Cyclooxygenase-2 Modulates the Insulin-Like Growth Factor Axis in Non–Small-Cell Lung Cancer

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ABSTRACT

Constitutive overexpression of cyclooxygenase-2 (COX-2) occurs frequently in several different malignancies, including lung, colon, breast, and prostate cancer. Clinical studies have established elevated serum insulin-like growth factor (IGF-I) content and IGF-I:IGF-binding protein 3 (IGFBP-3) ratio as risk factors for these same malignancies. Therefore, we sought to determine the link between COX-2 expression and the IGF axis in COX-2–gene-modified human non–small-cell lung cancer (NSCLC) cells. Overexpression of COX-2 in NSCLC cells enhanced the antiapoptotic and mitogenic effects of IGF-I and IGF-II, facilitated the autophosphorylation of the type 1 IGF receptor, increased class IA phosphatidylinositol 3′-kinase activity, and decreased expression of IGFBP-3. Thus, these findings show that COX-2 augments the stimulatory arm of the IGF axis.

INTRODUCTION

Cyclooxygenase (COX) is the rate-limiting enzyme for the production of prostaglandins and thromboxanes from free arachidonic acid. Two isoforms of COX have now been described: a constitutively expressed enzyme COX-1, present in most cell lines, and an inducible form, COX-2, expressed in response to cytokines, growth factors, and various other stimuli (1). Tumor cells with elevated COX-2 levels are highly apoptotic resistant (2, 3), angiogenic (4), invasive (3, 5–7), and suppressive of host immunity (8, 9). We and others have reported previously that COX-2 is overexpressed in human non–small-cell lung cancer (NSCLC) and that inhibition of COX-2 leads to in vivo tumor reduction in various murine models for human cancer (10–15).

The insulin-like growth factor (IGF) axis is a signal transduction system composed of IGFs, IGF-binding proteins (IGFBPs), their receptors, and proteases that modulate the activity of IGFBPs. IGF-I and IGF-II activate the IGF axis via type 1 IGF receptor (IGF-IR), whereas the IGFBPs inhibit the IGF axis by a variety of IGF-dependent and -independent mechanisms (16–18). In brief, shifting the balance along the IGF axis affects proliferation, survival, and differentiation of normal and tumor cells. Epidemiologic studies indicate that events resulting in greater activation potential for the IGF axis, such as increased serum IGF-I and decreased IGFBP-3 levels, are risk factors for various malignancies, including lung (19), prostate (20), breast (21), and colon cancer (22). In support of increased systemic IGF-I as a risk factor, studies show that systemically administered recombinant IGF-I enhances tumor growth in athymic mice (23), whereas the reduced systemic IGF-I levels delayed the onset of chemically and genetically induced mouse mammary tumors (24).

Like COX-2, IGF-IR is also associated with in vitro and in vivo malignant transformation (25–30). Studies show that IGF-IR significantly enhances apoptosis resistance (28), proliferation (29), and metastasis (30). Hence, we speculated that studying tumor phenotypes associated with COX-2 and various members of the IGF axis would help delineate how tumor COX-2 expression regulates the malignant phenotype in NSCLC.

IGF-IR is a protein tyrosine-kinase cell surface receptor composed of an extracellular ligand binding α-subunit and an intracellular β-subunit (31). In normal conditions, the access of IGF-I and IGF-II to IGF-IR, as well as the activity of IGF-IR, are tightly controlled by a number of factors, including IGFBPs (16, 17). Activation of IGF-IR up-regulates phosphatidylinositol 3′-kinase/akt kinase (PI3k/akt) signaling and increases proliferation and survival (32–34). As previous studies show, (1) NSCLC cells frequently harbor high levels of COX-2 (10, 11) and PI3k/akt (35); (2) up-regulation of COX-2 in tumor cells partially depends on PI3k/akt (36); and (3) a COX-2 inhibitor drug, celecoxib, down-regulates AKT activity (37). Thus, a functional link between COX-2, IGF-IR, and PI3k/akt signaling is suggested by these studies.

To determine the effect of COX-2 on IGF signaling in NSCLC cells, we used an experimental strategy previously used by Tsujii and DuBois (3). Namely, we expressed COX-2 in sense and antisense orientations in an NSCLC cell line, A549. Our study shows that COX-2 strengthens the IGF-I– and IGF-II–related survival and proliferation of A549 cells by modulating the autophosphorylation of IGF-IR, the class IA PI3k activity, and expression of IGFBP-3. Overexpression of COX-2 also enhanced the sensitivity of A549 cells to the antiproliferative effect of PI3k inhibitors. We also found that celecoxib down-regulated the class IA PI3k activity in a number of COX-2–expressing NSCLC cell lines, thus, supporting the role of COX-2 as a modulator of the IGF axis. This is the first report linking NSCLC COX-2 expression with the events that augment the stimulatory arm of the IGF axis.

MATERIALS AND METHODS

Cells. A549-S and A549-AS overexpressing the human COX-2 cDNA in sense and antisense orientations, respectively, have been described by us elsewhere (5, 6). NSCLC cell lines H460 (large cell pleural effusion carcinoma), A-427 (adenocarcinoma), and SK-LU-1 (adenocarcinoma) were obtained from the American Type Culture Collection (Manassas, VA). RH2 (squamous cell carcinoma) was propagated in our laboratory as described previously (38).

Drugs. SC58236 (provided by Searle/Pharmacia, Peapack, NJ) was dissolved in EtOH at 100 mg/mL. Celecoxib (Pfizer, New York, NY) was dissolved in DMSO at 25 mmol/L. NS398 (Sigma, St. Louis, MO) was dissolved in EtOH at 10 mmol/L. IGF-1 and IGF-II (Chemicon, Temecula, CA) and Long R′IGF-I (Cell Sciences, Canton, MA) were dissolved at 100 μg/mL in water, 10 mmol/L acetic, and 10 mmol/L hydrochloric acid, respectively. LY294,002 (Alexis Pharmaceutical, San Diego, CA) was dissolved at 20 mmol/L in EtOH. Wortmannin (Calbiochem, La Jolla, CA) was dissolved at 2 mmol/L in DMSO. 16,16-Dimethyl-prostaglandin E2 (dm-PGE2; Sigma) was dissolved at 10 μg/mL in EtOH.
Flow Cytometry. Annexin V-FITC and propidium iodide double-staining (API) of A549 cells was measured using FACScan (Becton Dickinson, San Jose, CA) at the UCLA Flow Cytometry Lab. From each sample, ~10,000 cells were analyzed for API, and results presented as dot plots where the FL1 and FL2 values indicated the intensity of annexin V-FITC and propidium iodide staining, respectively (Fig. 1). The viable portion of cells was determined as a population of cells with low annexin V-FITC and low propidium iodide staining (Fig. 1A and D). The WinMDI 2.8 (http://facs.scripps.edu/software.html) and CellQuest (Becton Dickinson, Franklin Lakes, NJ) software were used to analyze results. Cells were stained with annexin V-FITC and propidium iodide using ApoTarget kit (BioSource International Inc., Camarillo, CA). Cells were plated in six-well plates. For API, supernatants were collected in 5-mL polystyrene tubes (Fisher, Hampton, NH), and cells were trypsinized for 4 to 5 minutes in 0.2 mL of trypsin/EDTA. Trypsin was neutralized by and cells were resuspended in 2.0 mL of 10% fetal bovine serum (FBS)-RPMI 1640. The resuspended cells were collected in the same tube as their respective supernatants and pelleted at 2400 rpm (1500 x g) for 5 minutes in Clay Adams Sero-Fuge 2002 (Becton Dickinson). The cells then were washed once with 2.0 mL of PBS, collected by centrifugation as described previously, and resuspended gently in 0.1 mL of annexin binding buffer containing annexin V-FITC and propidium iodide as specified by the manufacturer and incubated in the dark at room temperature for 15 minutes. At the end of this 15-minute incubation, the volume of cell suspensions was increased to 0.5 mL by annexin binding buffer, and cells were analyzed for API within 15 to 30 minutes.

Viability and Cell Death of A549 Cells by API. A distribution of the API signal characteristic to viable A549-S and A549-AS and representing low annexin V-FITC and low propidium iodide staining was determined separately for A549-S and A549-AS in each experiment. The double low API staining was defined as the predominant API signal produced by the control group of cells grown in 10% FBS-RPMI 1640 for the duration of the experiment (Figs. 1 and 2). As determined by trypan blue, this control group was always ~95% percent viable. In fluorescence-activated cell sorting (FACS) analysis, these control cells produced an API signal that formed a single major elliptically
COX-2 AUGMENTS THE IGF SIGNALING

The COX-2-enhanced mitogenicity of IGFs was calculated as follows:

\[ P = (T - B)(C - B), \]

where

- \( T \) = average final cell count or \( A_{900} \) of treated group;
- \( C \) = average final cell count or \( A_{900} \) of untreated control group; and
- \( B \) = average baseline cell count or \( A_{900} \) at the start of treatment.

The COX-2-enhanced mitogenicity of IGFs was calculated as follows:

\[ E = P_S - P_A, \]

where

- \( P_S \) = proliferation of A549-S in response to IGFs;
- \( P_A \) = proliferation of A549-AS in response to IGFs.

In the IGF proliferation assays (see below), the cell counts as determined by hemacytometer were used to calculate the aforementioned values. In the MTS assay (see below), the absorption values at 490 nm (\( A_{490} \)) were used to determine proliferation of A549-S and A549-AS.

**IGF Proliferation Assay.** Confluent A549-S and A549-AS were harvested, counted by hemacytometer, plated in six-well plates (Nunc, Rochester, NY) at 50,000 cells per well in 10% FBS-RPMI 1640, and grown for 48 hours. At this 48-hour time point, cells were washed twice with PBS and then supplemented with serum-free RPMI 1640 containing either IGF-I or IGF-II at various concentrations. Control cells were supplemented with SFM alone. At this time point, the baseline (B) values for A549-S and A549-AS were determined as an average of cell counts in six wells. All of the groups were grown in triplicate for an additional 72 hours. The cells were counted by hemacytometer at the end of experiment.

**MTS Assay.** The MTS assay was carried out using CellTiter AQueous One Solution Cell Proliferation Assay (Promega Corp., Madison, WI). The forma- zan formation in MTS assay is nearly proportional to the cell number and thus can be used to determine proliferation and cytotoxicity (18). Cells were plated at 2500 to 3000 cells in 0.1 mL of 10% FBS-RPMI 1640 in well-96-well plates, grown for 36 to 48 hours, washed twice with PBS, and supplemented with SFM containing IGF-I, IGF-II, or combination of one of the IGFs with a COX-2 inhibitor drug, SC58236. The cells then were grown for an additional 48 hours, and the MTS assay was carried out using the manufacturer’s specifications. The A490 values were determined using Benchmark Microplate Reader (Bio-Rad, Hercules, CA). All of the groups were tested in sets of six wells. Individual baseline (B) values for plates at the beginning of IGF/drug treatments were determined as an average \( A_{900} \) of 12 wells. In experiments in which the growth inhibitory effect of LY294,002 and Wortmannin in 10% FBS-RPMI 1640 was determined, the cells were plated as described previously and grown for 48 hours, at which time point they were washed once with PBS and then supplemented with fresh 10% FBS-RPMI 1640 containing PI3k inhibitor drugs at various concentrations (Fig. 5A). In experiments with combined use of the PI3k inhibitor drug LY294,002 and IGFs, cells were plated at 6000 cells per well in 0.1 mL of 10% FBS-RPMI 1640, grown overnight, washed once with PBS, provided with IGFs and LY294,002, and assayed 48 hours after the introduction of treatments.

Adjusting the COX-2-Enhanced Mitogenicity of IGFs for Viability. The proliferation (P) of A549-S and A549-AS as determined by three independent IGF proliferation assays was adjusted for viability as determined by three independent API double-staining/flow experiments as follows: \( P_V = P \times V \) (see above). The viability-adjusted COX-2–enhanced mitogenicity (\( E_V \)) of IGF-II was calculated as follows: \( E_V = P_{SV} - P_{AV} = 19.4\% \), where \( P_{SV} \) and \( P_{AV} \) are the viability-adjusted proliferation of A549-S and A549-AS, respectively. \( E_V = 19.4\% \) is in good agreement with two independent series of MTS assays, which determined that the COX-2–enhanced mitogenicity of IGF-II was 22.7 to 26% (Figs. 3B and 4C).

**Immunoprecipitation and Western Blot Analysis of IGF-IR.** For IGF-IR immunoprecipitation, the original proliferation assay was scaled up in T25 flasks. Confluent A549-S and A549-AS cells were trypsinized, and 1.2 to 1.5 \( \times 10^6 \) cells were plated in 4.0 mL of 10% FBS-RPMI 1640 in T25 flasks for 48 hours. At this 48-hour time point, the cells were washed twice with 4.0 mL of PBS, provided with 4.0 mL of SFM containing IGF-II at 25 ng/mL, and grown for an additional 48 hours. The SC58236 and dm-PGE2 treatment at 12.5 \( \mu \)g/mL and 1.0 \( \mu \)g/mL, respectively, was started at plating, and the fresh drug was added simultaneously with an SFM. For immunoprecipitation of IGF-IR, cells were washed twice with PBS and then lysed in ice-cold RIPA buffer [50 mMol/L Tris-HCl (pH 7.4), 150 mMol/L NaCl, 1% NP40, and 0.25% Na-deoxycholate] containing protease (Complete Mini; Roche, Basel, Switzerland) and phosphatase inhibitors (NaF and NaVO3 at 1 mMol/L; Sigma, St. Louis, MO). Protein concentrations were determined using BCA Protein Assay Kit (Pierce, Rockford, IL). Fifty micrograms of total protein were used...
to immunoprecipitate IGF-IR by 2 µg of rabbit polyclonal anti-IGF-IR β-subunit antibody (SC-713; Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. Then, 40 µL of protein A-agarose (SC-2001; Santa Cruz Biotechnology) slurry were added to the lysates for an additional 6 hours at 4°C. Protein A-agarose beads were washed twice with 0.8 mL of ice-cold PBS. PBS was removed carefully from the tubes, and 100 µL of 2× sample loading buffer were added to the protein A-agarose beads. Samples were boiled for 5 to 7 minutes, and 20 µL were loaded on 8% polyacrylamide gel. Western hybridization was carried out using a rabbit anti-phospho-IGF-IR antibody (SC-713; Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C, and Western hybridization was carried out with anti-phospho-IGF-IR-Tyr1131 antibody. IGF-II, SC58236, and dm-PGE2 were used at 25 ng/mL, 5.0 µg/mL (12.5 µmol/L), and 20 mmol/L NaCl supplemented with ATP at 25 µmol/L.

PI3k Class IA Activity by ELISA. PI3k activity was determined using PI3k ELISA (Echelon Biosciences Inc., Salt Lake City, UT). This kit measures PI3k activity as a conversion of PI(4,5)P2 into PI(3,4,5)P3. We used this kit in conjunction with anti-p85 PI3k antibody (Upstate Biotechnology, Lake Placid, NY). Thus, we specifically determined the class IA activity of PI3k. Briefly, cells were washed three times with ice-cold buffer A (see the manufacturer’s specifications) and then lysed in buffer A containing 1% NP40 and 1 mmol/L phenylmethylsulfonyl fluoride. Lysates were incubated on ice for 20 to 30 minutes and then cleared at 16,000 × g for 20 minutes. PI3k was immunoprecipitated by polyclonal anti-p85 PI3k antibody at 4°C for 1 hour. Binding of the immunoprecipitated p85/p110 PI3k complexes to protein A-agarose beads was carried out at 4°C for 1 hour, and the beads then were washed three times with buffer A containing 1% NP40, three times with buffer B, and twice with buffer C as specified by the manufacturer. The kinase reaction was carried out for 1 hour at room temperature in 4 mmol/L MgCl2, 20 mmol/L Tris-HCl (pH 7.4), and 20 mmol/L NaCl supplemented with ATP at 25 µmol/L.

IGFBP-3 ELISA. IGFBP-3 in the supernatants and cell lysates of A549-S and A549-AS was determined by Active ELISA kit for IGFBP-3 (Diagnostic System Laboratories, Webster, TX). Twenty-five microliters of a supernatant or cell lysate of A549-S and A549-AS diluted in 50 µL of assay buffer were used to quantify IGFBP-3 as specified by the manufacturer.

Statistics. P values were calculated using two-tailed Student’s t test assuming equal variance and paired t test.

RESULTS

Overexpression of Tumor Cell COX-2 Enhances the Antiproapoptotic Effect of IGFs. IGF-II significantly protected A549-S (Figs. 1 and 2A, black bars) and A549-AS (Figs. 1 and 2A, white bars) against SFM-induced cell death. Similar data were obtained with IGF-I (data not shown). Importantly, COX-2 overexpression enhanced IGF-mediated protection against SFM-induced cell death by 27% (Fig. 2A, red connectors). As depicted in Figs. 1 and 2C–F, the prevailing type of cell death in our experimental model was apoptosis. Consistent with these findings, a COX-2 inhibitor drug, SC58236, also reduced the antia apoptotic effect of IGF-II (Fig. 2B, red connectors) and IGF-I (data not shown) in A549-S.

Inhibition of Tumor Cell COX-2 Increases Susceptibility to Apoptosis. As evident in Fig. 2C and D, the COX-2 inhibitor drug SC52368 did not alter the viability of A549-S cells in serum-containing medium (red numerals). In contrast, as depicted in Fig. 2E and F, the viability of SC52368-treated A549-S was significantly decreased in SFM compared with the untreated control A549-S (64.3 ± 3.2% versus 78.7 ± 3.4% as measured in quadruplicate; P = 0.0003). Consistent with the data in COX-2 gene-modified cells (Fig. 2A, dashed connectors), the SFM-induced cell death was significantly greater in the SC52368-treated A549-S compared with the untreated control A549-S cells (Fig. 2B, dashed connectors).

Fig. 3. A and B, COX-2 enhances mitogenicity of IGFs. The IGF-I– and IGF-II–stimulated proliferation in the IGF proliferation, and MTS assay was calculated as an average ± SEM of three, and four or five independent experiments, respectively. Numerals below x-axis indicate concentrations of IGF-I and IGF-II (µg/mL). C–E, IGF-IR is a COX-2 downstream target. Fifty micrograms of total protein were immunoprecipitated by anti–IGF-IR β-subunit antibody, and Western hybridization was carried out with anti-phospho-IGF-IR-Tyr1131 antibody. IGF-II, SC58236, and dm-PGE2 were used at 25 ng/mL, 5.0 µg/mL (12.5 µmol/L), and 1.0 µg/mL, respectively. ■ A549-S; □ A549-AS; ■ A549-S + SC58236. * P < .05.

Fig. 4. Overexpression of COX-2 enhances the antiproliferative effect of PI3k inhibitors. A, Cells were treated with LY294,002 and Wortmannin in 10% FBS-RPMI 1640. B and C, Cells were plated and grown overnight in 10% FBS-RPMI160 and then treated with 10 µmol/L LY294,002 and IGF-II in SFM. The results are expressed as mean average ± SEM of three independent experiments. ■ A549-S; □ A549-AS.
Overexpression of Tumor Cell COX-2 Enhances Mitogenicity of IGFs on A549 Cells. Compared with A549-AS, the IGF-mediated proliferation of A549-S was significantly increased (Figs. 3A and B and 4C). Consistent with these findings, SC58236 significantly decreased the proliferation of A549-S in response to both IGFs (Fig. 3B). Similar results also were obtained with another COX-2 inhibitor drug, celecoxib (data not shown). Thus, COX-2 enhances the proliferation of A549 cells in response to IGFs. In summary, our viability and proliferation data indicate that high-level COX-2 expression in A549 cells increases IGF-dependent and -independent survival.

Overexpression of Tumor Cell COX-2 Expression Facilitates the Autophosphorylation of IGF-IR. On the basis of our findings indicating a COX-2–dependent sensitivity to IGFs, we speculated that tumor cell COX-2 expression could modulate the phosphorylation of IGF-IR. We studied the phosphorylation of Tyr1131 of IGF-IR in A549-S and A549-AS cells. Phosphorylation of the triple tyrosine cluster Tyr1131/1135/1136 in the intracellular kinase domain of IGF-IR is caused by autophosphorylation and required for activation of the intracellular kinase domain of IGF-IR (39). As determined by immunoprecipitation of the β-subunit of IGF-IR, the pIGF-IR-Tyr1131 level in A549-S, but not in A549-AS, was distinctly elevated following 24 hours of IGF-II treatment (Fig. 3C and D). Consistent with these data, SC58236 decreased the IGF-II–stimulated levels of pIGF-IR-Tyr1131 in A549-S (Fig. 3C), whereas the pIGF-IR-Tyr1131 levels of A549-AS did not respond to the SC58236 treatment (Fig. 3D).

As shown in Figs. 3D and E, PGE2 appears to mediate the COX-2–dependent effects on IGF-IR. Namely, a chemically stable analog of PGE2, dm-PGE2, reproduced the effect of elevated COX-2 expression on IGF-IR autophosphorylation in A549-AS (Fig. 3D). In addition, the pIGF-IR-Tyr1131 levels of A549-S, but not A549-AS, were responsive to SC58236 in 10% FBS-RPMI 1640 (Fig. 3E), suggesting an important role for tumor cell COX-2 expression in the regulation of autophosphorylation of IGF-IR. In conclusion, our results indicate that COX-2 facilitates the autophosphorylation of IGF-IR.

Overexpression of Tumor Cell COX-2 Increases Class IA PI3k Activity. PI3k signaling has been implicated in regulation of proliferation and apoptosis signaling downstream of IGF-IR (32–34). Using a polyclonal anti-PI3Kp85 antibody to immunoprecipitate the PI3Kp85/p110 complex, we measured the class IA PI3k catalyzed conversion of P(l,4,5)P2 into P(3,4,5)P3. A549-S was plated at 1 to 1.2 million cells per 10-cm2 Petri dish, grown for 24 hours, and then treated with celecoxib at 5 μmol/L overnight. (A) NSCLC cell lines were plated at 0.5 to 1 million cells per T75 flask and treated with celecoxib at 5 μmol/L for 48 hours.

Fig. 5. Celecoxib inhibits the class IA PI3k activity. A. Class I PI3k activity was determined as a conversion of P(l,4,5)P2 into P(3,4,5)P3. A549-S was plated at 1 to 1.2 million cells per 10-cm2 Petri dish, grown for 24 hours, and then treated with celecoxib at 5 μmol/L overnight. (B) NSCLC cell lines were plated at 0.5 to 1 million cells per T75 flask and treated with celecoxib at 5 μmol/L for 48 hours.

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vival activities of IGF-I and IGF-II and thus are consistent with and serve to explain our initial findings (Figs. 1, 2, and 3).

COX-2 (12) and IGF-IR kinase inhibitors (26, 27) have produced significant antitumor effects in mouse models for human cancer. Therefore, our current data indicating that COX-2 facilitates the autophosphorylation of IGF-IR (Fig. 3C–E), combined with the previous findings showing the antitumor effects of IGF-IR and COX-2 inhibitors, suggest that in future NSCLC-targeted therapies, COX-2 and IGF-IR kinase inhibitors could be combined. Simultaneous and alternate administration of COX-2 and IGF-IR kinase could produce benefits. Although the former can potentially maximize the suppression of IGF-IR–mediated NSCLC tumor growth, the latter can alleviate the toxicity of these drugs while also preventing a substantial refacilitation of the IGF-IR kinase activity (Fig. 3C–E). The rationale for the use of combination of COX-2 and IGF-IR inhibitors is further supported by our current and previous (2) findings indicating that, rather than inducing cell death in serum-containing medium, inhibition of COX-2 increases the susceptibility of NSCLC cells to apoptosis (Figs. 1 and 2C–F).

Autophosphorylation of receptor tyrosine kinases including IGF-IR is required for the class IA PI3k activity. An autophosphorylated IGF-IR facilitates translocation of PI3k to the cytoplasmic membrane where the lipid substrates of PI3k reside. Specifically, an autophosphorylated β-subunit of IGF-IR binds the class IA PI3k, a complex of p85 adaptor/p110 catalytic subunits, whereby it translocates this p85/p110 complex to the cytoplasmic membrane, and subsequently activates the conversion of PI(4,5)P2 into PI(3,4,5)P3. The accumulation of PI(3,4,5)P3 then activates the PI3k downstream kinases, such as PDK1 and AKTK (31). Thus, the inability of IGF-IR to maintain autophosphorylation, as we found to have occurred in response to COX-2 inhibition (Fig. 3), leads to a reduced ability of IGF-IR to control the activity of downstream kinases, including PI3k (Fig. 2). This limited upstream control over PI3k in turn results in strengthening of the inhibitory arm of the IGF axis and thus shifts the balance toward growth inhibition and cell death (Fig. 6D).

Our current data also show that PI3k is another potential target for the future NSCLC therapies. Most interestingly, the genetic overexpression of COX-2 enhanced the sensitivity of A549 proliferation to PI3k inhibitors LY294,002 and Wortmannin (Fig. 2). On the basis of these data, we hypothesize that the high levels of COX-2 increase the dependency of proliferation of A549 cells on PI3k signaling (Fig. 3). In conclusion, an important course of COX-2–related events can be envisioned. Namely, as COX-2 increases the IGF-related survival and proliferation via up-regulation of PI3k signaling, it also increases the vulnerability of NSCLC tumor growth to the abrogation of this pathway.

Our current results also are compatible with earlier studies linking high levels of COX-2 to up-regulation of the stimulatory arm of the IGF axis. Similar to our current findings, these previous studies show COX-2 up-regulates the PI3k-AKT pathway (36, 37) and down-regulates IGFBP-3 mRNA expression (47). Celecoxib also has been shown to down-regulate the expression of IGF-IR and IGF-II–related tumor growth (48). A link between the IGF axis and COX-2 also can be inferred from the previous publications showing that IGF-I increases COX-2 mRNA expression and PGE2 production (49) and that IGF-I amplifies the interleukin 1β–induced PGE2 production (50).

In summary, our current findings are of particular interest when viewed in the context of previous studies indicating that elevated levels of the systemic, liver-derived IGF-I and serum IGF-1:IGFBP-3 ratio might be risk factors for the malignancies (19–22) that also frequently express high levels of COX-2 (10, 11, 42–44). Namely, overexpression of COX-2 appears to improve the capacity of NSCLC cells to take advantage of liver-derived systemic, as well as tumor-produced, IGF-I and IGF-II. This is the first report to link tumor COX-2 expression with the antiapoptotic and mitogenic effects of IGFBP-3. Our findings suggest that a combination of elevated serum IGFBP-3 and tumor COX-2 expression promotes NSCLC progression.

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