Cyclooxygenase-2-Dependent Activation of Signal Transducer and Activator of Transcription 3 by Interleukin-6 in Non–Small Cell Lung Cancer

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Abstract

Purpose: Cyclooxygenase-2 (COX-2), phosphorylated signal transducers and activators of transcription 3 (STAT3), and interleukin-6 (IL-6) are elevated in non–small cell lung cancer (NSCLC). These molecules affect numerous cellular pathways, including angiogenesis and apoptosis resistance, and, therefore, may act in concert in NSCLC.

Experimental Design: We examined IL-6 and phosphorylated STAT3 in COX-2-overexpressing [COX-2 sense-oriented (COX-2-S)] NSCLC cells and control cells. The effect of IL-6, STAT3, phosphatidylinositol 3-kinase, and mitogen-activated protein/extracellular signal-regulated kinase kinase on vascular endothelial growth factor (VEGF) production and apoptosis resistance was assessed in COX-2-overexpresing cells.

Results: We report that NSCLC cells overexpressing COX-2 (COX-2-S) have increased IL-6 and phosphorylated STAT3 expression compared with control cells. IL-6 induced expression of VEGF in NSCLC cells. Moreover, blocking IL-6, mitogen-activated protein/extracellular signal-regulated kinase kinase, or phosphatidylinositol 3-kinase decreased VEGF production in COX-2-S cells. The addition of IL-6 to NSCLC cells resulted in increased apoptosis resistance. Furthermore, the inhibition of STAT3 or IL-6 induced apoptosis and reduced survivin expression, a member of the inhibitor of apoptosis protein family in COX-2-S cells.

Conclusions: Overall, these findings suggest a novel pathway in which COX-2 activates STAT3 by inducing IL-6 expression. This pathway could contribute to tumor formation by promoting survivin-dependent apoptosis resistance and VEGF production. These findings provide a rationale for the future development of STAT3, IL-6, and/or COX-2-targeted therapies for the treatment of lung cancer.

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Cyclooxygenase (COX) is a rate-limiting enzyme that is essential for the conversion of free arachidonic acid into prostaglandins (PG) and thromboxanes (1). There are two isoforms of COX: constitutively expressed COX-1 and an inducible COX-2 (2). COX-2 is elevated in a variety of cancers (3, 4), including non–small cell lung cancer (NSCLC; ref. 5). Elevated expression of COX-2 renders tumor cells more invasive via increased CD44 and matrix metalloproteinase 2 expression (6, 7). Furthermore, COX-2 expression in tumors increases angiogenesis by the induction of vascular endothelial growth factor (VEGF; ref. 8), ENA-78/CXC ligand 5 (9), and IL-8/CXC ligand 8 (9). Finally, apoptosis resistance in COX-2-overexpressing [COX-2 sense-oriented (COX-2-S)] tumor cells is due to Bcl-2 (10), Mcl-1 (11), and survivin expression (12).

Cytokines are essential polypeptide factors that are produced by multiple tissues and cell types (13). In cancer, cytokines are often overexpressed and act as autocrine or paracrine growth factors that promote tumor cell survival and metastasis (14). Interleukin-6 (IL-6) plays an antiapoptotic role in esophageal carcinoma (15), gastric cancer (16), cervical cancer (17), and myeloma (18) by inducing Mcl-1 and/or Bcl-xL. IL-6 also increases angiogenesis by inducing VEGF expression in cervical

cancer (19). Elevated serum IL-6 (20) and IL-6-dependent proliferation (21) has been observed in lung cancer, suggesting an important biological role for IL-6 in this disease.

IL-6 exerts its biological activity by binding to a heterotrimeric receptor complex of a ligand-specific binding chain (IL-6R-α) and two signal-transducing subunits (gp130; ref. 22). IL-6-activated Janus kinases phosphorylate the gp130 subunit, which leads to the activation of signal transducer and activator of transcription (STAT), Ras/mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K)/Akt signaling cascades (23). A major IL-6-driven signaling pathway involves Janus-activated kinase 1–dependent STAT1 and STAT3 activation (24).

STATs are a family of transcription factors, including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 (25). Signaling through receptor tyrosine kinases or through cytokine receptors via Janus-activated kinases cause phosphorylation and dimerization of STAT monomers, resulting in STAT activation (26). Constitutive activation of STATs, especially STAT3, has been observed in a variety of human cancers, including NSCLC (reviewed in ref. 26). Expression of a number of cell cycle control genes, such as *cyclin D1*, *p21*, and *c-Myc*, has been shown to be affected by STAT3 (27–29). Phosphorylated STAT3 can also increase antiapoptotic gene expression, namely Bcl-xL (18), Mcl-1 (30), and survivin, a member of the inhibitor of apoptosis protein family (31). Finally, STATs play a role in tumor angiogenesis by regulating VEGF expression (19, 32, 33). In lung cancer, IL-6 can activate STAT3, thereby promoting cell survival (34) and proliferation in human NSCLC (21, 35). Collectively, these studies suggest an important role for IL-6 and STAT3 in lung cancer.

Thus, COX-2, STAT3, and IL-6 are highly expressed in many malignancies; however, studies have not yet fully defined how these proteins work together to promote malignancy. In this study, we investigated the role of COX-2, IL-6, and STAT3 in NSCLC. We show that IL-6 is highly expressed in COX-2-overexpressing NSCLC cells but absent in COX-2 antisense-oriented (COX-2-AS) cells. Moreover, phosphorylated STAT3 expression was elevated in A549-S, and was in part due to IL-6. Additionally, VEGF expression in A549-S cells was IL-6 and PI3K dependent. We found that IL-6 and STAT3 mediated apoptosis resistance by reducing survivin expression in A549-S. Overall, these findings suggest a novel pathway in which COX-2 activates STAT3-mediated cell survival and VEGF production by inducing IL-6 expression.

Materials and Methods

Cell lines. NSCLC cells, A549 (human lung adenocarcinoma), and H157 (squamous cell carcinoma) were obtained from the American Type Culture Collection (Manassas, VA) and National Cancer Institute, NIH (Bethesda, MD), respectively. COX-2-S, COX-2-AS, and pLNCX (vector alone) clones were generated for A549 and H157 cell lines using retroviral transfection as previously described (6, 7). Briefly, COX-2-S cells had an approximate 10-fold higher level of COX-2 expression and PGE2 production compared with controls (6, 7). In contrast, 4-fold less COX-2 and PGE2 was observed in COX-2-AS cells. Moreover, IL-1β induced COX-2 and PGE2 production in parental, vector, sense, but not antisense cells, indicating that antisense cells lacked COX-2 expression. The RH2 (lung squamous cell carcinoma) cell line was previously established in our laboratory from a lung cancer resection specimen (36).

Cell conditions for gene expression. Biological triplicates were used for gene expression analysis on the U133A Human Genome array (Affymetrix, Santa Clara, CA). Cell lines were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) until 70% confluent. Cell lines were examined for PGE2 to ensure that the COX-2-S and COX-2-AS clones were expressing appropriate levels of PGE2. Briefly, COX-2-S, COX-2-AS, vector control, and parental control (A549 and H157) cells were stimulated with or without IL-1β (280 units/mL; BD PharMingen, San Diego, CA) for 24 hours. PGE2 levels were measured by PGE2 EIA kit according to instructions of the manufacturer (Cayman Chemicals, Ann Arbor, MI).

RNA and probe preparation. Total RNA from 2×10^6 cells was prepared using Qiagen RNeasy Mini (Qiagen, Valencia, CA) with on-column DNase digestion. Ten micrograms of total RNA was used for probe preparation. The University of California at Los Angeles DNA Microarray Core was used for probe preparation and chip hybridization. Specifically, double-stranded cDNA was extracted from 10 μg of total RNA. Biotin-labeled antisense cRNA was produced using *in vitro* transcription reaction by using the ENZO BioArray HighYield RNA Transcript Labeling kit (ENZO, Farmingdale, NY). The labeled cRNA target was fragmented and 15 μg of fragmented cRNA was used for U133A chip hybridization.

Data analysis. Data from the chip was analyzed for clustering and visualization using the Harvard dCHIP version 1.3. Data from each chip was normalized using the chip with the median mean intensity. After normalization, the data was modeled using PM-model. For hierarchical clustering and gene selection, genes were identified that were 2-fold greater or less than in COX-2-S cells compared with COX-2-AS cells using a P value of ≤ 0.05.

Apoptosis assays. Cells (0.3×10^6) were seeded into six-well plates in 10% FBS containing RPMI. After 24 hours of culture, the cells were washed with PBS and then replaced with RPMI containing 2% FBS with or without IL-6 (50-100 ng/mL). After 4 hours of culture with IL-6, 0.1 μmol/L of staurosporine was added to the culture for another 18 hours. Apoptosis was assayed using the BD PharMingen Apo-BrdUrd kit (BD PharMingen, San Diego, CA). Briefly, both floating and adherent cells were harvested, washed with PBS, and fixed with 1% paraformaldehyde for 1 hour. After fixation, the cells were washed twice with PBS and stored in 70% ethanol until flow cytometry analysis. After fixation and permeabilization, cells were washed and resuspended with reaction buffer, terminal deoxynucleotidyl transferase enzyme, and br-dUTP for 2 hours at 37°C. Cells were then washed and labeled with fluorescein-labeled anti-BrdUrd in the dark for 30 minutes at room temperature followed by propidium iodide and RNase staining for 30 minutes. Cells were then analyzed by flow cytometry and 10,000 events were collected.

In small interfering RNA (siRNA) experiments, apoptosis was visualized by fluorescence microscopy (Leica Microsystems, Wetzlar, Germany) using 0.1 μmol/L apoptotic stain YO-PRO-1 (Molecular Probes, Eugene, OR) for 30 minutes. For each sample, photographs were taken from several random fields.

Western blot analysis. Protein (10-25 μg) was loaded for SDS-PAGE and Western blot analysis. Primary antibodies used in this study were actin (Santa Cruz Biotechnology, Santa Cruz, CA), phosphorylated STAT3 (Ser^{727}; Santa Cruz Biotechnology), phosphorylated STAT3 (Try^{705}; Cell Signaling, Beverly, MA), STAT3 (Cell Signaling), and survivin (Novus, Littleton, CO). Horseradish peroxidase–conjugated secondary antibodies (Santa Cruz Biotechnology) and enhanced chemiluminescence (Pierce, Rockford, IL) was used for protein detection.

Cytokine assays. Cells (0.3×10^6) were seeded into six-well plates in 10% FBS-containing RPMI. After cells were adherent, cells were washed with PBS twice and then replaced with RPMI containing 0% FBS with or without IL-6 (50 ng/mL) for 24 hours at 37°C in 5% CO_2 humidified air. After 24 hours of incubation, culture supernatants were collected and assayed by capture ELISA for IL-6 (R&D, Minneapolis, MN) and

VEGF according to the instructions of the manufacturer (Peprotech, Rocky Hill, NJ). The limit of detection for the cytokine assays were 62 and 31 pg/mL (IL-6 and VEGF, respectively).

In certain experiments, cells were treated with 10 μmol/L of LY294002 (Alexis, San Diego, CA) or U0126 (Calbiochem, San Diego, CA) for 24 hours. Culture supernatants were analyzed for VEGF ELISA.

Small interfering RNA inhibition of signal transducers and activators of transcription 3 and interleukin-6. Cells (6×10^4) were seeded into 24-well plates in 10% FBS-containing RPMI. On the next day, TransMessenger transfection reagent (Qiagen) was used to transfect validated STAT3, predesigned IL-6, or negative control Silencer siRNA (Ambion, Austin, TX). Serum-free RPMI using 1.6 μg STAT3, IL-6, or negative control siRNA; 3.2 μL Enhancer R; and 8 μL TransMessenger reagent for 3 hours were the conditions used for the transfection. After the 3-hour incubation, the transfection medium was replaced with fresh RPMI containing 10% FBS for another 24 hours. Subsequently, apoptosis was induced and ELISA or Western blot was used to assess the effectiveness of the suppression of STAT3 or IL-6.

Statistical analysis. A two-tailed nonpaired Student's *t* test was used to calculate the *P* values.

Results

Interleukin-6 is highly expressed in non–small cell lung cancer cells in a cyclooxygenase-2-dependent manner. COX-2 is elevated in NSCLC (5). Tumor COX-2 overexpression seems to affect numerous cellular pathways; however, the exact mechanisms of these cellular changes are presently unclear. In this study, we did Affymetrix microarray (U133A chip) to elucidate COX-2-dependent gene expression in NSCLC. We examined 15,000 genes in A549 (human lung adenocarcinoma cell line) and H157 (squamous cell carcinoma cell line) either overexpressing COX-2 (COX-2-S) or expressing little or no COX-2 (COX-2-AS; Fig. 1). One hundred fifty-one genes were differentially expressed (either 2-fold increase or decrease) in A549-S cells compared with A549-AS cells (Fig. 1A). In the H157 NSCLC cell line, 2,074 genes were differentially expressed (either 2-fold increase or decrease) in H157-S cells when compared with H157-AS (Fig. 1B). From these two gene subsets, only 37 genes were increased (Fig. 1C; Table 1) and eight genes were decreased (data not shown) in both A549-S and H157-S cell lines. Many of the

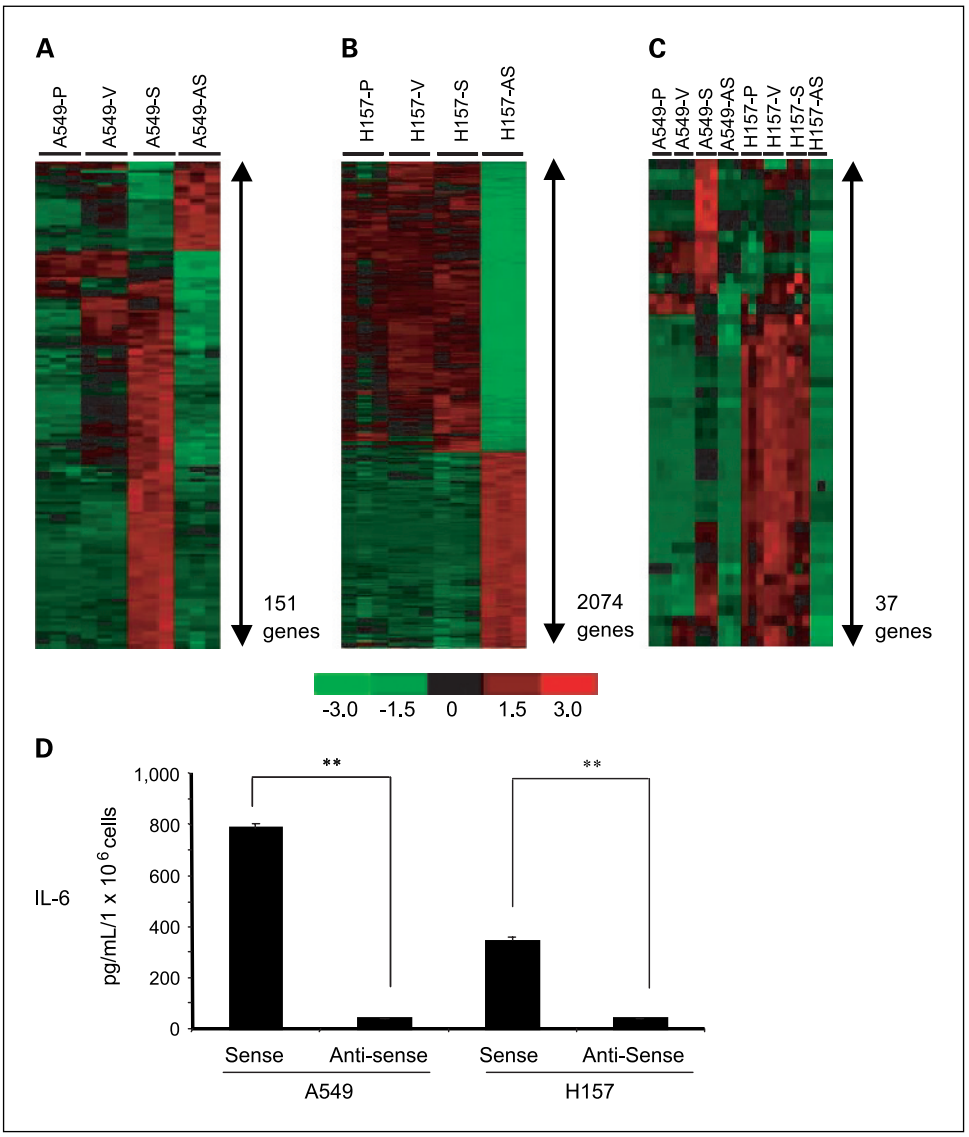

Fig. 1. Differential gene expression in A549 and H157 COX-2-S, and COX-2-AS NSCLC. For hierarchical clustering and gene selection, genes were identified that were changed >2-fold (increase or decrease) in COX-2-S cells compared with COX-2-AS cells. We used dCHIP programs for clustering and visualization of the data set. *A,* 151 genes were differentially expressed (2-fold increase or decrease) in A549 cells expressing high COX-2 compared with A549 cells with no COX-2 expression. *B,* 2,074 genes were differentially expressed (2-fold increase or decrease) in H157 cells expressing high COX-2 when compared with COX-2 absent cells. *C,* from these two gene subsets, only 37 genes were increased and eight genes (data not shown) were decreased in both cell lines (A549 and H157) expressing high COX-2. Black horizontal bar, samples assayed in triplicates. Differentially expressed genes of $P \leq 0.05$ are shown. Green, low expression; red, high expression. (*P,* parental; *V,* vector; *S,* sense; *AS,* antisense). *D,* cells were plated and cultured for 24 hours. Supernatants were collected and human IL-6 ELISA was done. Values are adjusted for cell number. Columns, mean; bars, SD. One representative experiment out of three independent experiments. **, $P \leq 0.001$.

genes identified in this microarray study were relevant to tumorigenesis and can be classified into various categories, such as angiogenesis, chemotaxis, cell proliferation, cell adhesion, apoptosis, and cell growth (Table 2). Additionally, we show that previously defined COX-2-dependent genes, such as *CD44* and *MMP2* (6, 7), *IL-8* (9), *ENA78/CXC ligand 5* (9), and *VEGF* (8), were identified by this array analysis (Table 2). Interestingly, IL-6 was one of the genes that was highly expressed in COX-2-S cells and absent in COX-2-AS cells (Table 2). To determine if protein levels were also elevated, we did IL-6-specific ELISA on

Table 1. List of genes differentially expressed in COX-2-S and COX-2-AS (A549 and H157) cells

Gene	Accession no.	Fold change					
		A549			H157		
		S-AS	S-P	S-V	S-AS	S-P	S-V
PGE-endoperoxide synthase 2 (PGE G/H synthase and COX)	NM 000963.1	19.92	18.53	12.98	2.1	1.25	−1.3
Solute carrier family 16 (monocarboxylic acid transporters), member 3	NM 004207.1	7.59	7.57	6.31	10.83	−1.27	−1.75
IL-8	NM 000584.1	7.59	4.94	6.38	50.86	1.09	−1.14
IL-6 (INF, β2)	NM 000600.1	6.72	9.43	9.99	3.65	1.96	−2.05
IL-8 C-OOH variant (IL-8)	AF043337.1	5.88	4.35	4.62	6.27	1.14	−1.21
Pentaxin-related gene, rapidly induced by IL-1β	NM 002852.1	5.65	15.54	9.67	22.1	−1.01	−1.08
Proteasome (prosome, macropain) subunit, β type, 8 (large multifunctional protease 7)	U17496.1	5.28	5.51	5.65	5.16	−1.15	−1.2
Phospholipase A2, group IVA (cytosolic, calciumdependent)	M68874.1	4.86	2.21	1.66	3.91	−1.02	−1.15
Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, α)	NM 001511.1	4.64	4.63	5.62	16.46	1.11	−1.32
ATP-binding cassette, subfamily A (ABC1), member 1	NM 005502.1	4.21	4.73	3.78	2.01	1.41	1.01
Solute carrier family 16 (monocarboxylic acid transporters), member 3	AL513917	4.18	4.08	3.63	3.55	−1.33	−1.92
Six transmembrane epithelial antigen of the prostate	NM 012449.1	3.45	1.14	1.37	4.19	−1.07	−1.17
Nidogen 2 (osteonidogen)	NM 007361.1	3.39	2.78	2.53	3.88	1.03	−1.38
Annexin A10	AF196478.1	3.29	7.85	10.08	4.6	1.02	−1.36
Epidermal growth factor – containing fibulin-like extracellular matrix protein 1	AI826799	3.28	2.11	2.07	5.29	−1.58	−1.51
INF-induced transmembrane protein 2 (1-8D)	NM 006435.1	3.27	2.72	2.49	3.88	−1.05	−1.13
INF, γ-inducible protein 16	NM 005531.1	2.67	4.15	2.63	26.5	1.21	−1
Dual specificity phosphatase 4	NM 001394.2	2.64	1.86	1.51	4.27	1.43	−1.18
Zinc finger protein	AA349848	2.64	1.32	1.78	2.03	−1.29	−1.5
Adipose differentiation-related protein	BC005127.1	2.53	3.95	6.6	4.44	−1.09	−1.27
INF, γ-inducible protein 16	AF208043.1	2.49	3.86	2.48	19.35	1.25	−1.06
Chemokine (C-X-C motif) ligand 2	M57731.1	2.48	3.57	4.52	34.88	1.13	1.07
GLI pathogenesis-related 1 (glioma)	U16307.1	2.46	2.22	2.73	8.64	−1.03	−1.31
Phosphodiesterase 4B, cyclic AMP-specific (phosphodiesterase E4 dunce homologue, Drosophila)	NM 002600.1	2.43	3.01	1.6	8.3	1.02	−1.61
Tumor necrosis factor, α-induced protein 3	NM 006290.1	2.36	2.88	3.3	2.5	−1.34	−1.39
Chemokine (C-C motif) ligand 20	NM 004591.1	2.3	2.28	3.06	3.62	1.07	1.32
Chemokine (C-X-C motif) ligand 3	NM 002090.1	2.29	2.2	3.78	25.3	1.05	−1.19
Cytochrome P450, family 1, subfamily B, polypeptide 1	NM 000104.2	2.26	1.07	1.45	6.71	1.51	−1.07
Transforming growth factor β – stimulated protein TSC-22	AK027071.1	2.24	1.41	1.18	2.3	1.43	−1.25
HIV-1 rev binding protein 2	AI912583	2.15	2.12	2.7	7.05	1.06	−1.3
Runt-related transcription factor 3	AA541630	2.13	1.35	−1.43	5.83	1.02	−1.56
Nucleotide-sugar transporter similar to C. elegans sqv-7	AJ005866.1	2.09	1.6	1.17	10.38	1.11	1.24
Brain-derived neurotrophic factor	NM 001709.1	2.08	2.16	1.81	14.21	−1.04	−1.28
Tumor necrosis factor receptor superfamily, member 21	NM 016629.1	2.06	1.94	1.35	2.44	1.84	−1.23
Dual specificity phosphatase 10	N36770	2.06	2.49	2.34	3.49	1.09	−1.46
Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	AF021834.1	2.05	1.88	1.86	2.6	−1.01	3.47
Runt-related transcription factor 3	NM 004350.1	2.01	1.5	−1.32	5.03	−1.26	−1.6

NOTE: Thirty-seven genes were differentially expressed (2-fold increase) in A549 cells and H157 cells expressing high COX-2 as compared with cells with no COX-2 expression. Differentially expressed genes of $P = 0.05$ are shown. Fold changes are shown as a ratio of sense/antisense, sense/parental, and sense/vector mean expression. Positive values mean fold increase; negative values mean fold decrease.
Abbreviations: P, parental; V, vector; S, sense; AS, antisense.

Table 2. Classification of genes differentially expressed in COX-2-S and COX-2-AS (A549 and H157) cells

Cluster	Accession no.	Fold change, S-AS
A549 NSCLC		
Apoptosis		
Secreted frizzled-related protein 1	NM.003012.2	2.7
Tumor factor, α-induced protein 3	NM.006290.1	2.5
p8 (candidate of metastasis 1)	AF135266.1	−2.5
Chemotaxis		
IL-8	NM.000584.1	7.8
Chemokine ligand 1	NM.001511.1	4.6
Chemokine ligand 20	NM.004591.1	2.2
Cell proliferation		
IL-6	NM.000600.1	7.1
MAPK kinase 6	NM.002758.1	2.3
CDC-like kinase	AI251890	−2.2
H157 NSCLC		
Angiogenesis		
Epiregulin	NM.001432.1	66.5
IL-8	NM.000584.1	57.6
ENA78/CXCL5	NM.002994.1	29.5
Tumor necrosis factor receptor, 12A	NM.016639.1	18.8
VEGF	AF022375.1	2.5
Cell growth		
Tumor protein p53	NM.000546.2	−8.3
Cyclin G2	AW134535	−2.7
Insulin-like growth factor binding protein 5	L27560.1	−5.5
Cell adhesion		
CD44	BE903880	38.4
Integrin, α3	NM.002204.1	17.9
Integrin, α6	NM.000210.1	4.2
Matrix metalloproteinase 2	NM.004530.1	4.2

NOTE: Classification of differentially expressed genes in A549S compared with A549-AS and H157-S compared with H157-AS. Fold changes are shown as a ratio of sense/antisense. Positive values denote fold increase, whereas negative values denote fold decrease. Differentially expressed genes of $P = 0.05$ are shown.

supernatants of COX-2-S and COX-2-AS cells and showed that IL-6 is significantly increased in COX-2-S compared with the COX-2-AS in both A549 and H157 cells (Fig. 1D).

Constitutive signal transducers and activators of transcription 3 activation in cyclooxygenase-2-overexpressing non–small cell lung cancer cells is interleukin-6 dependent. Several studies suggest that IL-6 may exert an important biological role in lung cancer (20, 21, 34). Therefore, we next sought to determine the functional consequence of IL-6 in COX-2-dependent regulation of the malignant phenotype in NSCLC. Because IL-6 is a cytokine involved in STAT3 activation (26), we determined the levels of phosphorylated STAT3 in A549-S and A549-AS by Western blotting using anti–phosphorylated STAT3 antibodies for phosphorylated Tyr^{705} and phosphorylated Ser^{727} residues. We show that A549 cells overexpressing COX-2 have constitutive STAT3 (Tyr^{705} and Ser^{727}) activation, whereas A549 cells with limited COX-2 lacked this activation (Fig. 2A). Total STAT3 and actin protein levels remained at similar levels in A549 cells, regardless of COX-2 expression. Moreover, STAT3 phosphorylation (Tyr^{705} and Ser^{727}) could be induced by the addition of IL-6 in both A549-S and A549-AS cells. We show that increased STAT3 phosphorylation (Tyr^{705} and Ser^{727}) in A549-S cells is in part due to IL-6 because knocking down IL-6 in A549-S cells by siRNA resulted in reduction of STAT3 phosphorylation of both Tyr^{705} and Ser^{727} residues (Fig. 2B and C). IL-6 production was effectively inhibited by siRNA treatment as shown by IL-6-specific ELISA. Negative control siRNA treatment of A549-S cells did not affect the IL-6 or phosphorylated STAT3 levels, confirming the STAT3 phosphorylation was IL-6 dependent. Furthermore, actin levels were not altered by IL-6 or negative control siRNA treatment, indicating that the decease in phosphorylated STAT3 is not due to a reduction in total protein expression. These results show that constitutive STAT3 (Tyr^{705} and Ser^{727}) activation in A549-S cells is partly mediated by elevated IL-6 expression.

Inhibition of interleukin-6, phosphatidylinositol 3-kinase, and mitogen-activated protein/extracellular signal-regulated kinase kinase reduces vascular endothelial growth factor expression in cyclooxygenase-2-overexpressing non–small cell lung cancer cells. Several studies suggest that IL-6 and STAT3 play an important role in cancer by promoting angiogenesis through the induction of VEGF (19, 32, 33). We next addressed the role of IL-6 in VEGF expression in NSCLC. Addition of IL-6 for

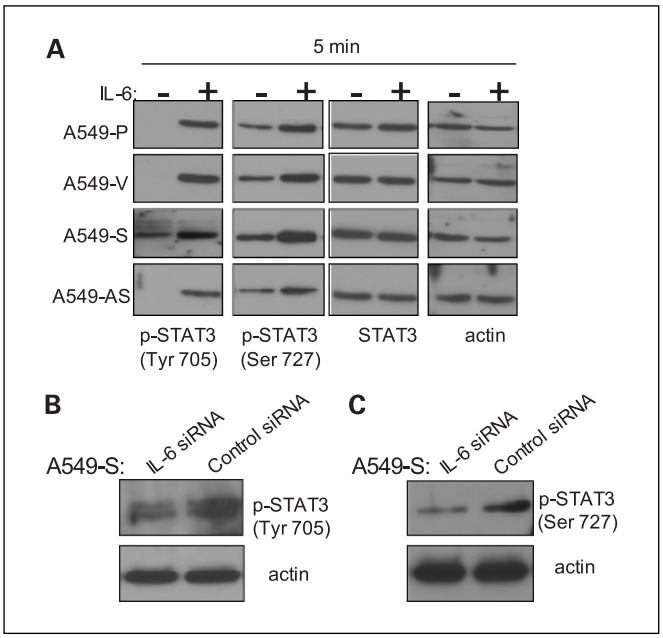

Fig. 2. Constitutive STAT3 activation in A549-S cells is IL-6 dependent. *A,* cells were treated with IL-6 (50 ng/mL) for 5 minutes and cell lysates were prepared for Western blotting using antiphosphorylated STAT3 (*p-STAT3*, Tyr^{705} and Ser^{727}), anti-STAT3, and antiactin. *B* and *C,* A549-S cells were transfected with IL-6, STAT3, or negative control siRNA for 3 hours, and then incubated for 24 hours. Cell lysates were prepared for Western blotting for phosphorylated STAT3 (Tyr^{705} and Ser^{727}) and actin expression. One representative experiment out of three independent experiments.

24 hours to A549-V or AS cells resulted in significant VEGF induction (Fig. 3A). Interestingly, constitutive levels of VEGF expression were increased in A549-S cells compared with vector or antisense and were comparable with what was observed in IL-6-stimulated A549-V cells. The latter finding suggests that VEGF expression may be dependent on IL-6. To address this possibility, we inhibited IL-6 in A549-S cells using the siRNA technique and showed that VEGF levels were indeed reduced by IL-6 inhibition, thus confirming our hypothesis (Fig. 3B). In contrast, siRNA-mediated inhibition of STAT3 did not reduce VEGF levels in A549-S cells, indicating that IL-6-regulated VEGF production occurs via a STAT3-independent pathway. IL-6 ELISA (Fig. 3C) and STAT3 Western blotting were done to ensure that IL-6 and STAT3 were effectively inhibited by siRNA treatment, respectively. Negative control siRNA treatment of A549-S cells did not affect VEGF levels. Actin levels were not altered in STAT3, IL-6, and negative control siRNA-treated cells, confirming the specificity of VEGF response on IL-6 and STAT3 siRNA treatment.

Because IL-6 can exert its biological activity by the activation of the PI3K/Akt and Ras/MAPK signaling cascades (23), we sought to determine the role of these pathway in the IL-6-dependent VEGF production in A549-S cells. The addition of PI3K inhibitor, LY294002, and mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK) 1/2 inhibitor, U0126, were able to reduce VEGF levels in A549-S NSCLC cells (Fig. 3D). Collectively, these results show that the inhibition of IL-6 reduces VEGF expression in A549-S cells in a PI3K- and MEK-dependent, but a STAT3-independent, manner.

Apoptosis induction and reduction of survivin expression by signal transducers and activators of transcription 3 or interleukin-6 inhibition in cyclooxygenase-2-overexpressing non–small cell lung cancer cells. IL-6 and STAT3 have been shown to play a critical role in apoptosis resistance in cancer cells via induction of antiapoptotic proteins, such as survivin, Bcl-xL, or Mcl-1 (18, 30, 31). Therefore, we examined the role of IL-6 and STAT3 in apoptosis resistance in NSCLC. Staurosporine was used to induce apoptosis with or without the addition of IL-6 in A549 and RH2 NSCLC cells for 18 hours. Terminal deoxynucleotidyl transferase–mediated nick end labeling assays and flow cytometry showed that the addition of IL-6 reduced staurosporine-induced apoptosis levels in A549 and RH2 cells (Fig. 4A), thus supporting the antiapopotic role of IL-6 in NSCLC.

Interestingly, compared with cells expressing limited COX-2, NSCLC cells overexpressing COX-2 have increased survivin expression and are more resistant to apoptosis (12, 37). We, therefore, sought to determine if this finding was due to IL-6 and STAT3 expression. To address this possibility, we inhibited STAT3 or IL-6 by siRNA in A549-S cells and determined the effect of inhibition of these molecules on staurosporine-induced apoptosis using the nuclear YO-PRO dye, which stains brightly for apoptotic cells. Inhibition of IL-6 or STAT3 expression in A549-S cells resulted in increased staurosporine-induced apoptosis, suggesting that the apoptosis resistance observed in A549-S cells is due, at least in part, to the expression of IL-6 and STAT3 (Fig. 4B). Furthermore, survivin levels were also reduced in A549-S cells by STAT3 or IL-6 inhibition (Fig. 4C and D). As before, IL-6 ELISA (data not shown) and STAT3 Western blotting (Fig. 4C) were done to ensure that IL-6 and STAT3 were effectively inhibited by siRNA treatment. Negative control siRNA treatment of A549-S cells did not affect apoptosis or survivin levels, and actin levels were similar in all conditions tested, thus confirming the specificity of IL-6 and STAT3 siRNA treatment. These results suggest that the IL-6/STAT3 pathway mediate COX-2-dependent regulation of survivin and apoptosis resistance in NSCLC. Overall, these

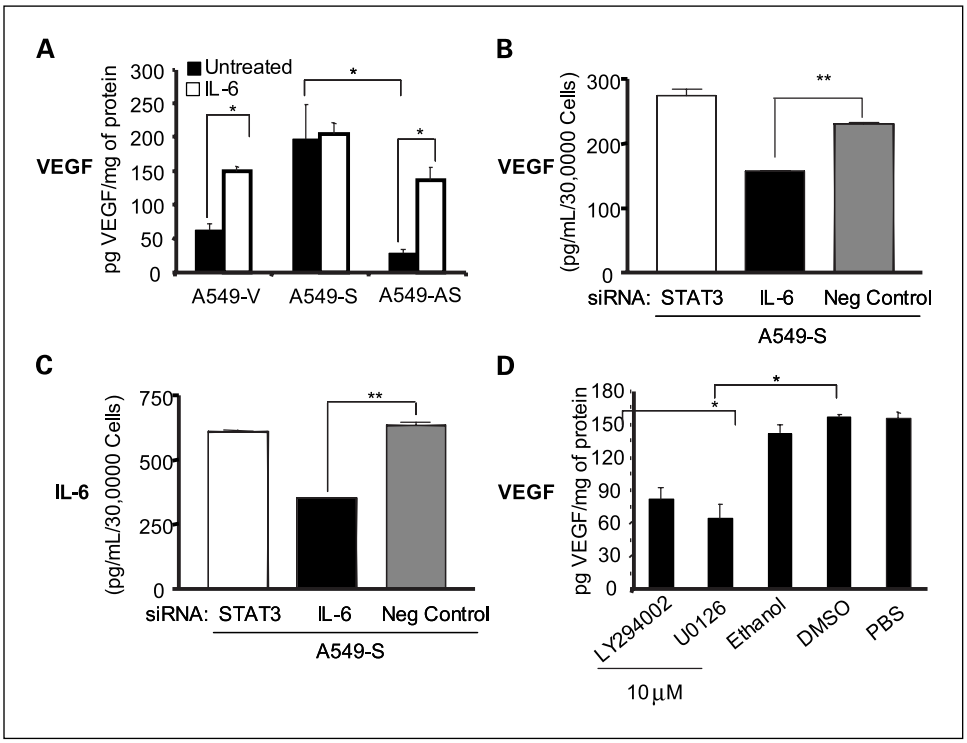

Fig. 3. Inhibition of IL-6, PI3K, and MEK reduces VEGF expression in A549-S cells. *A,* A549 NSCLC cells were treated with IL-6 (50 ng/mL) for 24 hours. Supernatants were collected and human VEGF ELISA was done. Values are in picograms of VEGF per milligram of total protein. Columns, mean; bars, SD. One representative experiment out of three independent experiments. *, $P \leq 0.05$. *B*, A549-S cells were transfected with IL-6, STAT3, or negative control siRNA for 3 hours and then incubated for 24 hours with new media containing 10% FBS. Supernatants were collected and analyzed for IL-6 (*B*) and VEGF by ELISA (*C*). Values are adjusted for cell number. Columns, mean; bars, SD. One representative experiment out of three independent experiments. **, $P \leq 0.001$. *D*, A549-S and A549-V NSCLC cells were treated with LY294002 or U0126 (10 μmol/L) for 24 hours and supernatants were collected and analyzed for VEGF ELISA. Columns, mean; bars, SD. *, $P \leq 0.05$.

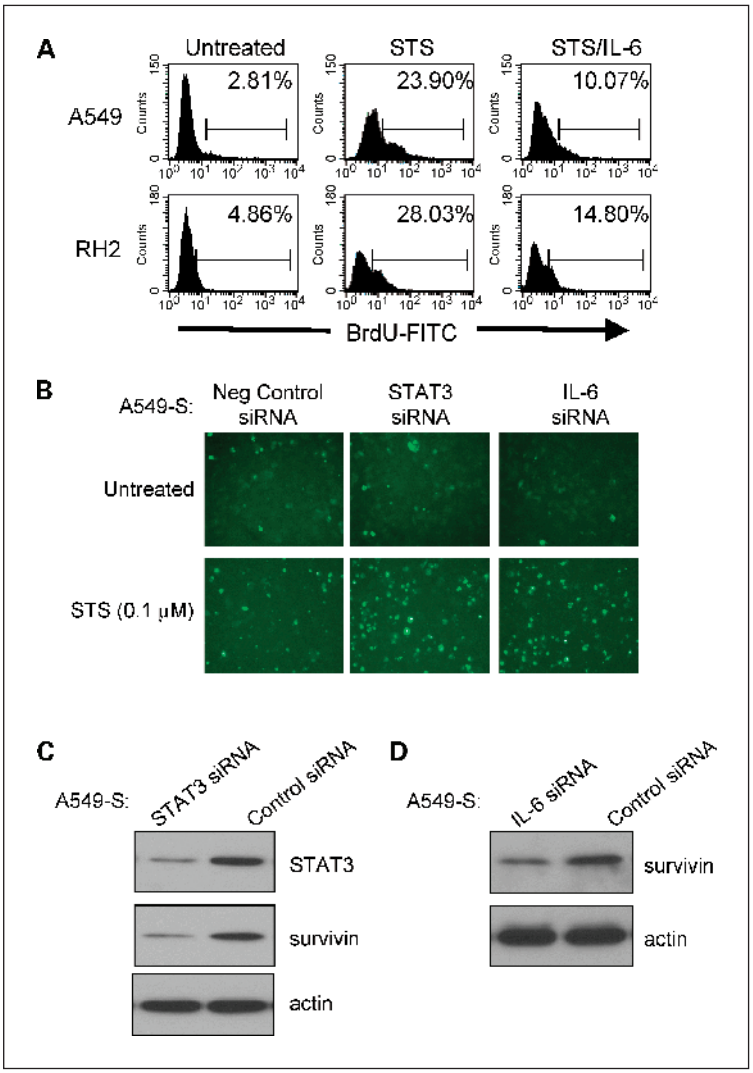

Fig. 4. Apoptosis induction and reduction of survivin expression by STAT3 or IL-6 inhibition in A549-S cells. *A,* apoptosis was induced in A549 and RH2 NSCLC cells with 0.1 μmol/L staurosporine with IL-6 (100 ng/mL) for 24 hours and was detected by Apo-BrdUrd staining and flow cytometry. One representative experiment out of three independent experiments. *B,* A549-S NSCLC cells were treated with IL-6, STAT3, or negative control siRNA for 3 hours and replaced with fresh media containing 10% FBS for 24 hours. On the next day, apoptosis was induced in A549-S with 0.1 μmol/L staurosporine for 12 hours and was detected by YO-PRO-1 dye. A549-S NSCLC cells were transfected with STAT3 (*C*) and IL-6 or negative control siRNA (*D*) for 3 hours, and then incubated for 24 hours. Cell lysates were prepared for Western blotting for STAT3, survivin, and/or actin expression. One representative experiment of three independent experiments.

findings suggest a role for COX-2 in the activation of STAT3 by IL-6 in tumor formation by promoting apoptosis resistance and VEGF production.

Discussion

In this study, we show the role of IL-6 and STAT3 in the COX-2 mediated regulation of VEGF and apoptosis resistance in NSCLC cells. Our results show the COX-2-dependent expression of IL-6 and phosphorylated STAT3 in NSCLC. Furthermore, the phosphorylated STAT3 observed in A549-S cells is, in part, dependent on IL-6 expression. These findings are consistent with a study showing that increased expression of COX-2 and PGE2 contributes to prostate cancer development by the activation of the IL-6/GP130/STAT3 signaling pathway (38). Moreover, COX-dependent expression of IL-6 has been observed in human oropharyngeal carcinoma (39). In addition to IL-6, receptor tyrosine kinases, such as epidermal growth factor receptor (40), and nonreceptor tyrosine kinases, including Src (28), and Janus-activated kinase (41) have been implicated in STAT3 activation and may also be involved. At this time, the mechanism by which COX-2 regulates IL-6 remains unclear. One possibility currently under investigation is that PGE2 and/or other COX-2-dependent products may regulate IL-6 production in NSCLC.

IL-6-dependent STAT3 activation has been shown to increase tumor angiogenesis by inducing VEGF in cervical cancer (19). Also, VEGF expression has been shown to be COX-2 dependent (8) and thus may be modulated by IL-6 and STAT3. In the current study, we show that IL-6 is able to induce VEGF expression in A549 NSCLC cells. Additionally, our RNA interference data show that VEGF expression in A549-S cells is dependent on IL-6 but is STAT3 independent. Pharmacologic inhibition of PI3K and MEK showed that these pathways are involved in the VEGF expression in A549-S cells. Overall, these results suggest that COX-2-dependent regulation of VEGF is partly mediated by IL-6 expression, MEK, and PI3K. These results give further support to current efforts to target MEK and PI3K in lung cancer clinical trials (42, 43).

The regulation of VEGF by IL-6 in COX-2-overexpressing cells remains to be defined. Studies in gastric and cervical cancer cells show that IL-6 increases VEGF production via a STAT3-dependent pathway (19, 44). However, our experimental results suggest that the VEGF production in COX-2-overexpressing NSCLC cells is STAT3 independent, but is PI3K and MAPK dependent. Interestingly, PI3K/Akt signaling has

been shown to regulate VEGF expression by modulating hypoxia-inducible factor 1, a transcriptional activator for VEGF (45). Moreover, studies have shown that the MAPK pathway activates the VEGF promoter region where Sp-1/AP-2 transcriptional factors bind (46). In hypoxic conditions, MAPKs enhance VEGF expression by activating the VEGF promoter region, which contains hypoxia-inducible factor-1α and activator protein transcription factor–binding sites (46, 47). Putative DNA motifs for nuclear factor–IL-6 have also been defined in the 5′ untranslated region of VEGF (48). Furthermore, it has been reported that PI3K and p38 MAPK can regulate the activity of AU-binding proteins such as HuR, which have been shown to stabilize VEGF mRNA (49, 50). These pathways may be operative in our system and further studies will be required to address these possibilities.

The requirement for STAT3 and IL-6 in apoptosis resistance and the expression of antiapoptotic proteins, such as survivin, Bcl-xL, and Mcl-2, have been described in various malignancies (18, 30, 31). Importantly, NSCLC cells that have increased COX-2 are more resistant to apoptosis compared with cells that have low COX-2, and this increased apoptosis resistance is partly due to survivin expression (12, 37). In this study, we show that the inhibition of either STAT3 or IL-6 by siRNA in A549-S cells resulted in reduced apoptosis resistance and survivin expression, suggesting that COX-2-regulated apoptosis resistance and survivin expression is mediated in part by STAT3 and IL-6. Our finding that the addition of IL-6 to NSCLC cells results in increased apoptosis resistance also supports this notion. Our findings support the concept that the IL-6/STAT3 pathway is functional in the COX-2 dependent regulation of apoptosis resistance and survivin expression in lung cancer.

Currently, COX-2 inhibitors are being assessed in clinical trials for chemoprevention and as an adjuvant for conventional therapy in lung cancer (reviewed in ref. 51). Additionally, anti-IL-6 therapy has shown promising results in metastatic renal cell carcinoma and multiple myeloma (52, 53). Studies in mice that are STAT3 deficient show tumor growth reduction, suggesting that STAT3 inhibition may be beneficial for cancer treatment (54). Progress has been made on the development of small-molecule inhibitors to STAT3 for human cancer (55, 56). This study increases our understanding of the mechanisms of action of COX-2, IL-6, and STAT3 in angiogenesis and apoptosis resistance in lung cancer. New therapies targeting COX-2-dependent genes, such as *IL-6* and *STAT3*, may be beneficial in the treatment of lung cancer.

References

1. Herschman HR. Prostaglandin synthase 2. Biochim Biophys Acta 1996;1299:125–40.
2. Dubois RN, Abramson SB, Crofford L, et al. Cyclooxygenase in biology and disease. FASEB J 1998;12:1063–73.
3. Yip-Schneider MT, Barnard DS, Billings SD, et al. Cyclooxygenase-2 expression in human pancreatic adenocarcinomas. Carcinogenesis 2000;21:139–46.
4. Chan G, Boyle JO, Yang EK, et al. Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. Cancer Res 1999;59:991–4.
5. Huang M, Stolina M, Sharma S, et al. Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. Cancer Res 1998;58:1208–16.
6. Dohadwala M, Batra RK, Luo J, et al. Autocrine/paracrine prostaglandin E2 production by non-small cell lung cancer cells regulates matrix metalloproteinase-2 and CD44 in cyclooxygenase-2-dependent invasion. J Biol Chem 2002;277:50828–33.
7. Dohadwala M, Luo J, Zhu L, et al. Non-small cell lung cancer cyclooxygenase-2-dependent invasion is mediated by CD44. J Biol Chem 2001;276:20809–12.
8. Masferrer JL, Leahy KM, Koki AT, et al. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 2000;60:1306–11.
9. Pold M, Zhu LX, Sharma S, et al. Cyclooxygenase-2-dependent expression of angiogenic CXC chemokines ENA-78/CXC ligand (CXCL) 5 and interleukin-8/CXCL8 in human non-small cell lung cancer. Cancer Res 2004;64:1853–60.
10. Liu XH, Yao S, Kirschenbaum A, Levine AC. NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates bcl-2 expression in LNCaP cells. Cancer Res 1998;58:4245–9.
11. Lin MT, Lee RC, Yang PC, Ho FM, Kuo ML. Cyclooxygenase-2 inducing Mcl-1-dependent survival mechanism in human lung adenocarcinoma CL1.0 cells. Involvement of phosphatidylinositol 3-kinase/Akt pathway. J Biol Chem 2001;276:48997–9002.
12. Krysan K, Merchant FH, Zhu L, et al. COX-2-dependent stabilization of survivin in non-small cell lung cancer. FASEB J 2004;18:206–8.
13. Kelso A. Cytokines: principles and prospects. Immunol Cell Biol 1998;76:300–17.
14. Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. Nat Rev Cancer 2004;4:11–22.
15. Leu CM, Wong FH, Chang C, Huang SF, Hu CP. Interleukin-6 acts as an antiapoptotic factor in human esophageal carcinoma cells through the activation of both STAT3 and mitogen-activated protein kinase pathways. Oncogene 2003;22:7809–18.
16. Lin MT, Juan CY, Chang KJ, Chen WJ, Kuo ML. IL-6 inhibits apoptosis and retains oxidative DNA lesions in human gastric cancer AGS cells through up-regulation of anti-apoptotic gene mcl-1. Carcinogenesis 2001;22:1947–53.
17. Wei LH, Kuo ML, Chen CA, et al. The anti-apoptotic role of interleukin-6 in human cervical cancer is mediated by up-regulation of Mcl-1 through a PI3-K/Akt pathway. Oncogene 2001;20:5799–809.
18. Catlett-Falcone R, Landowski TH, Oshiro MM, et al. Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity 1999;10:105–15.
19. Wei LH, Kuo ML, Chen CA, et al. Interleukin-6 promotes cervical tumor growth by VEGF-dependent angiogenesis via a STAT3 pathway. Oncogene 2003;22:1517–27.
20. Yanagawa H, Sone S, Takahashi Y, et al. Serum levels of interleukin 6 in patients with lung cancer. Br J Cancer 1995;71:1095–8.
21. Bihl M, Tamm M, Nauck M, Wieland H, Perruchoud AP, Roth M. Proliferation of human non-small-cell lung cancer cell lines: role of interleukin-6. Am J Respir Cell Mol Biol 1998;19:606–12.
22. Hirano T, Ishihara K, Hibi M. Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. Oncogene 2000;19:2548–56.
23. Hirano T, Nakajima K, Hibi M. Signaling mechanisms through gp130: a model of the cytokine system. Cytokine Growth Factor Rev 1997;8:241–52.
24. Aaronson DS, Horvath CM. A road map for those who don't know JAK-STAT. Science 2002;296:1653–5.
25. Darnell JE, Jr. STATs and gene regulation. Science 1997;277:1630–5.
26. Yu H, Jove R. The STATs of cancer—new molecular targets come of age. Nat Rev Cancer 2004;4:97–105.
27. Odajima J, Matsumura I, Sonoyama J, et al. Full oncogenic activities of v-Src are mediated by multiple signaling pathways. Ras as an essential mediator for cell survival. J Biol Chem 2000;275:24096–105.
28. Turkson J, Bowman T, Garcia R, Caldenhoven E, De Groot RP, Jove R. Stat3 activation by Src induces specific gene regulation and is required for cell transformation. Mol Cell Biol 1998;18:2545–52.
29. Kiuchi N, Nakajima K, Ichiba M, et al. STAT3 is required for the gp130-mediated full activation of the c-*myc* gene. J Exp Med 1999;189:63–73.
30. Epling-Burnette PK, Liu JH, Catlett-Falcone R, et al. Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. J Clin Invest 2001;107:351–62.
31. Kanda N, Seno H, Konda Y, et al. STAT3 is constitutively activated and supports cell survival in association with survivin expression in gastric cancer cells. Oncogene 2004;23:4921–9.
32. Niu G, Wright KL, Huang M, et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. Oncogene 2002;21:2000–8.
33. Wei D, Le X, Zheng L, et al. Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. Oncogene 2003;22:319–29.
34. Song L, Turkson J, Karras JG, Jove R, Haura EB. Activation of Stat3 by receptor tyrosine kinases and cytokines regulates survival in human non-small cell carcinoma cells. Oncogene 2003;22:4150–65.
35. Chang KT, Tsai CM, Chiou YC, Chiu CH, Jeng KS, Huang CY. IL-6 induces neuroendocrine dedifferentiation and cell proliferation in non-small cell lung cancer cells. Am J Physiol Lung Cell Mol Physiol 2005;289:L438–45.
36. Huang M, Wang J, Lee P, et al. Human non-small cell lung cancer cells express a type 2 cytokine pattern. Cancer Res 1995;55:3847–53.
37. Krysan K, Dalwadi H, Sharma S, Pold M, Dubinett S. Cyclooxygenase-2 dependent expression of survivin is critical for apoptosis resistance in non-small cell lung cancer. Cancer Res 2004;64:6359–62.
38. Liu XH, Kirschenbaum A, Lu M, et al. Prostaglandin E(2) stimulates prostatic intraepithelial neoplasia cell growth through activation of the interleukin-6/

GP130/STAT-3 signaling pathway. Biochem Biophys Res Commun 2002;290:249–55.

39. Hong SH, Ondrey FG, Avis IM, et al. Cyclooxygenase regulates human oropharyngeal carcinomas via the proinflammatory cytokine IL-6: a general role for inflammation? FASEB J 2000;14:1499–507.

40. Zhong Z, Wen Z, Darnell JE, Jr. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 1994;264:95–8.

41. Garcia R, Bowman TL, Niu G, et al. Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. Oncogene 2001;20:2499–513.

42. Lorusso P, Krishnamurthi S, Rinehart JR, et al. A phase 1-2 clinical study of a second generation oral MEK inhibitor, PD 0325901 in patients with advanced cancer. ASCO Annual Meeting Proceedings 2005. J Clin Oncol Suppl 2005;23:194S.

43. Castillo SS, Brognard J, Petukhov PA, et al. Preferential inhibition of Akt and killing of Akt-dependent cancer cells by rationally designed phosphatidylinositol ether lipid analogues. Cancer Res 2004;64:2782–92.

44. Huang SP, Wu MS, Shun CT, et al. Interleukin-6 increases vascular endothelial growth factor and angiogenesis in gastric carcinoma. J Biomed Sci 2004;11:517–27.

45. Jiang BH, Jiang G, Zheng JZ, Lu Z, Hunter T, Vogt PK. Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. Cell Growth Differ 2001;12:363–9.

46. Pages G, Milanini J, Richard DE, et al. Signaling angiogenesis via p42/p44 MAP kinase cascade. Ann N Y Acad Sci 2000;902:187–200.

47. Xu L, Fukumura D, Jain RK. Acidic extracellular pH induces vascular endothelial growth factor (VEGF) in human glioblastoma cells via ERK1/2 MAPK signaling pathway: mechanism of low pH-induced VEGF. J Biol Chem 2002;277:11368–74.

48. Cohen T, Nahari D, Cerem LW, Neufeld G, Levi BZ. Interleukin 6 induces the expression of vascular endothelial growth factor. J Biol Chem 1996;271:736–41.

49. Winzen R, Kracht M, Ritter B, et al. The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. EMBO J 1999;18:4969–80.

50. Levy NS, Chung S, Furneaux H, Levy AP. Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR. J Biol Chem 1998;273:6417–23.

51. Riedl K, Krysan K, Pold M, et al. Multifaceted roles of cyclooxygenase-2 in lung cancer. Drug Resist Updat 2004;7:169–84.

52. Tassone P, Neri P, Burger R, et al. Combination therapy with interleukin-6 receptor superantagonist Sant7 and dexamethasone induces antitumor effects in a novel SCID-hu *in vivo* models of human multiple myeloma. Clin Cancer Res 2005;11:4251–8.

53. Trikha M, Corringham R, Klein B, Rossi JF. Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. Clin Cancer Res 2003;9:4653–65.

54. Chan KS, Sano S, Kiguchi K, et al. Disruption of Stat3 reveals a critical role in both the initiation and the promotion stages of epithelial carcinogenesis. J Clin Invest 2004;114:720–8.

55. Turkson J, Ryan D, Kim JS, et al. Phosphotyrosyl peptides block Stat3-mediated DNA binding activity, gene regulation, and cell transformation. J Biol Chem 2001;276:45443–55.

56. Turkson J, Kim JS, Zhang S, et al. Novel peptidomimetic inhibitors of signal transducer and activator of transcription 3 dimerization and biological activity. Mol Cancer Ther 2004;3:261–9.

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