mode of action of COX-2 inhibitors against cancer.

Acknowledgments

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References


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