Cyclooxygenase 2-Dependent Expression of Survivin Is Critical for Apoptosis Resistance in Non-Small Cell Lung Cancer

Kostyantyn Krysan,¹ Harnisha Dalwadi,¹ Sherven Sharma,¹,² Mehis Pöld,¹ and Steven Dubinett¹,²

¹Division of Pulmonary and Critical Care, University of California at Los Angeles Lung Cancer Research Program of the Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California at Los Angeles; and ²Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, California

Abstract

Elevated tumor cyclooxygenase 2 (COX-2) expression is associated with increased angiogenesis, tumor invasion, and promotion of tumor cell resistance to apoptosis. In our previous studies using non-small cell lung cancer (NSCLC) cell lines constitutively expressing COX-2 cDNA in sense and antisense orientations, we demonstrated that constitutive overexpression of COX-2 leads to stabilization of the inhibitor of apoptosis protein survivin resulting in the elevated apoptosis resistance of COX-2–overexpressing cells. Genetic or pharmacologic suppression of COX-2 activity increased proteasomal degradation of survivin and cellular response to apoptosis induction. Our data show that expression of survivin in non-small cell lung cancer cells can be significantly down-regulated by RNA interference. Whereas COX-2–overexpressing NSCLC cells have significantly higher apoptosis resistance than the parental cells, inhibition of survivin expression by small interfering RNA decreases apoptosis resistance to the level of the parental non-small cell lung cancer. We conclude that COX-2-dependent expression of survivin is critical for apoptosis resistance in non-small cell lung cancer.

Introduction

Cyclooxygenases are the rate-limiting enzymes in prostanooid synthesis, which convert arachidonic acid into prostaglandin (PG) H2, a substrate for specific PG synthases (1). Two isoforms of cyclooxygenase (COX) have been isolated and characterized: the ubiquitously expressed COX-1 and inducible COX-2 (2). Studies of human cancers have revealed frequent overexpression of COX-2 in a variety of different malignancies. High-level constitutive COX-2 expression has been detected in colorectal (3), head and neck (4), lung (3, 5, 6), breast (3), and other cancers. Overexpression of COX-2 and elevated production of the COX-2 metabolite PGE2 can stimulate epithelial cell growth and invasion and promote cellular survival (7).

Recent studies show that COX-2 promotes cell survival by modulation of antiapoptotic protein expression (8). Survivin is a member of the inhibitor of apoptosis protein (IAP) family and is able to bind caspases thus preventing their activation (9). Suppression of survivin expression leads to increased caspase-3 activation and apoptosis as well as mitosis deregulation and sensitization to anticancer drugs (10). Our previous results indicate that COX-2 overexpression leads to stabilization of survivin in non-small cell lung cancer that alters the balance between pro- and antiapoptotic proteins and facilitates the prosurvival phenotype, resulting in elevated resistance to apoptosis (11).

In the present study, we investigated the effect of survivin inhibition by RNA interference in COX-2–overexpressing non-small cell lung cancer cell lines. These cell lines were used to model the COX-2–rich tumor microenvironment. We found that inhibition of survivin expression overcame the prosurvival phenotype of COX-2–overexpressing non-small cell lung cancer (NSCLC) cells. Inhibition of survivin in COX-2 sense tumor cells reduced the apoptosis threshold to the level of the parental cell lines. These findings indicate the importance of survivin in mediating COX-2–dependent apoptosis resistance in NSCLC.

Materials and Methods

Stable Transduction of Non-Small Cell Lung Cancer Cells. NSCLC cells A549 (human lung adenocarcinoma) and H157 (human squamous cell carcinoma) were obtained from American Type Culture Collection (Rockville, MD) and the National Cancer Institute (Bethesda, MD), respectively. A 2.0-kb cDNA fragment of human COX-2 (generously provided by Dr. Harvey Herschman, University of California at Los Angeles) was cloned in sense and antisense orientations in the retroviral vector pLNCX (Clontech, Palo Alto, CA) as described previously (11). For each cell line, an approximately 10-fold higher level of COX-2 expression and PGE2 production was noted in sense compared with parental or vector controls (7).

Inhibition of Survivin Expression by Small Interfering RNA (siRNA). Stable transduction of non-small cell lung cancer cells. Cells were plated in 24-well plates at 6 × 10⁴ cells per well and grown overnight in RPMI + 10% fetal bovine serum. Cells were transfected with validated survivin or negative control Silencer siRNA (Ambion, Inc., Austin, TX) using TransMessenger transfection reagent (Qiagen, Valencia, CA) at different RNA/transfection reagent ratios. At all conditions, we observed a significant suppression of survivin expression without any signs of cytotoxicity or notable decrease of actin levels as assessed by Western blotting (data not shown). For additional experiments transfection was performed in serum-free RPMI using 1.6 μg of survivin or control siRNA, 3.2 μL of Enhancer R, and 12 μL of TransMessenger reagent for 3 hours followed by the replacement of transfection medium with fresh RPMI supplemented with 10% fetal bovine serum and incubation for an additional 24 hours. After 24 hours of incubation, apoptosis was induced, and the efficiency of survivin suppression was assessed by Western blotting or enzyme-linked immunosorbent assay (ELISA).

Apoptosis Induction and Detection Techniques. Apoptosis was induced by addition of 17 μmol/L Camptothecin (Sigma, St. Louis, MO) for 18 hours. After 18 hours, cells were stained with 0.1 μmol/L of propidium iodide (PI) and 0.1 μmol/L of Hoechst 33342 (Invitrogen, Carlsbad, CA) and analyzed by flow cytometry. PI and Hoechst staining was assessed by flow cytometry.

Requests for reprints: Steven M. Dubinett, David Geffen School of Medicine at University of California at Los Angeles Lung Cancer Research Program, 200 San Pedro Street, Suite 1030, Los Angeles, CA 90015. Phone: 310-794-6566; Fax: 310-267-2829; E-mail: sdbuinett@mednet.ucla.edu.

©2004 American Association for Cancer Research.
bodies (Novus Biologicals, Littleton, CO) diluted 1:1000 in blocking solution followed by treatment with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:10,000 in blocking solution and developed using a SuperSignal West Pico kit (Pierce, Rockford, IL).

Survivin ELISA was performed using the DuoSet Human Survivin ELISA kit (R&D Systems, Minneapolis, MN) in the conditions recommended by the manufacturer. Protein concentration was determined in the cell lysates, and survivin concentration was calculated per milligram of the total protein.

**Statistical Analysis.** Probability values were calculated using two-tailed nonpaired Student’s *t* test.

**Results and Discussion**

Survivin Expression Is Effectively Suppressed by Ribonucleic Acid Interference in Non-Small Cell Lung Cancer Cell Lines. RNA interference is a powerful tool for inhibiting the expression of specific genes (12). Using Western blotting (Fig. 1A) and ELISA (Fig. 1B), we found that survivin expression was strongly suppressed by specific siRNA and not affected by the control siRNA, whereas the actin levels were unaffected by either siRNA. We did not observe any signs of transfection reagent-dependent cytotoxicity after the treatment and upon the subsequent incubation. Our data demonstrate high efficiency of the RNA interference technique in suppression of survivin expression in NSCLC cells.

Inhibition of Survivin Expression by siRNA Sensitizes the Cyclooxygenase 2–Overexpressing Non-Small Cell Lung Cancer Cells to Apoptotic Stimuli. The overexpression of survivin in tumors has been linked to poor prognosis and diminished patient survival in prostate (13), lung (14), and other cancers. Previous studies have shown that COX-2 and its metabolite PGE2 promote cancer cell survival (8). We have found previously that COX-2 overexpression leads to survivin stabilization via decreased proteasomal degradation that renders the NSCLC cells more resistant to apoptotic stimuli. In contrast, pharmacologic inhibition of COX-2 by specific inhibitors significantly reduces survivin levels and thus enhances susceptibility of NSCLC cells to radiation and chemotherapy agents (11). Consistent with the pharmacologic inhibition, genetic inhibition of COX-2 expression led to enhanced apoptosis sensitivity, whereas overexpression increased the apoptosis threshold.

To determine the importance of survivin in mediating COX-2-
dependent apoptosis resistance, we tested the effect of survivin expression inhibition by siRNA on apoptosis resistance of COX-2–overexpressing NSCLC cells. Apoptosis was induced in survivin- or control-siRNA–transfected parental (P) or COX-2 sense (S) A549 and H157 cells by Camptothecin treatment for 18 hours. Apoptotic cells were detected by staining with the nuclear dye YO-PRO that selectively enters and brightly stains apoptotic cells. Consistent with our previous findings (11), both A549S and H157S cells were significantly more resistant to apoptosis induction than their respective parental cells. Inhibition of survivin expression by siRNA rendered NSCLC cells more susceptible to apoptosis and was able to overcome the effect of COX-2 overexpression in S cells. Survivin inhibition lowered the apoptosis threshold of S cells to a level that was comparable with that seen in P cells in A549 (Fig. 2) and H157 (Fig. 3). This effect was not observed in control-siRNA–transfected cells. Our data suggest that survivin is a critical COX-2–dependent modulator of apoptosis resistance in NSCLC cells.

Consistent with these findings, genetic targeting of survivin has been shown to be sufficient for the apoptosis induction in certain cancer cell types (15). Recent data support the suggestion that survivin is a potential target for anticancer therapy. Expression of a dominant-negative mutant of survivin triggers the apoptotic cascade in melanoma cells (16). Similar results showing reduction of tumor growth and suppression of tumor-derived angiogenesis by survivin targeting in vivo in breast cancer cells xenografts were recently shown by Blanc-Brude et al. (17). Recently, specific suppression of survivin gene expression by triplex-forming oligonucleotides significantly inhibited proliferation and induced apoptosis in lung cancer cells (18). Similarly, ribozyme-mediated survivin inhibition sensitized human melanoma (19) and prostate cancer (20) cells to spontaneous and drug-induced apoptosis.

Here, we questioned the role of survivin in COX-2–mediated apoptosis resistance. Our data show the critical significance of survivin for COX-2–overexpressing NSCLC cell survival. By specific inhibition of survivin, we were able to overcome the antiapoptotic effect of COX-2 overexpression. This finding is particularly important given the fact that survivin levels are modulated by COX-2 expression. As a COX-2–dependent factor of apoptosis resistance, survivin may be an important target for antitumor therapy. Additional studies are required to further define the importance of COX-2 and survivin in lung cancer. In particular, additional studies will be required to determine whether survivin can serve as a COX-2–dependent biomarker in lung cancer clinical trials that utilize COX-2 inhibition.

References

Cyclooxygenase 2-Dependent Expression of Survivin Is Critical for Apoptosis Resistance in Non-Small Cell Lung Cancer

Kostyantyn Krysan, Harnisha Dalwadi, Sherven Sharma, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/64/18/6359

Cited articles
This article cites 20 articles, 10 of which you can access for free at:
http://cancerres.aacrjournals.org/content/64/18/6359.full.html#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
/content/64/18/6359.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.