Redox Factor-1 Inhibits Cyclooxygenase-2 Expression via Inhibiting of p38 MAPK in the A549 Cells

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In this study, we evaluated the role of apurinic/apyrimidinic endonuclease1/redox factor-1 (Ref-1) on the tumor necrosis factor-α (TNF-α) induced cyclooxygenase-2 (COX-2) expression using A549 lung adenocarcinoma cells. TNF-α induced the expression of COX-2 in A549 cells, but did not induce BEAS-2B expression. The expression of COX-2 in A549 cells was TNF-α dose-dependent (5∼100 ng/ml). TNF-α-stimulated A549 cells evidenced increased Ref-1 expression in a dose-dependent manner. The adenoviral transfection of cells with AdRef-1 inhibited TNF-α-induced COX-2 expression relative to that seen in the control cells (Adβgal). Pretreatment with 10 μM of SB203580 suppressed TNF-α-induced COX-2 expression, thereby suggesting that p38 MAPK might be involved in COX-2 expression in A549 cells. The phosphorylation of p38 MAPK was increased significantly after 5 minutes of treatment with TNF-α, reaching a maximum level at 10 min which persisted for up to 60 min. However, p38MAPK phosphorylation was markedly suppressed in the Ref-1-overexpressed A549 cells. Taken together, our results appear to indicate that Ref-1 negatively regulates COX-2 expression in response to cytokine stimulation via the inhibition of p38 MAPK phosphorylation. In the lung cancer cell lines, Ref-1 may be involved as an important negative regulator of inflammatory gene expression.

Key Words: Redox factor-1, Cyclooxygenase-2, A549, Lung cancer, p38 MAPK

INTRODUCTION

Cyclooxygenase (COX) is an enzyme that is responsible for the formation of important biological mediators referred to as the prostanoids, a group that includes the prostaglandins, prostacyclin and thromboxane. Two COX isoenzymes have been identified thus far: COX-1 and COX-2. COX-1 is constitutively expression in most tissues, and is generally responsible for the production of prostaglandins that control normal physiological functions; on the other hand, COX-2 is undetectable in the majority of tissues. COX-2 is an inducible enzyme, which is detected abundantly in activated macrophages and other cells at inflammation sites. Recently, COX-2 has been shown to be upregulated in a variety of carcinomas, and to play a central role in tumor genesis [1].

COX-2 has been reported to be constitutively overexpressed in a variety of malignancies [2-5]. It has reported that COX-2 is frequently constitutively elevated in human non-small cell lung cancer [6-9]. The overexpression of COX-2 in tumors may be critically important to tumor invasion and angiogenesis, as well as the suppression of host immunity [9]. Preclinical studies suggest that treatment with a selective COX-2 inhibitor may enhance the antitumor effects of chemotherapy [10,11]. Perioperative COX-2 inhibition could reduce tumor cell adhesion and the metastatic potential of circulating tumor cells in non-small cell lung cancer [12].

Apurinic/apyrimidinic endonuclease/redox factor-1 (Ref-1) is a ubiquitous multifunctional protein involved in the base excision repair pathways and has reducing properties that promote the binding of redox-sensitive transcription factors, such as AP-1, NF-κB, p53, Egr-1, and cMyb to their cognate DNA sequences [13]. Ref-1 performs an important anti-oxidative stress function. The overexpression of Ref-1 repair function stimulates an increase in resistance to certain alkylating agents and oxidative stress [14]. It has been previously reported that Ref-1 inhibits TNF-α-induced inflammation in the vascular endothelial cells [15]. We recently noted that nuclear and cytoplasmic expressions of Ref-1 were upregulated profoundly in the non-small cell lung cancer specimens, and its upregulation was found to be closely related with impaired antioxidant systems in cas-

ABBREVIATIONS: Ref-1, apurinic/apyrimidinic endonuclease/redox factor-1; COX-2, cyclooxygenase-2; TNF-α, tumor necrosis factor-α; p38 MAPK, p38 mitogen-activated protein kinases.

es of lung cancer [16].

Ref-1 is regulated at both the transcriptional and post-translational levels. In terms of transcriptional regulation, reactive oxygen species enhance the Ref-1 expression, which correlates with an increase of its endonuclease activity and redox activities [17,18]. Also, inflammatory cytokines induced the increased Ref-1 expression as well as pro-inflammatory gene [19]. Therefore, it remains possible that Ref-1 performs certain roles in the induction of inflammatory genes in cases of cancer.

However, the role of Ref-1 on COX-2 expression and its underlying mechanism have yet to be precisely determined. In this study, we evaluated the role of Ref-1 on TNF-α induced COX-2 expression using the A549 lung adenocarcinoma cells.

METHODS

Materials-Human tumor necrosis factor-α (TNF-α) was purchased from Sigma. SB203580, a specific inhibitor of p38 MAPK, was purchased from Tocris Bioscience (Bristol, UK). All reagents were obtained from Sigma-Aldrich unless stated otherwise.

Cell culture

A549 derived from lung adenocarcinoma (ATCC #CCL-185) were maintained in RPMI 1640 containing 10% heat-inactivated fetal bovine serum (v/v) and 100 U/ml Penicillin/Streptomycin from Invitrogen (Carlsbad, CA, USA) in an atmosphere of 5% CO_2 at 37°C. BEAS-2B, normal bronchial epithelial cell lines were maintained and harvested using BEGM medium (Cambrex Bioscience, Walkersville, MD, USA). BEAS-2B cells were derived from human bronchial epithelium transformed with an adenovirus12-SV40 hybrid virus [20].

Western blotting

24 or 48 hr after infection with adenoviruses or TNF-α treatment, the expression and phosphorylation of proteins (50 μg) was evaluated via Western blotting as described previously [15]. A549 cells harvested in 100 μl of lysis buffer containing 20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 2 mM EDTA, 2 mM EGTA, 1 mM Na_3VO_3, 1 mM β-glycerophosphate, 4 mM Na pyrophosphate, 5 mM NaF and 1% Triton X-100 (v/v), and protease inhibitor cocktail. The lysate was then centrifuged for 20 min at 12,000 rpm and the supernatants were collected. Proteins (50 μg) were separated via denatured reducing SDS-PAGE, and were electrotransfered onto nitrocellulose membranes. After blocking with 5% skim milk (w/v) for 2 h at room temperature, the blots were incubated overnight at 4°C with antibodies (dilution 1 : 1000) to Ref-1 (SC 5572, Santa Cruz), COX-2 (SC-1745, Santa Cruz), ERK (SC-093, Santa Cruz), phospho-p44/p42 MAPK (Erk 1/2, Cell Signaling), phospho-p38 MAPK and p38 MAPK (Cell Signaling), and anti-β-actin (Sigma Co, USA) and subsequent detection was conducted using horseradish peroxidase-conjugated secondary antibody. Immunoreactivity was evaluated using an enhanced chemiluminescence (ECL) kit (Amersham Pharmacia Biotech, USA).

Adenoviral transfections

Adenoviruses encoding for β-galactosidase (Adβgal) and full-length Ref-1 (AdRef-1), were generated via homologous recombination in human embryonic kidney 293 cells, as previously described [15]. The A549 cells were infected with 0~500 multiplicities of infection (MOI; particle forming units per cell) of the specified adenovirus for 24 h.

Statistical analysis

Values are expressed as the means±S.E.M. Statistical evaluations were conducted using the Student's t-test, with $p < 0.05$ being considered significant.

RESULTS

TNF-α induced COX-2 expression in A549 cells

In order to determine whether TNF-α induces COX-2 expression in the normal epithelial cells and non-small cell lung cancer cell lines, we assessed the effects of TNF-α on COX-2 expression via Western blotting in 24 hr after exposure of TNF-α (30~100 ng/ml). As shown in Fig. 1A, no basal COX-2 expression was detected in BEAS-2B and A549 cell lines. However, TNF-α induced COX-2 expression in A549 cells, but not in BEAS-2B cells. The expression of COX-2 in A549 cells evidenced dose-dependency to TNF-α (5~100 ng/ml) (Fig. 1B). TNF-α induced COX-2 expression
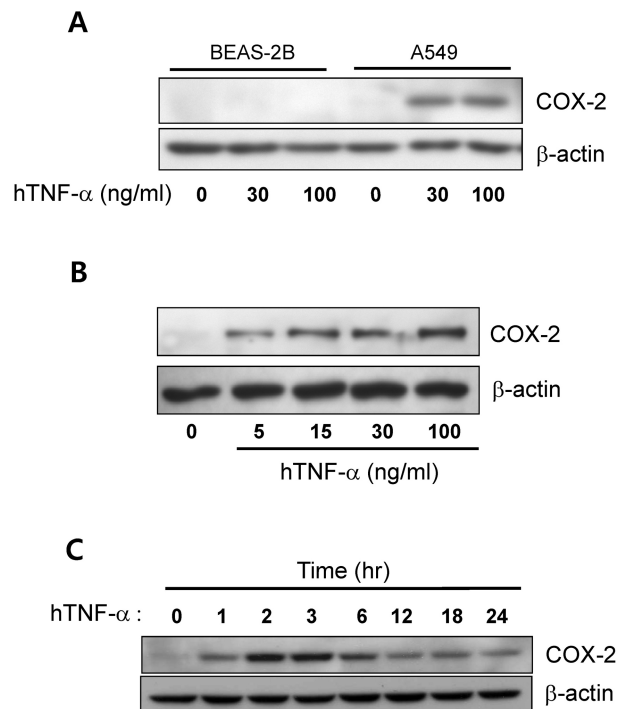

Fig. 1. Tumor necrosis factor-α (TNF-α) induced cyclooxygenase-2 (COX-2) expression in the A549 cells. (A) The effect of TNF-α on COX-2 expression in BEAS-2B and A549 cells. COX-2 expression was analyzed via Western blotting 24 hr after exposure to TNF-α (30~100 ng/ml). (B) COX-2 expression in A549 cells evidenced dose-dependency to TNF-α (5~100 ng/ml). (C) The time-dependent change of COX-2 expression in the response to TNF-α (30 ng/ml) in A549 cells.

reached a maximum level at 3 hr, and this level persisted for 24 hr in the A549 cells (Fig. 1C). This finding indicates that transformed cells (A549) were more sensitive than non-transformed cells (BEAS-2B) to TNF-α-induced COX-2 expression.

Ref-1 expression was increased by TNF-α in A549 cells

Human Ref-1 is regulated at both the transcriptional and translational levels [21] and is up-regulated in a reactive oxygen species-dependent manner [19]. Therefore, we examined the Ref-1 expression in response to TNF-α stimulation for 18 hr in A549. Therefore, we evaluated Ref-1 expression in response to 18 h of TNF-α stimulation in A549. As is shown in Fig. 2, TNF-α-stimulated A549 cells demonstrated the increase of Ref-1 expression in a dose-dependent manner. Densitometry analysis data was plotted at the bottom of Fig. 2B.

Overexpression of Ref-1 inhibited TNF-α-induced COX-2 expression

As previously established TNF-α induced the expression of both COX-2 and Ref-1 in A549 cells. Therefore, we attempted to determine whether Ref-1 affects COX-2 expression in response to TNF-α. After the treatment of cells with AdRef-1 (0～500 MOI), the cells were stimulated with TNF-α (30 ng/ml) for 18 hr. A549 cells were immunoblotted using anti-COX-2 and anti-Ref-1 antibody. As is shown in Fig. 3, the adenoviral transfection of cells with AdRef-1 inhibited TNF-α-induced COX-2 expression relative to control cells.

Ref-1 inhibited p38 MAPK activation in A549 cells

TNF-α can activate MAPKs in the signaling pathway, resulting in the expression of several cytokines [22]. As previously established Ref-1 inhibited COX-2 expression in A549 cells, we attempted to characterize the Ref-1-induced COX-2 expression in A549 cells. First, to evaluate the role of p38 MAPK on the expression of COX-2, the effects of SB203580, an inhibitor of p38 MAPK, were evaluated on the TNF-α-induced COX-2 expression. In this experiment, SB203580 was pretreated for 30 min prior to TNF-α treatment, and the cells were exposed to TNF-α for 6 hours. Pretreatment with 10 μM of SB203580 suppressed TNF-α-induced COX-2 expression, thereby suggesting that p38 MAPK may be involved in the expression of COX-2 in A549 cells, as shown in Fig. 4A.

Finally, we attempted to determine whether Ref-1 inhibited p38 MAPK and ERK activation in response to TNF-α treatment. After the treatment of cells with Adβgal (200 MOI) and AdRef-1 (200 MOI), the cells were stimulated with 30 ng/ml TNF-α for 0～60 min at the indicated times. As shown in Fig. 4B, p38 MAPK phosphorylation was increased significantly after 5 minutes of TNF-α treatment,
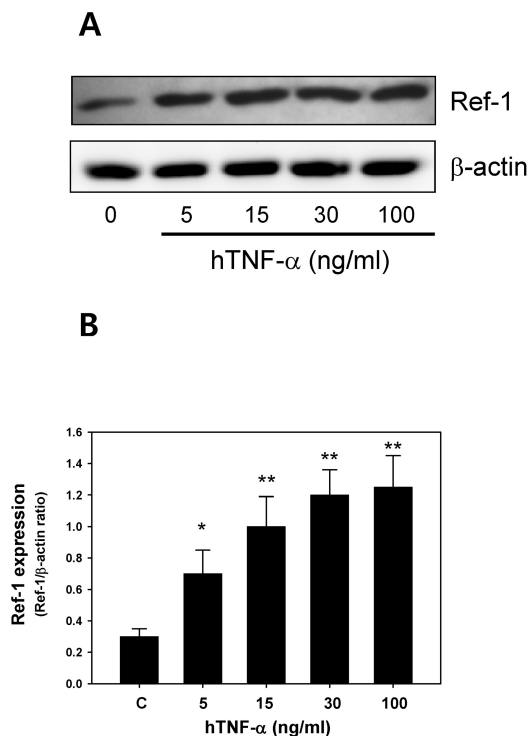

Fig. 2. Tumor necrosis factor-α (TNF-α) induced Ref-1 expression in the A549 cells. (A) Effect of TNF-α on the Ref-1 expression in the A549 cells. Ref-1 expression was analyzed via Western blotting 24 hr after exposure to TNF-α (30～100 ng/ml). β-actin was used as a loading control. (B) Densitometry analysis for Ref-1 expression. Each bar shows the mean±S.E. (n=4), *p＜0.05, **p＜0.01.
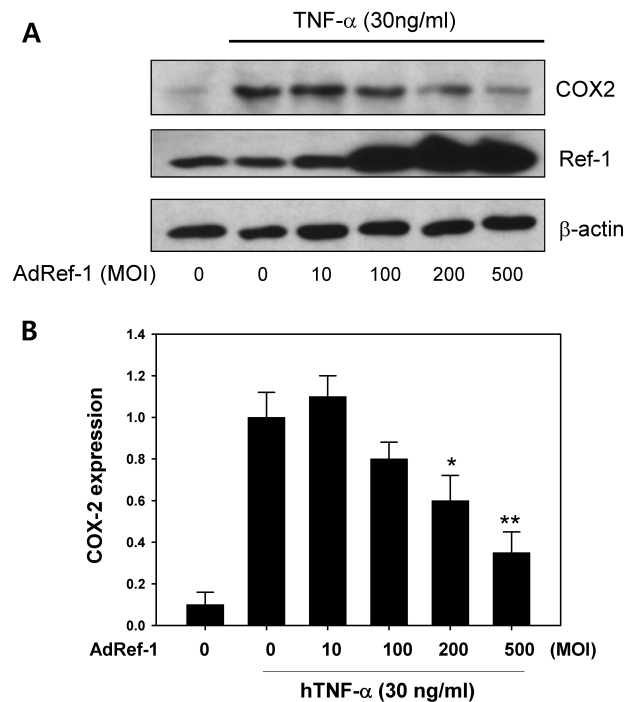

Fig. 3. Ref-1 inhibited COX-2 expression in the A549 cells. (A) Western blots for COX-2 and Ref-1. After the cells were treated with AdRef-1 (0～500 MOI), the cells were stimulated with TNF-α (30 ng/ml) for 18 hr. Western blotting was conducted using anti-COX-2 and anti-Ref-1 antibodies. The total adenovirus titer was balanced with Adβgal (500 MOI). (B) Densitometry analysis for COX-2 expression. Each bar showed the mean±S.E. (n=3), *p＜0.05, **p＜0.01.
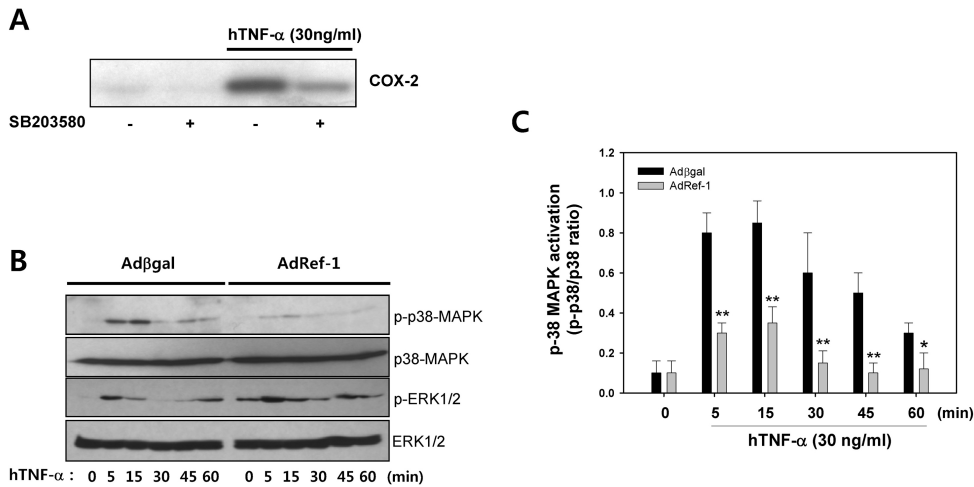
A

hTNF-α (30ng/ml)

COX-2

SB203580   -   +   -   +

B

Adβgal   AdRef-1

p-p38-MAPK

p38-MAPK

p-ERK1/2

ERK1/2

hTNF-α : 0 5 15 30 45 60   0 5 15 30 45 60 (min)

C

Fig. 4. Ref-1 inhibited p38 MAPK activation in the A549 cells. (A) TNF-α induced COX-2 expression was inhibited by SB203580 (10 μM), an inhibitor of p38 MAPK. (B) Time-dependent change of p38 MAPK activation (p-p38 MAPK) and ERK activation (p-ERK) in the response of TNF-α in the Adβgal or AdRef-1-transfected A549 cells. (C) Densitometry analysis for phospho-p38 MAPK expression. Each bar showed the mean±S.E. (n=4), **p$<$0.01.

reaching a maximum level at 15 min that persisted for up to 60 min. ERK phosphorylation was also increased by TNF-α. Only p38MAPK phosphorylation was suppressed markedly in the Ref-1-overexpressed A549 cells. The overexpression of Ref-1 suppressed p38 MAPK activation in the response to TNF-α, but it did not suppress ERK activation in A549 cells. Densitometry analysis for p38 MAPK was plotted at Fig. 4C. These results indicate that reduced p38 MAPK activation performs a Ref-1-inhibitory effect in the transcriptional regulation of TNF-α-induced COX-2 expression.

DISCUSSION

The principal objective of this study was to elucidate the mechanisms responsible for the overexpression of Ref-1 and COX-2 in non-small cell lung cancer. These studies demonstrated the novel inhibitory role of Ref-1 on tumor COX-2 expression in response to TNF-α treatment in A549 lung adenocarcinoma cells.

In this experiment, we employed an A549 cell line, which is known as a *ras*-transformed cell line among non-small cell lung cancer cell lines [23] and a normal epithelial cell line, the BEAS-2B cell line, was derived from human bronchial epithelium transformed by an Adenovirus12-SV40 hybrid virus [20]. In this experiment, A549 cells evidenced high sensitivity to the upregulation of COX-2 in response to TNF-α, as compared with BEAS-2B. This finding is consistent with a previous report showing increased COX-2 expression in transformed cells [24]. In the preliminary study we also confirmed that NCI-H460 and VMRC-LCD human lung carcinoma cell lines did not evidence COX-2 expression in response to TNF-α (Data not shown). Therefore, we selected A549 cell lines in this experiment.

COX-2 has been implicated in the control of cell growth in cases of human non-small cell lung carcinoma. In cases of lung cancer, the expression of COX-2 has been observed at most stages of tumor progression. Almost all non-small cell lung cancer pre-invasive precursor lesions, as well as invasive lung carcinomas, express COX-2 at higher levels than are measured in normal lung tissue [6,25-28]. Additionally, COX-2 inhibitors inhibit proliferation and induce apoptosis in human non-small cell lung carcinoma

[29]. The critical function of TNF-α in chronic inflammatory disease has previously been well established. TNF-α generated in a tumor microenvironment can function as an endogenous tumor promoter, and also as an inducer of the genes involved in inflammation and cell survival. It has been demonstrated that inflammatory cytokines were upregulated in specimens of human lung cancer [30,31]. In this study, we confirmed TNF-α induced COX-2 expression in A549 cells, suggesting abundant COX-2 expression in cancer environments.

TNF-α has also been demonstrated to contribute to the initiation of tumors by stimulating the production of genotoxic molecules--that is, molecules that can cause DNA damage and mutations, such as NO and ROS [32]. Ref-1 is a multifunctional protein that is involved in the regulation of reduction-oxidation, in addition to its DNA repair function. Our data demonstrate that Ref-1 is also upregulated in the TNF-α stimulated A549 cells. Previously, we noted that Ref-1 expression was upregulated in lung adenocarcinoma [16]. This data indicated that upregulated Ref-1 may be involved in cellular protection against TNF-α-stimulated environments.

COX-2 catalyzes the rate-limiting step in prostaglandin synthesis, usually as a part of the inflammatory process. The expression of COX-2 may be upregulated in cancer cells or during the inflammatory response. Most significantly, COX-2 inhibitors repress tumor development in the lungs of cancer-treated mice [12]. Both Ref-1 and COX-2 expressions were upregulated in the several kinds of human cancer specimens, however, the crosstalk between Ref-1 and COX-2 is not still defined. In this study, we attempted to determine whether Ref-1 is involved in the expression of COX-2 in a lung cancer cell line. In the present study we demonstrated the crosstalk between Ref-1 and COX-2 in lung cancer cell lines, A549 cells. Exposure of TNF-α increased the endogenous Ref-1 expression as well as the induction of COX-2 expression, however the overexpression of Ref-1 suppressed markedly TNF-α-induced COX-2 expression in A549 cells. Those data suggested the increased Ref-1 in the response to TNF-α may be compensatory up-regulation for the reduction of TNF-α- induced inflammatory process.

It is also worth noting that the upregulation of COX-2 expression induced by a variety of stimuli was involved in

the activation of the p38 MAPK and ERK pathways in A549 [33-35]. TNF-α-induced COX-2 expression was inhibited by the p38 MAPK inhibitor SB203580, thereby indicating that of p38 MAPK activation performs an important function in COX-2 expression. The overexpression of Ref-1 inhibited p38 MAPK activation in response to TNF-α, but did not detectably inhibit the activation of ERK. In addition to the DNA repair function of Ref-1, it has also been demonstrated that Ref-1 suppresses oxidative stress [36,37] and performs an anti-inflammatory function in endothelial cells [15], monocytes/macrophages [38] and neointima of balloon-injured carotid arteries [37].

Our results, therefore, appear to indicate that Ref-1 negatively regulates COX-2 expression in response to cytokine stimulation via the inhibition of p38 MAPK phosphorylation. In the lung cancer cell lines, Ref-1 may perform an important role as a negative regulator of inflammatory gene expression.

ACKNOWLEDGEMENTS

REFERENCES

1. **Fosslien E.** Molecular pathology of cyclooxygenase-2 in neoplasia. *Ann Clin Lab Sci.* 2000;30:3-21.

2. **Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, Koki AT.** COX-2 is expressed in human pulmonary, colonic, and mammary tumors. *Cancer.* 2000;89:2637-2645.

3. **Yip-Schneider MT, Barnard DS, Billings SD, Cheng L, Heilman DK, Lin A, Marshall SJ, Crowell PL, Marshall MS, Sweeney CJ.** Cyclooxygenase-2 expression in human pancreatic adenocarcinomas. *Carcinogenesis.* 2000;21:139-146.

4. **Shamma A, Yamamoto H, Doki Y, Okami J, Kondo M, Fujiwara Y, Yano M, Inoue M, Matsuura N, Shiozaki H, Monden M.** Upregulation of cyclooxygenase-2 in squamous carcinogenesis of the esophagus. *Clin Cancer Res.* 2000;6:1229-1238.

5. **Chan G, Boyle JO, Yang EK, Zhang F, Sacks PG, Shah JP, Edelstein D, Soslow RA, Koki AT, Woerner BM, Masferrer JL, Dannenberg AJ.** Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. *Cancer Res.* 1999;59:991-994.

6. **Hida T, Yatabe Y, Achiwa H, Muramatsu H, Kozaki K, Nakamura S, Ogawa M, Mitsudomi T, Sugiura T, Takahashi T.** Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. *Cancer Res.* 1998;58:3761-3764.

7. **Hida T, Kozaki K, Muramatsu H, Masuda A, Shimizu S, Mitsudomi T, Sugiura T, Ogawa M, Takahashi T.** Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytotoxicity of various anticancer agents in non-small cell lung cancer cell lines. *Clin Cancer Res.* 2000;6:2006-2011.

8. **Huang M, Stolina M, Sharma S, Mao JT, Zhu L, Miller PW, Wollman J, Herschman H, Dubinett SM.** 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;58:1208-1216.

9. **Dohadwala M, Luo J, Zhu L, Lin Y, Dougherty GJ, Sharma S, Huang M, Pold M, Batra RK, Dubinett SM.** Non-small cell lung cancer cyclooxygenase-2-dependent invasion is mediated by CD44. *J Biol Chem.* 2001;276:20809-20812.

10. **Altorki NK, Keresztes RS, Port JL, Libby DM, Korst RJ, Flieder DB, Ferrara CA, Yankelevitz DF, Subbaramaiah K, Pasmantier MW, Dannenberg AJ.** Celecoxib, a selective cyclooxygenase-2 inhibitor, enhances the response to preoperative paclitaxel and carboplatin in early-stage non-small-cell lung cancer. *J Clin Oncol.* 2003;21:2645-2650.

11. **Castelao JE, Bart RD, 3rd, DiPerna CA, Sievers EM, Bremner RM.** Lung cancer and cyclooxygenase-2. *Ann Thorac Surg.* 2003;76:1327-1335.

12. **Backhus LM, Sievers E, Lin GY, Castanos R, Bart RD, Starnes VA, Bremner RM.** Perioperative cyclooxygenase 2 inhibition to reduce tumor cell adhesion and metastatic potential of circulating tumor cells in non-small cell lung cancer. *J Thorac Cardiovasc Surg.* 2006;132:297-303.

13. **Evans AR, Limp-Foster M, Kelley MR.** Going APE over ref-1. *Mutat Res.* 2000;461:83-108.

14. **Tomicic M, Eschbach E, Kaina B.** Expression of yeast but not human apurinic/apyrimidinic endonuclease renders Chinese hamster cells more resistant to DNA damaging agents. *Mutat Res.* 1997;383:155-165.

15. **Kim CS, Son SJ, Kim EK, Kim SN, Yoo DG, Kim HS, Ryoo SW, Lee SD, Irani K, Jeon BH.** Apurinic/apyrimidinic endonuclease1/redox factor-1 inhibits monocyte adhesion in endothelial cells. *Cardiovasc Res.* 2006;69:520-526.

16. **Yoo DG, Song YJ, Cho EJ, Lee SK, Park JB, Yu JH, Lim SP, Kim JM, Jeon BH.** Alteration of APE1/ref-1 expression in non-small cell lung cancer: the implications of impaired extracellular superoxide dismutase and catalase antioxidant systems. *Lung Cancer.* 2008;60:277-284.

17. **Tell G, Damante G, Caldwell D, Kelley MR.** The intracellular localization of APE1/Ref-1: more than a passive phenomenon? *Antioxid Redox Signal.* 2005;7:367-384.

18. **Pines A, Perrone L, Bivi N, Romanello M, Damante G, Gulisano M, Kelley MR, Quadrifoglio F, Tell G.** Activation of APE1/Ref-1 is dependent on reactive oxygen species generated after purinergic receptor stimulation by ATP. *Nucleic Acids Res.* 2005;33:4379-4394.

19. **Lee HM, Yuk JM, Shin DM, Yang CS, Kim KK, Choi DK, Liang ZL, Kim JM, Jeon BH, Kim CD, Lee JH, Jo EK.** Apurinic/apyrimidinic endonuclease 1 is a key modulator of keratinocyte inflammatory responses. *J Immunol.* 2009;183:6839-6848.

20. **Reddel RR, Ke Y, Gerwin BI, McMenamin MG, Lechner JF, Su RT, Brash DE, Park JB, Rhim JS, Harris CC.** Transformation of human bronchial epithelial cells by infection with SV40 or adenovirus-12 SV40 hybrid virus, or transfection via strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. *Cancer Res.* 1988;48:1904-1909.

21. **Bhakat KK, Mantha AK, Mitra S.** Transcriptional regulatory functions of mammalian AP-endonuclease (APE1/Ref-1), an essential multifunctional protein. *Antioxid Redox Signal.* 2009;11:621-638.

22. **Baud V, Karin M.** Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol.* 2001;11:372-377.

23. **Mitsudomi T, Viallet J, Mulshine JL, Linnoila RI, Minna JD, Gazdar AF.** Mutations of ras genes distinguish a subset of non-small-cell lung cancer cell lines from small-cell lung cancer cell lines. *Oncogene.* 1991;6:1353-1362.

24. **Subbaramaiah K, Telang N, Ramonetti JT, Araki R, DeVito B, Weksler BB, Dannenberg AJ.** Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. *Cancer Res.* 1996;56:4424-4429.

25. **Hosomi Y, Yokose T, Hirose Y, Nakajima R, Nagai K, Nishiwaki Y, Ochiai A.** Increased cyclooxygenase 2 (COX-2) expression occurs frequently in precursor lesions of human adenocarcinoma of the lung. *Lung Cancer.* 2000;30:73-81.

26. **Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimaki A.** Expression of cyclooxygenase-2 in human lung carcinoma. *Cancer Res.* 1998;58:4997-5001.

27. **Fang HY, Lin TS, Lin JP, Wu YC, Chow KC, Wang LS.** Cyclooxygenase-2 in human non-small cell lung cancer. *Eur J Surg Oncol.* 2003;29:171-177.

28. **Ermert L, Dierkes C, Ermert M.** Immunohistochemical expression of cyclooxygenase isoenzymes and downstream enzymes in human lung tumors. *Clin Cancer Res.* 2003;9:1604-1610.

29. **Han S, Roman J.** COX-2 inhibitors suppress lung cancer cell

growth by inducing p21 via COX-2 independent signals. *Lung Cancer*. 2006;51:283-296.

30. **Dalaveris E, Kerenidi T, Katsabeki-Katsafli A, Kiropoulos T, Tanou K, Gourgoulianis KI, Kostikas K.** VEGF, TNF-alpha and 8-isoprostane levels in exhaled breath condensate and serum of patients with lung cancer. *Lung Cancer*. 2009;64:219-225.

31. **Derin D, Soydinc HO, Guney N, Tas F, Camlica H, Duranyildiz D, Yasasever V, Topuz E.** Serum levels of apoptosis biomarkers, survivin and TNF-alpha in nonsmall cell lung cancer. *Lung Cancer*. 2008;59:240-245.

32. **Hussain SP, Hofseth LJ, Harris CC.** Radical causes of cancer. *Nat Rev Cancer*. 2003;3:276-285.

33. **Lin CH, Kuan IH, Wang CH, Lee HM, Lee WS, Sheu JR, Hsiao G, Wu CH, Kuo HP.** Lipoteichoic acid-induced cyclooxygenase-2 expression requires activations of p44/42 and p38 mitogen-activated protein kinase signal pathways. *Eur J Pharmacol*. 2002;450:1-9.

34. **N'Guessan PD, Etouem MO, Schmeck B, Hocke AC, Scharf S, Vardarova K, Opitz B, Flieger A, Suttorp N, Hippenstiel S.** Legionella pneumophila-induced PKCalpha-, MAPK-, and NF-kappaB-dependent COX-2 expression in human lung epithe-lium. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L267-277.

35. **Wei J, Yan W, Li X, Chang WC, Tai HH.** Activation of thromboxane receptor alpha induces expression of cyclooxy-genase-2 through multiple signaling pathways in A549 human lung adenocarcinoma cells. *Biochem Pharmacol*. 2007;74:787-800.

36. **Angkeow P, Deshpande SS, Qi B, Liu YX, Park YC, Jeon BH, Ozaki M, Irani K.** Redox factor-1: an extra-nuclear role in the regulation of endothelial oxidative stress and apoptosis. *Cell Death Differ*. 2002;9:717-725.

37. **Lee HM, Jeon BH, Won KJ, Lee CK, Park TK, Choi WS, Bae YM, Kim HS, Lee SK, Park SH, Irani K, Kim B.** Gene transfer of redox factor-1 inhibits neointimal formation: involvement of platelet-derived growth factor-beta receptor signaling via the inhibition of the reactive oxygen species-mediated Syk pathway. *Circ Res*. 2009;104:219-227.

38. **Yuk JM, Yang CS, Shin DM, Kim KK, Lee SK, Song YJ, Lee HM, Cho CH, Jeon BH, Jo EK.** A dual regulatory role of apurinic/apyrimidinic endonuclease 1/redox factor-1 in HMGB1-induced inflammatory responses. *Antioxid Redox Signal*. 2009; 11:575-588.