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ORIGINAL ARTICLE

A comparison of the effectiveness of selected non-steroidal anti-inflammatory drugs and their derivatives against cancer cells in vitro

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Abstract
Purpose Previously, we reported in vitro observations suggesting that ibuprofen is an effective non-prescription non-steroidal anti-inflammatory drug (NSAID) to reduce the survival of human prostate cancer cells (Andrews et al. in Cancer Chemother Pharmacol 502:77–284, 2002), and that this observed effectiveness is mediated by an up-regulation of the $p75^{NTR}$ tumor suppressor (Khwaja et al. in Cancer Res 646:207–6213, 2004). However, other NSAIDs and their derivatives have received significant attention with regard to their anti-cancer effectiveness and have been selected for clinical trials to treat a variety of human cancers. In this investigation, we compared celecoxib, sulindac sulfone, nitric oxide linked NSAIDs, and R-flurbiprofen with ibuprofen in their ability to inhibit the growth of a variety of human cancer cells lines including cells lines with multi-drug resistance. We also evaluated whether, like ibuprofen, an up-regulation of $p75^{NTR}$ is a molecular mechanism that mediates the anti-growth effectiveness of these drugs.
Materials and methods Selected dosages for each drug were evaluated for their ability to reduce the growth (MTT analysis) and induce apoptosis (Hoechst staining) of a variety of different cancer cell lines, including an ovarian cell line expressing multidrug resistance-1 glycoprotein (MDR-1). The drugs were then analyzed using immunoblot, RT-PCR and siRNA to study the role of $p75^{NTR}$ in their anti-growth effectiveness.
Results Our study revealed consistency in the drug dosages that inhibit the survival of different human cancer cell lines. While NO-linked aspirin and celecoxib were most effective in decreasing cell growth and inducing apoptosis at the lowest dosages, R-flurbiprofen and ibuprofen were most effective at clinically relevant dosages. A multidrug resistant ovarian cell line is more resistant to growth inhibition by the drugs tested than its non-drug resistant parental counterpart. There was no correlation between the expression of cyclooxygenase-2 (COX-2) and the ability of the drugs to reduce cancer cell survival. All the drugs tested induced an up-regulation in $p75^{NTR}$ tumor suppressor gene expression in concert with their observed growth inhibiting ability. Inhibition of $p75^{NTR}$ expression with *siRNA* reduced the cell growth inhibiting effects of all the drugs tested.
Conclusions The method of chemotherapy (i.e., intravascular, intrathecal, oral) might dictate the choice of NSAID/NSAID derivative used to treat/prevent a given type of cancer. Also, the $p75^{NTR}$ tumor suppressor appears to be a common molecular mechanism that mediates the growth inhibiting effectiveness of these drugs.

Keywords Cancer · COX-2 · Drug resistance · Nitric oxide · NSAIDs · $p75^{NTR}$

Introduction

In vitro, in vivo and epidemiological studies have all demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) appear to be effective in the chemoprevention and possible treatment of many cancers including colorectal, breast, prostate, pancreatic, squamous cell carcinoma of the head and neck, ovarian, lung, and bladder cancers [57, 62,

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70]. Recently, we studied the comparative effectiveness of common non-prescription NSAIDs and reported that ibuprofen appears to be better than other non-prescription NSAIDs (i.e., aspirin, neproxen, etc.) in its ability to reduce the survival of prostate cancer cells [3]. However, other NSAIDs and NSAID derivatives have also been purported to have superior anti-tumorigenic effects both in vitro and in vivo and have been selected for clinical trials (i.e., exsulindac, R-flurbiprofen, celecoxib and nitric oxide linked NSAIDs). Unlike ibuprofen, these compounds either have little cyclooxygenase-1 inhibiting activity (i.e., R-flurbiprofen, celecoxib, exsulindac) or compensate for this activity (i.e., nitric oxide linked NSAIDs) in order to reduce the gastrointestinal toxicity otherwise associated with ibuprofen and other common NSAIDs [1]. Celecoxib, a selective cyclooxygenases-2 (COX-2) inhibitor, has been shown to be effective in animal models of colon cancer [47], breast cancer [2, 5, 20], bladder cancer [18], prostate cancer [21], lung cancer [13], head and neck cancer [72], and skin cancers [65] and has been in clinical trials for prostate, breast, ovarian, non-small cell lung and even skin cancer. Sulindac sulfone (Exisulind) is a metabolite of the NSAID sulindac sulfoxide that does not inhibit cyclooxygenases activity [42, 43]. Sulindac sulfone has been reported to reduce tumor growth in murine models of colon [34, 42, 48], bladder [44], breast [60], prostate [17], and lung cancers [35] and has also been selected for clinical trials in patients with advanced solid tumors [66]. R-flurbiprofen, the R-enantiomer of the NSAID flurbiprofen, has been reported to be effective in murine models of prostate [64] and colon cancers [33, 63] and was selected for clinical trials to determine if it can prevent the progression of prostate cancer [30, 45]. Nitric oxide linked NSAIDs (i.e., NO-NSAIDs) represent traditional NSAIDs to which a nitrogen oxide-releasing fragment has been linked in order to reduce the gastrointestinal toxicity otherwise associated with the cyclooxygenases inhibiting activity of NSAID treatment [10]. In studies of a variety of cancer cell lines, the NO-linked NSAIDs have been reported to be significantly more effective than the original NSAID [23, 25, 68]. In the present study, we have evaluated the effectiveness of the foregoing drugs together with ibuprofen in reducing the growth of human cancer cell lines including colon cancer, prostate cancer, bladder cancer, squamous cell carcinoma, ovarian cancer, neuroglioma and neuroblastomas. Some of the cancer cell lines tested are COX-2 deficient while others express COX-2. Since multi-drug resistance is a major factor in cancer chemotherapy, we evaluated the effectiveness of these NSAIDs/derivatives in drug-resistant cell lines as compared with their non-drug resistant counterparts. A range of dosages were evaluated in order to address the fact that clinically acceptable dosages associated with oral administration of some NSAIDs result in blood serum concentrations that are significantly different than other NSAIDs.

The molecular basis for the tumorigenic effectiveness of NSAIDs was initially attributed to inhibition of cyclooxygenases [67]. Although cyclooxygenase inhibition of prostaglandin synthesis can explain part of the anti-tumor activity of certain NSAIDs, there is overwhelming evidence indicating that the ability of NSAIDs to inhibit the survival of cancer cells is due to cyclooxygenase independent mechanisms. Recently, we have shown that the glycoprotein surface receptor and tumor suppressor $p75^{NTR}$ is significantly upregulated in cancer cells following treatment with ibuprofen [26]. We further demonstrated that there is a causal relationship between $p75^{NTR}$ up-regulation and the ability of ibuprofen to reduce cancer cell survival [26]. $P75^{NTR}$ is a 75 kD cell surface receptor glycoprotein that shares both structural and sequence homology with the tumor necrosis factor receptor super-family of proteins [8, 9]. The expression pattern of $p75^{NTR}$ is widespread extending outside of the nervous system to numerous peripheral organs and tissues, where it negatively regulates cell survival, proliferation and growth [22]. In the present investigation, we have accessed whether an up-regulation of the $p75^{NTR}$ tumor suppressor gene represents a molecular mechanism common to the diverse NSAIDs and their derivatives that are included in this study.

Materials and methods

Cell cultures

The DU145, HCT 116, H4, PC-3, SH-SY5Y, SK-N-SH and T24 cell lines were purchased from the American Type Culture Collection (Rockville, MD). The CaOv3 and $CaOv3^{TaxR}$ cell lines were obtained from Dr. Christopher Taylor (Georgetown University Medical Center, Washington, DC). The PCI 13 and SCC25/CP cell lines were obtained from Dr Kevin Cullen (Lombardi Cancer Center, Washington, DC). All cells were grown in 10% fetal calf serum-supplemented Delbecco's Modified Eagle's Medium (DMEM from Mediatech, Herndon, VA) in a humidified atmosphere of 10% CO_2 in air at a temperature of 37°C

NSAIDs/derivatives/chemicals

Ibuprofen and sulindac sulfone were obtained from Sigma Chemical Co. (St Louis, Mo). R-flurbiprofen was a generous gift from Myriad Pharmaceuticals, Inc (Salt Lake City, Utah). Celecoxib was a generous gift from the Pharmacia Corporation (St Louis, Missouri). Nitric oxide linked aspirin (NCX-4050), flurbiprofen (NCX-2131), ibuprofen (NCX-2111) and sulindac (NCX-1102) were generous gifts from

NicOx S.A. (Sophia Antipolis, France). All compounds were made up in stock solutions of dimethyl sulfoxide (DMSO, Sigma Chemical Co., St Louis, Mo) that were diluted prior to use. Controls were treated with vehicle alone (i.e., not greater than 1% DMSO). For each compound, we tested a range of dosages that included high and low ends of the clinically relevant spectrum. The MDR-1 inhibitor verapamil was obtained from Calbiochem (La Jolla, CA).

Cell growth assay

Cellular survival was determined using the 3-[4,5-dimethylthiazol-2-yl]-2,55-diphenyltetrazolium bromide (MTT) colorimetric assay according to the published procedure [37] as provided by the manufacturer (Roche Diagnostics Corporation, Indianapolis, IN). The principle of the assay is that when MTT, a nontoxic pale yellow substrate, is taken up by living cells it is converted into a dark blue formazan product that requires active mitochondria; thus, dead cells will not form formazan. The formed formazan is read spectrophotometrically (570 nm absorbance) and is directly proportional to the number of viable cells. Since no media or cells are removed from the culture wells during the MTT analysis, there is no loss of cells (i.e., dead or floating) as a result of these procedures. For this assay, cells (2×10^3 per 100 µl) were seeded in 96-well plates and incubated for 24 h in a humidified atmosphere of 5% CO^2 at 37°C. Media containing selected concentrations of the different NSAIDs/derivatives were added to the wells and the cells incubated for an additional 48 h. Following incubation in NSAIDs/Derivatives, 10 ul of labeling reagent (MTT) was added to each well, and the cells further incubated for an additional 4 h at 37°C. The cells were then be solubilized by incubation overnight at 37°C in 100 ul of a solubilization solution (10% sodium dodecyl sulfate in 0.01 M HCl). The absorbance at 570 nm was determined in a microtiter plate reader (Bio-Rad Laboratories, Richmond, CA). The results of the NSAID/Derivatives treated cultures were expressed as the percentage of viable cells compared with non-treated controls. When there is a significant reduction in survival (MTT analysis), we determined to what extent this is due to apoptosis using Hoechst stain (see "Apoptosis assay").

Apoptosis assay

The cells were preserved for microscopic analysis by the addition of 10% neutral formalin to each of six-well plates (2 ml per well). The fixed cells were harvested (by scrapping) and stained using Hoechst 33258 stain (Molecular Probes, Eugene, Oregon). Apoptotic cells were distinguished by their intensely stained fragmented nuclei and counted and photographed using an BH-2 Olympus fluorescent microscope equipped with a Canon PowerShot G5 digital camera. Two independent observers undertook cell counts blindly. Statistical analysis performed as described below (see "Data analysis").

Immunoblot analysis

Cells grown on six-well plates were harvested by treatment with 200 µl of cold (4°C) lysis buffer (1% Igepal Ca-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate in PBS) containing 2 mg/ml aprotinin. After scraping cells from the plate, lysates were centrifuged for 10 min at $12{,}000 \times g$ at 4°C. Cells intended for immunoblotting were placed in less stringent NP40 lysis buffer (10 mM Tris–Cl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP40) containing 2 mg/ml aprotinin. After incubation on a gentle rocker for 20 min, the lysates were centrifuged for 20 s at $12{,}000 \times g$ at 4°C. Supernatants were collected for estimation of protein concentration according to the manufacturer's protocol (BioRad Laboratories, Hercules, CA). Lysates were evaluated for specific protein expression by immunoblotting with 50 µg protein loaded on 12% SDS-PAGE gels, followed by transfer to nitrocellulose membranes, blocking for 1 h in 5% milk in PBS, rinsed in PBS followed by the addition of specific primary antibody in PBS overnight at 4°C. Membranes were then washed three times in PBS followed by addition of appropriate secondary antibody in PBS for 1 h at room temperature. After rinsing three times in PBS, immunoreactivity was assessed using SuperSignal West Femto Maximum Sensitivity substrate (Pierce, Rockford, IL) and an Fujifilm LAS-1000 imaging system. The results were quantified using ImageJ software (downloaded from internet). A monoclonal antibody for $p75^{NTR}$ was obtained from Upstate/Chemicon (Temecula, CA) and used at a dilution of 1:1,000. A polyclonal antibody for COX-2 was obtained from Santa Cruz Biotechnologies (Santa Cruz, CA) and used at a dilution of 1:500. Appropriate positive controls and molecular weight ladder (i.e., Biotinylated Protein ladder from Cell Signaling Technology, Danvers, MA) were used to confirm antibody specificity. Polyclonal HRP-conjugated goat anti-rabbit and polyclonal HRP conjugated goat anti-mouse secondary antibodies (BioRad, Hercules, CA) were used at a dilution of 1:2,000.

RNA extraction and real-time quantitative RT-PCR

RNA was extracted using TRIzol reagent (Invitrogen, Gaithersburg, MD) according to the manufacturer's protocol. TRIzol extraction was repeated a second time on the resulting RNA pellet to increase the RNA purity. Final RNA was quantified spectrophotometrically at a 260-nm wavelength. cDNA synthesis and real-time RT-PCR were performed as described in Latil et al. [29], with some modifications.

Briefly, RNA was reverse transcribed with Superscript III RNase H-reverse transcriptase (Invitrogen, Gaithersburg, MD), according to the manufacturer's manual, with ten units of RNasin RNase inhibitor (Promega, Madison, WI), 50 units of Superscript III, 150 ng of random hexamer (Amersham Biosciences, Pittsburgh, PA), and 1.5 ug of total RNA. PCR was performed using a SYBR Green PCR Core Reagents kit (Applied Biosystems, Foster City, CA) and quantitated by an ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) according to the manufacturer's manual. Relative mRNA expression level was calculated using the ΔΔCt method, where the calibrator is the *RPLPO* gene. *RPLPO* encodes human acidic ribosomal phosphoprotein P0 and was used as the endogenous RNA control to correct for the amount of total RNA used in each PCR reaction. The primer sequences used were as follows: *RPLPO*: F- 5′-GGCGACCTGGAAGTC CAACT-3′, R- 5′-CCATCAGCACCACAGCCTTC-3′ product size 149 bp, *p75NTR*: F- 5′-CTGTGGTTGTGG GTCTTGTG-3′, R- 5′-TCGCTGTGGAGTTTTTCTCC-3′ product size 130 bp.

siRNA experiments

siRNA for human for $p75^{NTR}$ (Sense sequence: 5′-CUACCAGCCCGAGCACAUAUU-3′, and antisense: 5′-UAUGUGCUCGGGCUGGUAGUU-3′) and the siControl (non-targeting siRNA) were synthesized at Dharmacon (Chicago, IL). The siRNAs for human for $p75^{NTR}$ and siControl (non-targeting) siRNAs were diluted in Opti-MEM I and mixed with Lipofectamine™ 2000 as per the manufacturer's instructions (Invitrogen, Gaithersburg, MD). Human bladder cancer T24 cells diluted in antibiotic free media were plated onto 96 well plates and six well plates and grown overnight to 40–50% confluency. The T24 cells were then transfected for 4 h with the targeting ($p75^{NTR}$) or non-targeting (control) siRNAs, after which time the transfection media was replaced with fresh media. Twenty-four hours following transfection, the cells were treated with 5 μM of NO-Aspirin; 25 μM celecoxib; 500 μM ibuprofen, 500 μM R-flurbiprofen or 100 μM sulindac sulfone. Following 48 h of treatment with the NSAIDs/Derivatives, the cell protein was harvested from the six well plates for immunoblot analysis of $p75^{NTR}$ expression (see "Immunoblot analysis" above). The cells grown and treated in the 96 well plates underwent MTT analysis to determine the effects of the siRNA transfection on cell growth inhibition induced by the NSAIDs/Derivatives (see "Cell growth assay" above).

Data analysis

Data analysis was performed with a minimum of three replicates per experiment. The statistical differences between data sets and/or means was analyzed by ANOVA using the Prizm program (GraphPad Software) and the data expressed as the mean ± SEM. A P value < 0.05 was considered statistically significant.

Results

Comparing NSAIDs/NSAID derivatives in inhibiting survival of cancer cells in vitro

In this series of experiments, we compared ibuprofen, R-flurbiprofen, sulindac sulfone, celecoxib and nitric oxide linked-aspirin (i.e., NO-aspirin) in their abilities to reduce the survival of selected cancer cell lines in vitro. NO-linked aspirin was selected over other NO-linked NSAIDs because of our preliminary studies (data not shown) as well as past studies by others [23, 25, 68] revealing it to be more effective than other NO-linked NSAIDs in reducing the survival of cancer cells.

Although different lines of cancer cells varied in the extent to which their survival was inhibited by the NSAIDs/NSAID derivatives tested, there was consistency in the minimal effective dosage at which each drug induced statistically significant cell death following 48 h of treatment (see Table 1). Table 1 lists the cell lines in order of increasing resistance to the drugs tested together with the minimal dosage which induced a statistically significant decrease in cell growth over 48 h of incubation (i.e., minimal effective dosage determined by MTT analysis). As indicated in Table 1, for ibuprofen and R-flurbiprofen the minimal effective dose was 500 μM, for sulindac sulfone it was 100 μM, for celecoxib it was 50 or 25 μM, and for NO-aspirin it was 25 or 5 μm, depending on cell line. Therefore, when equivalent dosages are considered, NO-aspirin and celecoxib appear to be the most potent inhibitors of cell survival. Hoechst staining confirmed our previous observations indicating that the reduced survival is due in a large part to apoptotic cell death (Fig. 1) [3]. As indicated by the representative photo, there was a correlation between the amount of apoptosis observed and the cell survival as determined by the MTT assay (Table 1). When analyzed for the expression of cyclooxygenase-2 (i.e., COX-2), immunoblot analysis revealed that while some cell lines strongly express COX-2, others exhibited little or no COX-2 expression (see Table 1; Fig. 2). There was no correlation between the expression of COX-2 and the sensitivity of the cells to the drugs being tested.

Comparative effectiveness of NSAIDs/NSAID derivatives in reducing the growth of a drug-resistant cancer cell line

In this series of experiments, we compared the abilities of ibuprofen, R-flurbiprofen, sulindac sulfone, celecoxib and

Table 1 Minimal effective dosages (uM) of the drugs tested

Cell line[a]	Cancer type	Ibuprofen	R-flurbiprofen	Sulindac sulfone	Celecoxib	NO-Aspirin	COX-2 expression[b]
PCI-13	Squamous Cell	500	500	100	25	5	Strong
CaOv3	Ovarian	500	500	100	25	5	None
SCC25/CP	Squamous Cell	500	500	100	25	5	Strong
HCT116	Colon	500	500	100	25	5	None
T24	Bladder	500	500	100	25	5	Weak
DU145	Prostate	500	500	100	25	5	None
SK-N-SH	Neuroblastoma	500	500	100	50	5	None
H4	Glioblastoma	500	500	100	50	25	Strong
PC-3	Prostate	500	500	100	50	25	None

Minimal dose that induced a statistically significant (i.e., $P < 0.05$) decrease in cell survival following 48 h treatment (MTT analysis)

[a] Listed in order of decreasing sensitivity to drugs when compared with controls (i.e., Following treatment with the drugs, PCT-13 exhibited the greatest inhibition of growth and PC-3 the least inhibition of growth)

[b] As determined using Immunoblot analysis (see Fig. 2)

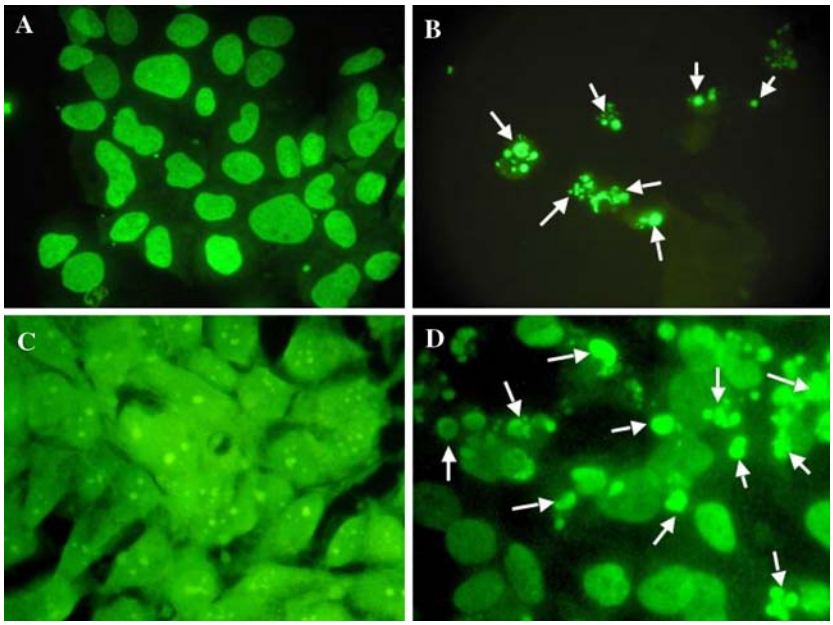

Fig. 1 Representative light microscopic images of CaOV3 cells (**a**, **b**) and H4 cells(**c**, **d**) that have been stained with Hoechst stain (following fixation) before (**a**, **c**) and following (**b**, **d**) exposure to 50 μM celecoxib for 48 h. Note the characteristic nuclear condensation and fragmentation indicative of apoptosis in the celecoxib treated cells (*arrows* in **b**, **d**). Also, while all CaOV3 cells exhibited apoptosis (**b**), H4 cells (**d**) appeared to be more resistant to the drugs tested (see Table 1)

NO-aspirin to reduce the growth of a drug-resistant cancer cell line compared with the parental cell line. The CaOV3^{TaxR} is a drug resistant cell line derived from the CaOV3 cell line that has been used as an in vitro model for drug-resistant cells [16]. Unlike the parental cell line (CaOV3), CaOV3^{TaxR} is resistant to paclitaxel and cisplatin and over-expresses multidrug resistance-1 (MDR-1)/P-glycoprotein [16]. An evaluation of COX-2 expression reveals that CaOV3^{TaxR} cells exhibits significant COX-2 expression, while the parental cell line exhibits none (Fig. 3). Our study indicates that the CaOV3^{TaxR} cell line is significantly more resistant to treatment with the drugs tested than the parental cell line (Fig. 4). Previously, the Taylor laboratory [16] found that pharmacological blocking MDR-1 with an inhibitor of the ATP-dependent drug efflux pumps (i.e., verapamil) resensitized CaOV3^{TaxR} cells to paclitaxel [16]. However, when we treated CaOV3^{TaxR} cells with the same and higher concentrations of verapamil (i.e., 1, 2 and 4 μM verapamil), we found no resensitization of these cells to the drugs being tested (data not shown). It would appear, therefore, that MDR-1 is not be involved in the reduced sensitivity to the drugs being tested in this study.

We should also note that of the two head and neck squamous cell carcinoma cell lines shown in Table 1, the SCC25/CP cell line has been reported to be cisplatin resistance (SCC25/CP), while the PCI-13 cell line is cisplatin sensitive [11]. Again, the cisplatin resistant cell line (SCC25/CP) was found to be significantly more resistant to the NSAIDs/NSAID derivatives being tested and than its non-drug resistant counterpart (i.e., PCI-13) (see Table 1).

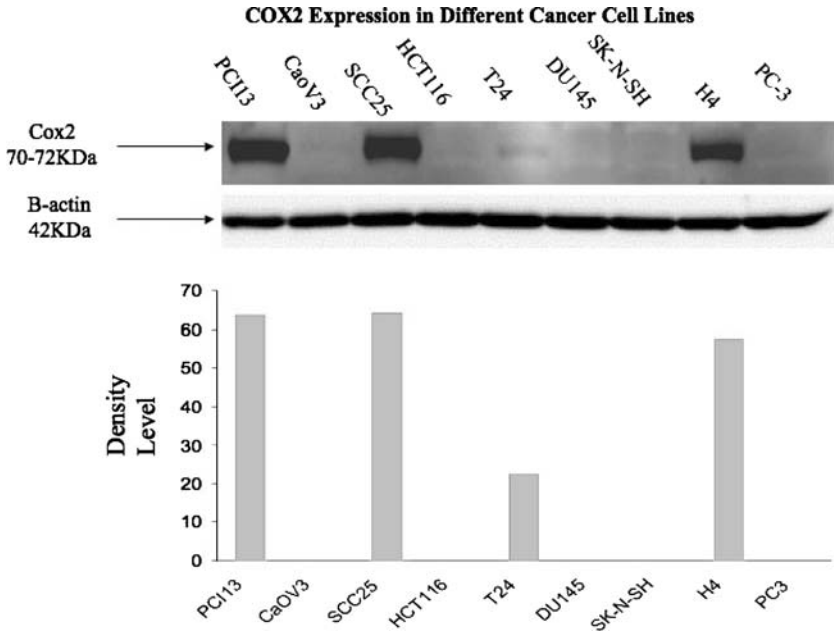

Fig. 2 Immunoblot of COX-2 protein expression in the nine cell lines tested. The cell lines are represented on the immunoblot in the increasing order of sensitivity to the NSAIDS/derivatives. It appears that there is no correlation between COX-2 expression and sensitivity to the drugs studied (see Table 1)

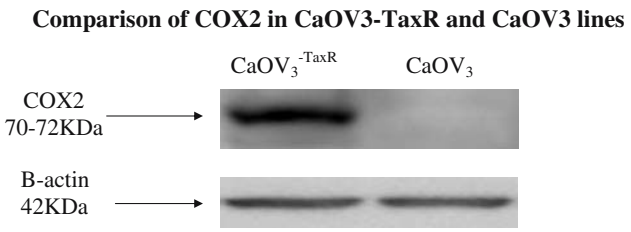

Fig. 3 Immunoblot showing expression of COX-2 protein expression in CaOV3^{TaxR} cells while CaOV3 cells exhibit no detectable COX-2 protein

An evaluation of p75^{NTR} expression by NSAIDs/NSAID derivatives

In this study, we evaluated whether the NSAIDs/NSAID derivatives induced an upregulation in the expression of p75^{NTR} in concert with the observed inhibition of cell growth. Using immunoblot, we analyzed p75^{NTR} expression in T24, DU-145, CaOV3 and CaOV3^{TaxR} cell lines following 48 h treatment with dosages of the NSAIDs/NSAID derivatives that induced significant inhibition of in cell growth (i.e., MTT analysis). Although all the cell lines showed an increased expression of p75^{NTR} in response to all the drugs tested, the expression levels varied in different cell lines. Figure 5 is a representative immunoblot illustrating the significant increase in expression of p75^{NTR} in T24 cells in response to minimal drug dosages that result in a significant decrease in cell growth over the same time period (i.e., see Table 1). Figure 6 shows immunoblot results comparing the expression of p75^{NTR} expression in the CaOV3^{TaxR} versus the CaOV3 parental cell line. The greater up-regulation of p75^{NTR} expression in CaOV3 cells versus the CaOV3^{TaxR} cell line (Fig. 6) correlates with the observed increase inhibition of cell growth in CaOV3 versus the CaOV3^{TaxR} cell line (Fig. 4). The correlation between the expression of p75^{NTR} (Fig. 6) and the percentage of growth inhibition induced by a particular drug (Fig. 4), was not always evident perhaps due to apoptotic degradation of the p75^{NTR} protein. RT-PCR of the DU-145 cell line supported the immunoblot studies showing an upregulation of p75^{NTR} gene expression following treatment with the NSAIDs/Derivatives (Fig. 7).

In order to demonstrate a causal relationship between p75^{NTR} gene expression and drug inhibition of cell growth, we inhibited p75^{NTR} expression in T24 cells with siRNA directed to p75^{NTR} prior to treatment with the NSAIDs/Derivatives. The T24 cell line was chosen because of preliminary studies showing the ease of transfecting this particular cell line (data not shown). Immunoblots of these treated cells indicated a significant reduction in p75^{NTR} expression in cells transfected with p75^{NTR} targeted siRNA compared with the non-targeted siRNA controls (Fig. 8). This reduction in p75^{NTR} expression resulted in less growth inhibition of the T24 cells treated with p75^{NTR} targeted siRNA following exposure to the NSAIDs/derivatives when compared with the non-targeted siRNA treated controls ($P < 0.05$) (Fig. 9).

Discussion

In this investigation we compared the ability of selected NSAIDs and NSAID derivatives to reduce the survival of different cancer cell lines, including drug resistant cancer cells. The NSAIDs and NSAID derivatives where chosen

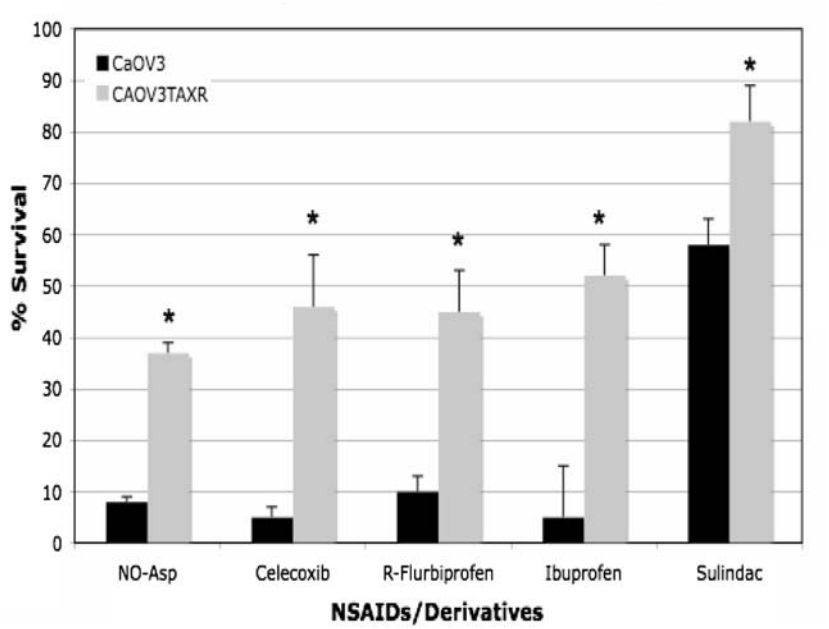

Fig. 4 This graph depicts the growth inhibition of CaOV3 cells (*black*) versus CaOV3^{TaxR} cells (*gray*) following 48 h incubation in 12.5 μM of NO-aspirin, 25 μM celecoxib, 500 μM R-flurbiprofen, 500 μM ibuprofen or 500 μM sulindac sulfone. Error bars = standard error. *Asterisk* signifies that the NSAIDS/Derivatives were significantly more effective in reducing the survival of CaOV3 cells than CaOV3^{TaxR} cells treated with the same dosage of the drug indicated (i.e., $P < 0.05$)

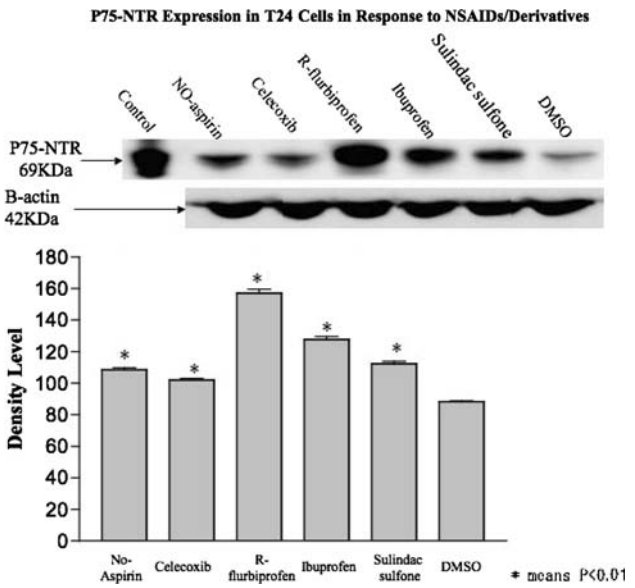

Fig. 5 Immunoblot (*top*) and quantification of the immunoblot (*below*) showing upregulation of p75^{NTR} protein expression in T24 cells following treatment with 5 μM of NO-Aspirin; 25 μM celecoxib; 500 μM ibuprofen, 500 μM R-flurbiprofen or 100 μM sulindac sulfone. *Asterisk* indicates significant differences between the treated cells and the DMSO control (i.e., $P < 0.01$)

based on studies indicating that they showed promise as anti-cancer agents. Other than ibuprofen, the compounds tested had in common reduced gastrointestinal cytotoxicity that is otherwise associated with NSAID treatment [1]. Since these NSAID and NSAID derivatives appear to target cancer cells and are significantly less toxic than traditional chemotherapeutic agents, they represent promising future alternatives for both the treatment and prevention of a variety of cancers.

While all the drugs tested successfully reduced the survival of the cell lines studied, the effective dosages differed significantly. NO-linked aspirin and celecoxib induced apoptosis at the lowest dosages. Therefore, if used for intravesicular (i.e., for superficial bladder cancer [46]) or intrathecal (i.e., for solid tumors [56]) treatment modalities, these NSAIDs might represent the most potent inducers of apoptosis. However, pharmacokinetic data indicates that less than 1 μM of a similar NO-linked aspirin derivative (i.e., NCX 4016 [6]) and less than 5 μM of celecoxib [12] are found in the blood of humans following oral administration of acceptable dosages of these drugs. Therefore, despite their effectiveness, when clinically relevant oral administration is taken into consideration, they may not be as effective as some of the other compounds tested. Specifically, R-flurbiprofen [38] and the over-the-counter NSAID ibuprofen [32] appear to be more effective at close to clinically relevant concentrations. R-flurbiprofen is attractive because it has minimal cyclooxygenase inhibiting activity and has been proven safe in ongoing clinical trials [30, 38, 45]. It should be added that we also found that significantly reduced dosages of these two drugs (i.e., 100 μM) can be effective over longer periods of treatment (i.e., 2 weeks, data not published). Therefore, these compounds might also represent a potential approach to cancer prevention. Indeed, epidemiological data supports the fact that individuals on NSAIDs have significantly reduced incidences of a variety of cancers [4, 49, 51].

To date, NSAIDs and NSAID derivatives used in this study have not been evaluated for their ability to reduce the survival of drug-resistant cancer cell lines. The present study indicates that the NSAIDs/NSAID derivatives that we evaluated appear to be less effective in reducing the survival

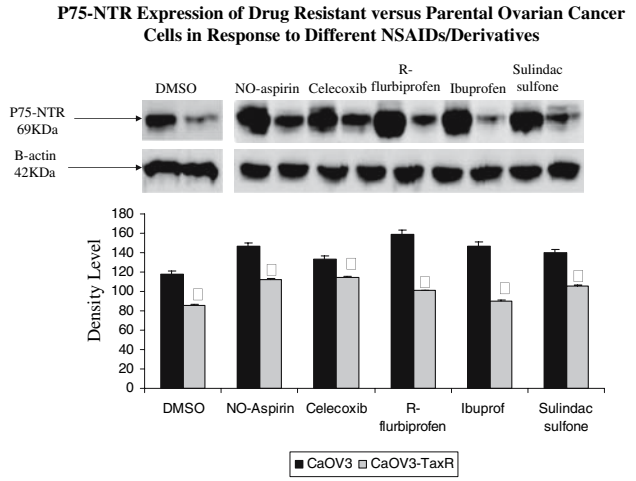

Fig. 6 Immunoblot (*top*) and quantification of the immunoblot (*below*) showing $p75^{NTR}$ protein expression in CaOV3 cells (*black*) versus $CaOV3^{TaxR}$ cells (*gray*) following treatment with 12.5 μM of NO-Aspirin; 25 μM celecoxib; 500 μM ibuprofen, 500 μM R-flurbiprofen and 500 μM sulindac sulfone. All the drugs tested induced a significantly greater expression of $p75^{NTR}$ in CaOV3 cells than in $CaOV3^{TaxR}$ cells (i.e., *small boxes* indicate $P < 0.01$)

of drug-resistant cell lines than the parental cell lines. Also, pharmacological inhibition of MDR-1 with verapamil, failed to restore sensitivity of the drug-resistant ovarian cell line to the drugs. Therefore, other strategies that might increase the susceptibility of drug resistant cells to chemotherapy may be applicable to the NSAIDs/NSAID derivatives evaluated in the present investigation.

Another purpose of the present investigation was to determine if the $p75^{NTR}$ tumor suppressor [28] might represent an anti-growth molecular pathway common to the variety of drugs tested. The molecular basis for the chemoprotective effects of NSAIDs was initially attributed to inhibition of cyclooxygenases [14, 50, 67] since the product of cyclooxygenase activity are thought to contribute to tumor growth by inhibiting apoptosis [53] and by inducing the formation of new blood vessels needed to sustain tumor growth [36, 61]. Although this view was supported by the observations that expression of COX-2 is elevated in tumors [15, 52] and by genetic data indicating that the COX-2 gene in mice is associated with tumorigenesis [40], there is overwhelming evidence to indicate that the ability of NSAIDs to induce apoptosis and inhibit the progression of the cell cycle of cancer cells are due to cyclooxygenase independent mechanisms. Studies of cyclooxygenase-null cells have shown that the antiproliferative and antineoplastic effects of NSAIDs are independent of the inhibition of either COX-1 or COX-2 [71]. Indeed, although two of the NSAID derivatives evaluated in the present study (i.e. R-flurbiprofen, sulindac sulfone) have little cyclooxygenase activity, they have been shown to inhibit tumor growth both in vivo and in vitro as much as the NSAIDs from which they were derived [18, 43–45, 31, 32, 34–36, 49, 62, 66]. Also, although a number of the cell lines studied in our and other studies [41, 54] lack cyclooxygenase-2 activity; they were still responsive to the drugs tested. Furthermore, the NSAIDs studied in this and other investigations inhibit COX and thus prostaglandin synthesis at concentrations that are 10–100-fold less than the concentrations that are associated with anti-tumor activity [59]. Finally, others have shown that in cells producing COX, the COX produced prostaglandins cannot rescue cells from NSAID-associated growth arrest in vivo or in vitro [7, 19, 39]. Taken together, the foregoing strongly suggested that COX-independent mechanisms are involved in the anticancer effects of NSAIDs and NSAID derivatives.

Recently, we proposed that the p75 neurotrophin receptor ($p75^{NTR}$) appears to play a significant role in the anti-growth effectiveness of ibuprofen [26]. Significantly, the $p75^{NTR}$ is an upstream surface receptor whose signaling

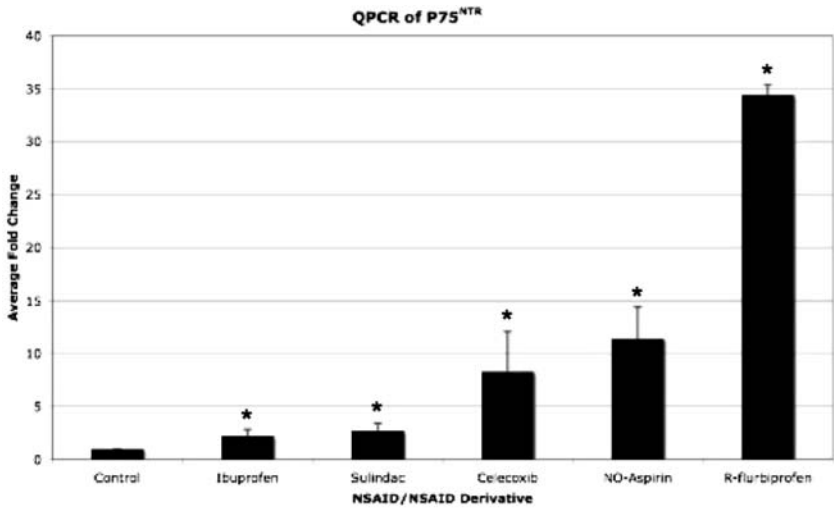

Fig. 7 Real-time Quantitative PCR for $p75^{NTR}$ in DU145 cells following treatment with vehicle (*control*); 5 μM of NO-Aspirin; 25 μM celecoxib; 500 μM ibuprofen, 500 μM R-flurbiprofen and 100 μM sulindac sulfone. *Error bars* represent standard errors. *Asterisks* signify $P < 0.01$ when compared with control

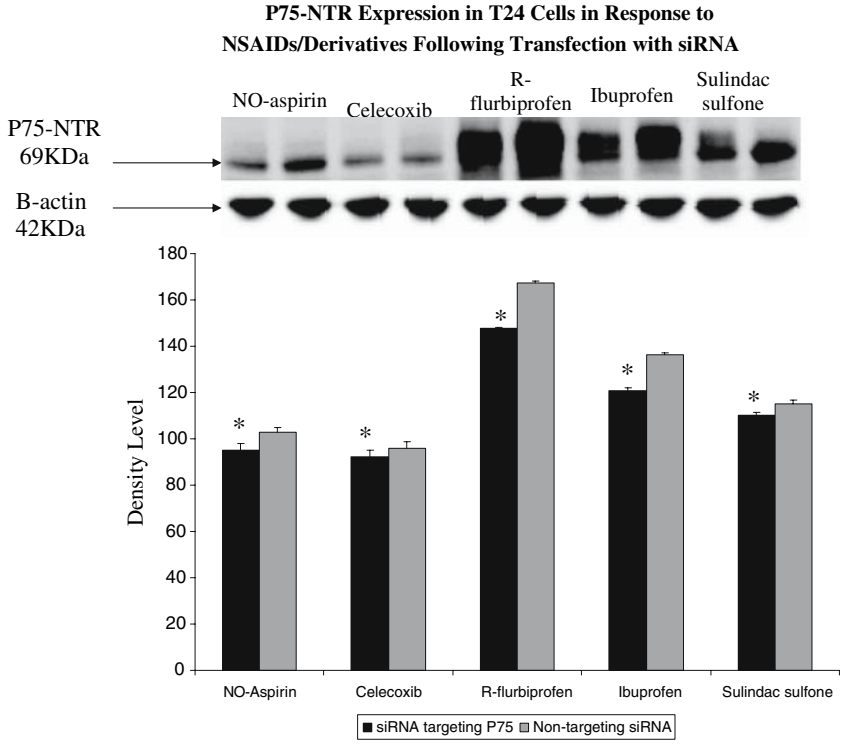

Fig. 8 Immunoblot (*top*) and quantification of the immunoblot (*below*) showing a reduction in $p75^{NTR}$ protein expression in T24 cells which were transfected with $p75^{NTR}$ targeted siRNA (*black*) versus non-targeted siRNA transfected controls (*gray*) and then incubated for 48 h in the presence of 5 μM of NO-aspirin, 25 μM celecoxib, 500 μM R-flurbiprofen, 500 μM ibuprofen, or 100 μM sulindac sulfone. *Asterisk* signifies $P < 0.01$ in $p75^{NTR}$ targeted siRNA (*black*) cells versus non-targeted siRNA controls (*gray*)

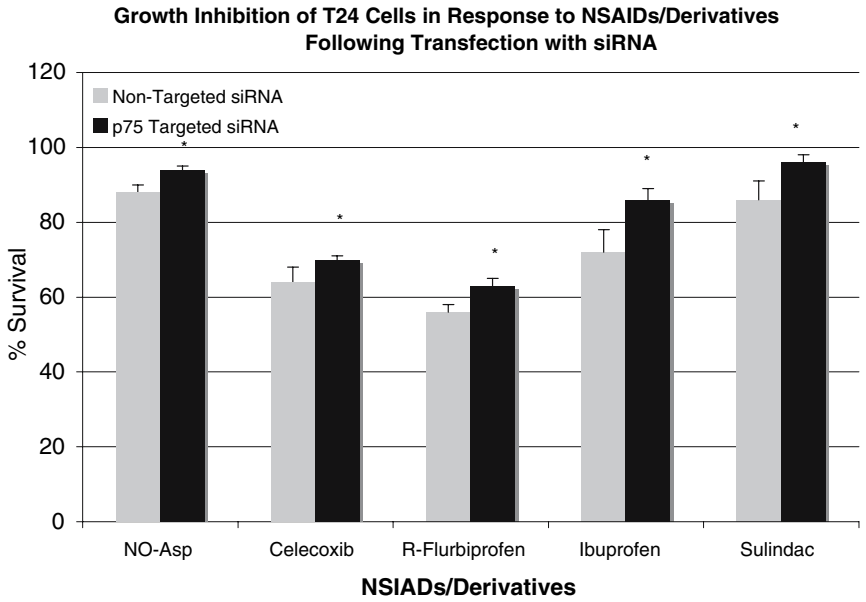

Fig. 9 This graph depicts growth inhibition of T24 cells which had been transfected with non-targeted siRNA (*gray*) or $p75^{NTR}$ targeted siRNA (*black*) and then incubated for 48 h in the presence of 5 μM of NO-aspirin, 25 μM celecoxib, 500 μM R-flurbiprofen, 500 μM ibuprofen, or 100 μM sulindac sulfone. Error bars = standard error. *Asterisk* signifies that the T24 cells pretreated with siRNA targeting $p75^{NTR}$ (*black*) exhibited a significantly less growth inhibition than T24 cells pretreated with non-targeted siRNA (*gray bars*) (i.e., $P < 0.05$)

cascade has been shown to include changes in many of the signaling molecules so far associated with NSAID treatment including down-regulation of NF-kappaB [69], Akt [24], COX-2 production (via its effects on NF-kappa B [27]), an increase in the mitochondrial pro-apoptotic effector proteins Bax and Bik, [58] a decrease in the mitochondrial pro-survival effector proteins phospho-Bad, Bcl-2 and Bcl-xL [58], and an increase in ceramide production [55]. As expected of an upstream signaling molecule, we recently found that $p75^{NTR}$ is expressed very early following treatment with ibuprofen (within a few hours, unpublished observation). In the present investigation, we found that all the NSAIDs and NSAID derivatives that were selected for study also induce an up-regulation of the $p75^{NTR}$ tumor suppressor in concert with their anti-tumorigenic activity. Also, the drugs induced a significantly greater expression of $p75^{NTR}$ in CaOV3 cells then in the drug resistant derivative of this cell line (i.e., $CaOV3^{TaxR}$ cells). Furthermore, selective inhibition of $p75^{NTR}$ with siRNA resulted in a decrease in the ability of the NSAIDs/derivatives to reduce survival

of cancer cells in vitro. Therefore, $p75^{NTR}$ is a molecular pathway that is responsible at least in part for the anti-cancer effectiveness of NSAIDs and their derivatives tested in the present investigation. In view of these findings, the $p75^{NTR}$ tumor suppressor pathway probably deserves attention in the structuring of newer and more effective chemotherapeutic treatments for cancer treatment.

References

1. Allison MC, Howatson AG, Torrance CJ, Lee FD, Russell RI (1992) Gastrointestinal damage associated with the use of non-steroidal anti-inflammatory drugs. N Engl J Med 327:749–754
2. Alshafie GA, Abou-Issa HM, Seibert K, Harris RE. (2000) Chemotherapeutic evaluation of celecoxib, a cyclooxygenase-2 inhibitor, in a rat mammary tumor model. Oncol Rep 7:1377–1381
3. Andrews J, Djakiew D, Krygier S, Andrews PM (2002) Superior effectiveness of ibuprofen compared with other NSAIDs for inhibition of human prostate cancer cell growth. Cancer Chemother Pharmacol 50:277–284
4. Baron JA (2003) Epidemiology of non-steroidal anti-inflammatory drugs and cancer. Prog Exp Tumor Res 37:1–24
5. Basu GD, Pathangey LB, Tinder TL, Lagioia M, Gendler SJ, Mukherjee P (2004) Cyclooxygenase-2 inhibitor induces apoptosis in breast cancer cells in an in vivo model of spontaneous metastatic breast cancer. Mol Cancer Res 2:632–642
6. Carini M, Aldini G, Orioli M, Piccoli A, Tocchetti P, Facino RM (2004) Chemiluminescence and LC–MS/MS analyses for the study of nitric oxide release and distribution following oral administration of nitroaspirin (NCX 4016) in healthy volunteers. J Pharm Biomed Anal 35:277–287
7. Chan TA, Morin PJ, Vogelstein B, Kinzler KW (1998) Mechanisms underlying nonsteroidal anti-inflammatory drug-mediated apoptosis. PNAS 95:681–689
8. Chao MV (1994) The p75 neurotrophin receptor. J Neurobiol 25:1373–1385
9. Chapman BS (1995) A region of the 75kD neurotrophin receptor homologous to the death domains of TNFR-1 and Fas. FEBS Lett 374:216–220
10. Chiroli V, Benedini F, Ongini E, Del Soldato P (2003) Nitric oxide-donating non-steroidal anti-inflammatory drugs: the case of nitroderivatives of aspirin. Eur J Med Chem 38:441–446
11. Cullen KJ, Newkirk KA, Schumaker LM, Aldosari N, Rone JD, Haddad BR (2003) Glutathione S-transferase pi amplification is associated with cisplatin resistance in head and neck squamous cell carcinoma cell lines and primary tumors. Cancer Res 63:8097–8102
12. Davies NM, McLachlan AJ, Day RO, William KM (2000) Clinical pharmacokinetics and pharmacodynamics of celecoxib: a selective cyclooxygenase-2 inhibitor. Clin Pharmacokinet 38:225
13. Diperna CA, Bart RD, Sievers EM, Ma Y, Starnes VA, Bremner RM (2003) Cyclooxygenase-2 inhibition decreases primary and metastatic tumor burden in a murine model of orthotopic lung adenocarcinoma. J Thorac Cardiovasc Surg 126:1129–1133
14. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE (1998) Cyclooxygenase in biology and disease. FASEB J 12:1063–1073
15. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN (1994) Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 107:1183–1188
16. George JA, Chen T, Taylor CC (2005) Src tyrosine kinase and multidrug resistance protein-1 inhibitions act independently but coorperatively to restore pacilitaxel sensitivity to paclitaxel-resistant ovarian cancer cells. Cancer Res 65:10381–10388
17. Goluboff ET, Shabsigh A, Saidi JA, Weinstein IB, Mitra N, Heitjan D, Piazza GA, Pamukcu R, Buttyan R, Olsson CA (1999) Exisulind (sulindac sulfone) suppresses growth of human prostate cancer in a nude mouse xenograft model by increasing apoptosis. Urology 53:440–445
18. Grubbs CJ, Lubet RA, Koki AT, Leahy KM, Masferrer JL, Steele VE, Kelloff GJ, Hill DL, Seibert K (2000) Celecoxib inhibits N-butyl-N-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancers in male B6D2F1 mice and female Fischer-344 rats. Cancer Res 15:5599–5602
19. Hanif R, Pittas A, Feng Y, Koutsos MI, Qiao L, Staiano-Coico L, Shiff SI, Rigas B (1996) Effects of nonsteroidal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-independent pathway. Biochem Pharmacol 52:237–245
20. Harris RE, Alshafie GA, Abou-Issa H, Seibert K (2000) Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. Cancer Res 60:2101–2103
21. Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS (2000) The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking akt activation in human prostate cancer cells independently of Bcl-2. J Biol Chem 275:11397–11400
22. Huber LJ, Chao MV (1995) Mesenchymal and neuronal cell expression of the p75 neurotrophin receptor gene occur by different mechanisms. Develop Biol 167:227–238
23. Huguenin S, Vacherot F, Kheuang L, Fleury-Feith J, Jaurand MC, Bolla M, Riffaud JP, Chopin DK (2004) Antiproliferative effect of nitrosulindac (NCX 1102), a new nitric oxide-donating non-steroidal anti-inflammatory drug, on human bladder carcinoma cell lines. Mol Cancer Ther 3:291–298
24. Ito H, Nomoto H, Furukawa S (2003) Growth arrest of PC12 cells by nerve growth factor is dependent on the phosphatidylinositol 3-kinase/Akt pathway via p75 neurotrophin receptor. J Neurosci Res 72:211–217
25. Kashfi K, Ryan Y, Qiao LL, Williams JL, Chen J, Del Soldato P, Traganos F, Rigas B, Ryann Y (2002) Nitric oxide-donating non-steroidal anti-inflammatory drugs inhibit the growth of various cultured human cancer cells: evidence of a tissue type-independent effect. J Pharmacol Exp Ther 303:1273–1282
26. Khwaja F, Allen J, Lynch J, Andrews P, Djakiew D (2004) Ibuprofen inhibits survival of bladder cancer cells by induced expression of the $p75^{NTR}$ tumor suppressor protein. Cancer Res 64:6207–6213
27. Konturek PC, Nikiforuk A, Kania J, Raithel M, Hahn EG, Muhldorfer S 2004 Activation of NFkappaB represents the central event in the neoplastic progression associated with Barrett's esophagus: a possible link to the inflammation and over expression of COX-2, PPARgamma and growth factors. Dig Dis Sci 49:1075–1083
28. Krygier S, Djakiew D (2002) Neurotrophin Receptor $p75^{NTR}$ suppresses growth and nerve growth factor-mediated metastasis of human prostate cancer cells. Int J Cancer 98:1–7
29. Latil A, Bieche I, Vidaud D, Lidereau R, Berthon P, Cusenot O, Vidaud M (2001) Evaluation of androgen, estrogen (ER alpha and ER beta), and progesterone receptor expression in human prostate cancer by real-time quantitative reverse transcription-polymerase chain reaction assays. Cancer Res 61:1919–1926
30. Lilley M, Ruckle H, Quiggle D, Gutierrez I, Murray ED, Gibson K, Leipold D, Wechter WJ, Loughman B (2001) Multi-dose phase I-II trial of E-7869 in prostate cancer patients: Safety and time to PSA progression (TPSAP). Proc of AACR 42:142
31. Lim JT, Piazza GA, Han EK, Delohery TM, Li H, Finn TS, Buttyan R, Yamamoto H, Sperl GJ, Brendel K, Gross PH, Pamukcu R, Weinstein IB (1999) Sulindac derivatives inhibit

growth and induce apoptosis in human prostate cancer cell lines. Biochem Pharmacol 58:1097–1015
32. Lockwood GF, Albert KS, Gillespie WR, Bole GG, Harkcom TM, Szpunar GJ, Wagner JG (1983) Pharmacokinetics of ibuprofen in man. I. Free and total area/dose relationships. Clin Pharmacol Ther 34:97–103
33. McCracken JD, Wechter WJ, Lui Y, Chase RL, Kantoci D, Murray ED, Quiggle DD, Mineyama Y (1996) Antiproliferative effects of the enantiomers of flurbiprofen. J Clin Pharmacol 36:540–545
34. Mahmoud NN, Boolbol SK, Dannenberg AJ, Mestre JR, Bilinski RT, Martucci C, Newmark HL, Chadburn A, Bertagnolli MM (1998) The sulfide metabolite of sulindac prevents tumors and restores enterocyte apoptosis in a murine model of familial adenomatous polyposis. Carcinogenesis 19:87–91
35. Malkinson AM, Koski KM, Dwyer-Nield LD, Rice PL, Rioux N, Castonguay A, Ahnen DJ, Thompson H, Pamukcu R, Piazza GA (1998) Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced mouse lung tumor formation by FGN-1 (sulindac sulfone) Carcinogenesis 19:1353–1356
36. Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, Edwards DA, Flickinger AG, Moore RJ, Seibert K (2000) Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 60:1306–1311
37. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival. J Immunol Methods 65:560–567
38. Murray ED, Quiggle D, Gibson K, Leipold D, Loughman B, Wechter W (2000) Phase I single-dose pharmacokinetics and lack of bioinversion of E-7869 (R-flurbiprofen). Clin Pharma Ther 67:103
39. Narisawa T, Hermanek P, Habs M, Schmahl D (1984) Reduction of carcinogenicity of N-nitrosomethylurea by indomethacin and failure of resuming effect of prostaglandin E2 (PGE2) against indomethacin. J Cancer Res Clin Oncol 108:239–242
40. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM (1996) Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87:803–809
41. Park C, Kim GY, Kim GD, Choi BT, Park YM, Choi YH (2006) Induction of G2/M arrest and inhibition of cyclooxygenase-2 activity by curcumin in human bladder cancer T24 cells. Oncol Rep 15:1225–1231
42. Piazza GA, Alberts DS, Hixson LJ, Paranka NS, Li H, Finn T, Bogert C, Guillen JM, Brendel K, Gross PH, Sperl G, Ritchie J, Burt RW, Ellsworth L, Ahnen DJ, Pamukcu R (1997) Sulindac sulfone inhibits azoxymethane-induced colon carcinogenesis in rats without reducing prostaglandin levels. Cancer Res 57:2909–2915
43. Piazza GA, Rahm AK, Finn TS, Fryer BH, Li H, Stoumen AL, Pamukcu R, Ahnen DJ (1997) Apoptosis primarily accounts for the growth-inhibitory properties of sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest, and p53 induction. Cancer Res 57:2452–2459
44. Piazza GA, Thompson WJ, Pamukcu R, Alila HW, Whitehead CM, Liu L, Fetter JR, Gresh WE Jr, Klein-Szanto AJ, Farnell DR, Eto I, Grubbs CJ (2001) Exisulind, a novel proapoptotic drug, inhibits rat urinary bladder tumorigenesis. Cancer Res 61:3961–3968
45. Quiggle DD, Lilly M, Murray ED, Gibson KM, Leipold DD, Gutierrez I, Loughman BE, Wechter WJ (2000) PK guided multi-dose, tolerance and safety of E-7869 in prostate cancer patients. Proc Am Assoc Cancer Res 41:609
46. Rath GD (1992) Bladder cancer, 1992. Postgrad Med 92:105–124
47. Reddy BS, Hirose Y, Lubet R, Steele V, Kelloff G, Paulson S, Seibert K, Rao CV (2000) Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. Cancer Res 60:293–297
48. Reddy BS, Kawamori T, Lubet RA, Steele VE, Kelloff GJ, Rao CV (1999) Chemopreventive efficacy of sulindac sulfone against colon cancer depends on time of administration during carcinogenic process. Cancer Res 59:3387–3391
49. Roberts RO, Jacbson DJ, Girman CJ, Rhodes T, Lieber MM, Jacobsen SJ (2002) A population-based study of daily nonsteroidal anti-inflammation drug use and prostate cancer. Mayo Clin Proc 77:219–225
50. Ruegg C, Zaric J, Stupp R (2003) Non steroidal anti-inflammatory drugs and COX-2 inhibitors as anti-cancer therapeutics: hypes, hopes and reality. Ann Med 35:476–487
51. Sabichi AL, Lippman SM (2004) COX-2 inhibitors and other nonsteroidal anti-inflammatory drugs in genitourinary cancer. Semin Oncol 31:36–44
52. Sano H, Kawahito Y, Wilder RL, Hashiramoto A, Mukai S, Asai K, Kimura S, Kato H, Kondo M, Hla T (1995) Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res 55:3785–3789
53. Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN (1998) Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res 58:362–366
54. Smith ML, Hawcroft G, Hull MA (2000) The effect of nonsteroidal anti-inflammatory drugs on human colorectal cancer cells: evidence of different mechanisms of action. Eu J Cancer 36:664–674
55. Song MS, Posse de Chaves EI (2003) Inhibition of rat sympathetic neuron apoptosis by ceramide. Role of p75NTR in ceramide generation. Neuropharmacology 45:1130–1150
56. Stewart DJ (1984) Novel modes of chemotherapy administration. Prog Exp Tumor Res 28:32–50
57. Taketo MM (1998) Cyclooxygenase-2 inhibitors in tumorigenesis. (Part II) J Natl Cancer Inst 90:1609–1620
58. Tabassum A, Khwaja F, Djakiew D (2003) The p75(NTR) tumor suppressor induces caspase-mediated apoptosis in bladder tumor cells. Int J Cancer 105:47–52
59. Tegeder I, Pfeilschifter J, Geisslinger G (2001) Cyclooxygenase-independent actions of cyclooxygenase, inhibitors. FASEB J 15:2057–2072
60. Thompson HJ, Jiang C, Lu J, Mehta RG, Piazza GA, Paranka NS, Pamukcu R, Ahnen DJ (1997) Sulfone metabolite of sulindac inhibits mammary carcinogenesis. Cancer Res 15:267–271
61. Tsujii M, Kawano S, Tsuiji S, Sawaoka H, Hori M, DuBois RN (1998) Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 93:705–716
62. Vainio H (2001) Is COX-2 inhibition a panacea for cancer prevention? Int J Cancer 1:613–614
63. Wechter WJ, Kantoci D, Murray ED Jr, Quiggle DD, Leipold DD, Gibson KM, McCracken JD (1997) R-flurbiprofen chemoprevention and treatment of intestinal adenomas in the APC(Min)/+ mouse model: implications for prophylaxis and treatment of colon cancer. Cancer Res 57:4316–4324
64. Wechter WJ, Leipold DD, Murray ED, Quiggle D, McCracken JD, Barrios RS, Greenberg NM (2000) E-7869 (R-Flurbiprofen) inhibits progression of prostate cancer in the TRAMP mouse. Cancer Res 60:2203–2208
65. Wilgus TA, Koki AT, Zweifel BS, Rubal PA, Oberyszyn TM (2003) Chemotherapeutic efficacy of topical celecoxib in a murine model of ultraviolet light B-induced skin cancer. Mol Carcinog 38:33–39
66. Witta SE, Gustafson DL, Pierson AS, Menter A, Holden SN, Basche M, Persky M, O'Bryant CL, Zeng C, Baron A, Long ME, Gibbs A, Kelly K, Bunn PA Jr, Chan DC, Pallansch P, Eckhardt SG (2004) A phase I and pharmacokinetic study of exisulind and docetaxel in patients with advanced solid tumors. Clin Cancer Res 1:7229–7237

67. Xu XC (2002) COX-2 inhibitors in cancer treatment and prevention, a recent development. Anticancer Drugs 13:127-137
68. Yeh RK, Chen J, Williams JL, Baluch M, Hundley TR, Rosenbaum RE, Kalala S, Traganos F, Benardini F, del Soldato P, Kashfi K, Rigas B (2004) NO-donating nonsteroidal antiinflammatory drugs (NSAIDs) inhibit colon cancer cell growth more potently than traditional NSAIDs: a general pharmacological property? Biochem Pharmacol 67:2197–2205
69. Yoon SO, Casaccia-Bonnefil P, Carter B, Chao MV (1998) Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival. J Neurosci 18:3273–3281
70. Zha S, Yegnasubramanian V, Nelson WG, Isaacs WB, De Marzo AM, Zha S, Yegnasubramanian V, Nelson WG, Isaacs WB, De Marzo AM (2004) Cyclooxygenases in cancer: progress and perspective. Cancer Lett 215:1–20
71. Zhang X, Morham SG, Langenbach R, Young DA (1999) Malignant transformation and antineoplastic actions of nonsteroidal antiinflammatory drugs (NSAIDs) on cyclooxygenase-null embryo fibroblasts. J Exp Med 190:451–459
72. Zweifel BS, Davis TW, Ornberg RL, Masferrer JL (2002) Direct evidence for a role of cyclooxygenase 2-derived prostaglandin E2 in human head and neck xenograft tumors. Cancer Res 62(22):6706–6711