Low-dose gamma-irradiation inhibits IL-6 secretion from human lung fibroblasts that promotes bronchial epithelial cell transformation by cigarette-smoke carcinogen

Wenshu Chen, Xiuling Xu, Lang Bai, Mabel T.Padilla, Katherine M.Gott, Shuguang Leng, Carmen S.Tellez, Julie A.Wilder, Steven A.Belinsky, Bobby R.Scott and Yong Lin*

Lovelace Respiratory Research Institute, 2425 Ridgecrest Dr. SE, Albuquerque, NM 87108, USA

*To whom correspondence should be addressed. Molecular Biology and Lung Cancer Program, Lovelace Respiratory Research Institute, 2425 Ridgecrest Dr. SE, Albuquerque, NM 87108, USA. Tel: +505-348-9645; Fax: +505-348-4990; Email: ylin@lrri.org

Despite decades of research in defining the health effects of low-dose (<100 mGy) ionizing photon radiation (LDR), the relationship between LDR and human cancer risk remains elusive. Because chemical carcinogens modify the tumor microenvironment, which is critical for cancer development, we investigated the role and mechanism of LDR in modulating the response of stromal cells to chemical carcinogen–induced lung cancer development. Secretion of proinflammatory cytokines such as interleukin-6 (IL-6), CXCL1 and CXCL5 from human lung fibroblasts was induced by cigarette-smoke carcinogen benzo[a]pyrene diol epoxide (BPDE), which was inhibited by a single dose of LDR. The activation of NF-κB, which is important for BPDE-induced IL-6 secretion, was also effectively suppressed by LDR. In addition, conditioned media from BPDE-treated fibroblasts activated STAT3 in the immortalized normal human bronchial epithelial cell line Beas-2B, which was blocked with an IL-6 neutralizing antibody. Conditioned medium from LDR-primed and BPDE-treated fibroblast showed diminished capacity in activating STAT3. Furthermore, IL-6 enhanced BPDE-induced Beas-2B cell transformation *in vitro*. These results suggest that LDR inhibits cigarette smoke–induced lung carcinogenesis by suppressing secretion of cytokines such as IL-6 from fibroblasts in lung tumor-prone microenvironment.

Introduction

Whereas high-dose ionizing photon radiation is carcinogenic, the effect of low-dose (<100 mGy) ionizing photon radiation (LDR) on human carcinogenesis is uncertain (1,2). There are two models regarding the relationship between LDR and cancer risk. The classic linear-no-threshold (LNT) model assumes that cancer risk is proportional to exposed dose without a threshold. According to this model, exposure to any dose of radiation will increase cancer incidence. The LNT model is the basis for radiation protection policy making. However, since LNT is an extrapolation from high- to low-dose radiation, its validity has been controversial for decades and is currently under critical reevaluation (1,3–5). Conversely, the radiation hormesis model argues that low-dose exposure has beneficial effects on human health that can reduce cancer risk (6). Like LNT, the hormesis model is still controversial and needs more scientific support (1,4).

Lung cancer is the leading cause of cancer-related mortality worldwide. Investigation of the role of low-dose radiation in lung cancer development is particularly important, considering that radiation diagnostics are used for lung cancer detection and monitoring treatment response and follow-up. For example, computed topography scanning and chest X-ray examination are regularly used for lung cancer

screening. One of the concerns in using these screening tools is the potential cancer risk of single or repeated low-dose radiation exposure (7).

Research on LDR has long been focused on direct effects, particularly DNA damage responses in the target cells. To better assess LDR's role in cancer risk, it is essential to gain mechanistic insight into the effect of LDR on tumor microenvironment, where epithelial cells interact with their stroma, which can be modified by carcinogens to contribute to cancer development (1,2,8). Most lung cancers are associated with tobacco smoking. Apart from direct mutagenic effects, cigarette-smoke carcinogens also induce chronic inflammation in the lung. Chronic inflammation, which results in sustained production of cytokines and chemokines and repeated tissue damage, is an important promoter of lung cancer (9). In support of this view, there is a strong link between chronic obstructive pulmonary diseases and lung cancer (10,11). In addition, recent studies using genetically altered animal models of spontaneous lung cancer highlight the pivotal role of the proinflammatory cytokine interleukin-6 (IL-6) in lung carcinogenesis (12,13). IL-6 activates signal transducer and activator of transcription 3 (STAT3) to execute its biological effects (14), and STAT3 has been shown to be an important mediator of inflammation-associated lung cancer (15).

Fibroblasts are an important stromal cell type that participates in inflammatory response and has been implicated in cancer initiation and progression through secretion of cytokines and chemokines and regulation of the homeostasis of extracellular matrix (16,17). For example, high-dose radiation-induced senescent fibroblasts facilitate the invasion of breast cancer and the growth of lung cancer cells (18,19). Although fibroblasts have been used to study the cellular effects of LDR, how LDR modulates carcinogen-induced responses in fibroblasts and impacts epithelial cells in cancer development is poorly understood.

In this study, we investigated the effect of LDR on cytokine secretion from human lung fibroblasts stimulated by benzo[a]pyrene diol epoxide (BPDE) and the underlying mechanisms and on bronchial epithelial cell transformation. We show that LDR suppresses BPDE-induced IL-6 secretion from fibroblasts by inhibiting activation of the NF-κB signaling pathway. Further, IL-6 activates STAT3 in human bronchial cells and enhances BPDE-mediated transformation of the cells. Importantly, LDR reduced the capacity of conditioned medium (CM) from BPDE-treated fibroblasts in activating STAT3 signaling. Because both NF-κB and STAT3 are master regulators of inflammation and have been shown to play pivotal roles in lung cancer formation (15,20–22), our results suggest that LDR prevents lung carcinogenesis by limiting cigarette smoke carcinogen–induced lung inflammation and epithelial cell transformation.

Materials and methods

Reagents and antibodies
BPDE was purchased from NCI Chemical Carcinogen Reference Standards Repository. Recombinant human IL-6 was from eBioscience (San Diego, CA). Extracellular signal-regulated kinase (ERK) inhibitor U0126 and IκB Kinase 2 inhibitor (SC-514) were from Calbiochem (La Jolla, CA). Antibodies used for western blot were the following: anti-IκBα, glyceraldehyde 3-phosphate dehydrogenase (GADPH, Santa Cruz Biotechnology, Santa Cruz, CA); anti-phospho-ERK, anti-ERK, anti-phospho-STAT3 (Tyr 705 and Ser 727), anti-STAT3 (Cell Signaling, Danvers, MA) and anti-β-actin (Sigma, St Louis, MO). Anti-human IL-6 receptor neutralizing antibody (AB-227-NA) was from R&D Systems (Minneapolis, MN).

Cell culture
Beas-2B and HBEC-2 are human bronchial epithelial cell lines immortalized by SV40 or by transfection of cyclin-dependent kinase 4 and human telomerase reverse transcriptase (23), respectively. Both cells are maintained in keratinocyte serum-free medium (K-SFM) with supplements (epidermal growth factor and bovine pituitary extract; Invitrogen, Carlsbad, CA). HFL1 is a human fetal lung fibroblast line cultured in F-12K medium with 10% bovine fetal serum

and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). THP-1 human monocytic leukemia cells were cultured in RPMI 1640 with antibiotics and differentiated to macrophages using 50 ng/ml of phorbol-12-myristate-13-acetate for 24 h before treatment. All cell lines and F-12K medium were purchased from American Type Culture Collection (Manassas, VA).

Irradiation of cells
Cells cultured in 35- or 60-mm dishes were irradiated at room temperature using a Gammacell 1000 Cs-137 irradiator (Atomic Energy of Canada Limited) at a dose rate of 0.216 Gy/min.

Antibody array and enzyme-linked immunosorbent assay
HFL1 plated in 60-mm dishes were left untreated or irradiated at 90 mGy and then treated with BPDE or dimethyl sulfoxide (DMSO) as indicated in figure legends. Cell culture medium was collected 24 h after treatment and subjected to human cytokine antibody array according to the manufacturer's instructions (RayBiotech, Norcross, GA). IL-6 concentration in culture medium was quantified using enzyme-linked immunosorbent assay (ELISA) kit from eBioscience (San Diego, CA).

Transfection and reporter assay
HFL1 cells were seeded in 35-mm dishes overnight and transfected with 0.6 µg NF-kB luciferase construct together with 0.4 µg pRSV-LacZ for monitoring transfection efficiency, using FuGENE™ HD according to the manufacturer's instruction (Roche, Indianapolis, IN). Twenty-four hours posttransfection, cells were left untreated or irradiated at 60 mGy and then the cells were treated with 0.2 µM BPDE or DMSO for 6 h. Luciferase reporter assays were carried out using a kit from Promega (Madison, WI), normalized to β-gal and expressed as fold change compared to control as 1.

Western blot
Total cell lysate was prepared using M2 buffer (20 mM Tris–HCl, pH 7.6; 0.5% NP-40; 250 mM NaCl; 3 mM ethylene glycol tetraacetic acid; 3 mM ethylenediaminetetraacetic acid; 2 mM dithiothreitol; 0.5 mM phenylmethylsulfonyl fluoride; 20 mM β-glycerophosphate; 1 mM sodium vanadate and 1 µg/ml leupeptin). Equal amount of protein from lysates was run in 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membrane and probed with specific antibodies. The signals were visualized by enhanced chemiluminescence according to the manufacturer's instructions (Millipore, Billerica, MA). The intensity of the individual bands was quantified by densitometry (NIH Image 1.62) and normalized to the corresponding input control (β-tubulin) bands. Fold changes were calculated with the control taken as 1.

Cytotoxicity assay
Beas-2B cells treated with STAT3 inhibitor X, BPDE or both were incubated with 20 µg/ml of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium

bromide (MTT) for about 2 h at the end of experiment. Cells were then rinsed two times with cold phosphate-buffered saline and dissolved with DMSO. The absorbance of the samples was read at 570 nm using a plate reader. Results were expressed as percentages relative to control as 100.

Cell transformation and colony formation assay
Beas-2B cells were transformed with BPDE using a protocol described by Okamoto *et al.* (24), with modification. Briefly, Beas-2B cells were treated once with 0.2 µM of BPDE or DMSO for 1 h. Twenty-four hours later, IL-6 (10 ng/ml) was added to the medium and the cells were cultured in the presence of IL-6 for 1 week. The cells were then seeded in triplicate at a density of 1×10^4 cells/well (12-well plate) in 0.2% agar in K-SFM with 0.6% agar-K-SFM as a support layer, and 0.5 ml of K-SFM containing IL-6 (10 ng/ml) was added over the solidified agar. Cell colonies were photographed and counted in three random fields per well 10 days later.

Statistical analysis
Data are expressed as mean ± SD. Statistics was performed using Student's *t*-test. $P < 0.05$ was considered statistically significant.

Results

LDR inhibits BPDE-induced secretion of cytokines and chemokines from human lung fibroblasts
To identify and characterize cytokines and chemokines secreted from fibroblast cells that may be involved in BPDE-induced lung cancer development, we treated the human lung fibroblast HFL1 cells with BPDE for 24 h. The culture media were collected and interrogated for expression of cytokines, chemokines and growth factors using an antibody array comprising 80 cytokines, chemokines and growth factors. BPDE treatment dramatically increased secretion of IL-6, CXCL1 and CXCL5 (Figure 1A and B). To examine the effect of LDR on BPDE-induced cytokine secretion, HFL1 cells were irradiated with γ-ray photons at a dose of 90 mGy prior to BPDE exposure. Although LDR alone did not result in noticeable changes in cytokine and growth factor secretion, it effectively suppressed BPDE-induced IL-6 secretion and, to a lesser extent, CXCL1 and CXCL5 secretion (Figure 1A and B).

ELISA was then used to validate the results from antibody array. BPDE induced IL-6 production in HFL1 in a dose-dependent manner (Figure 2A). Induction of IL-6 was first observed with treatment of cells with 0.1 µM of BPDE, and 4- to 5-fold induction can be seen with 0.4 µM of BPDE treatment (Figure 2A and B and Figure 3A).
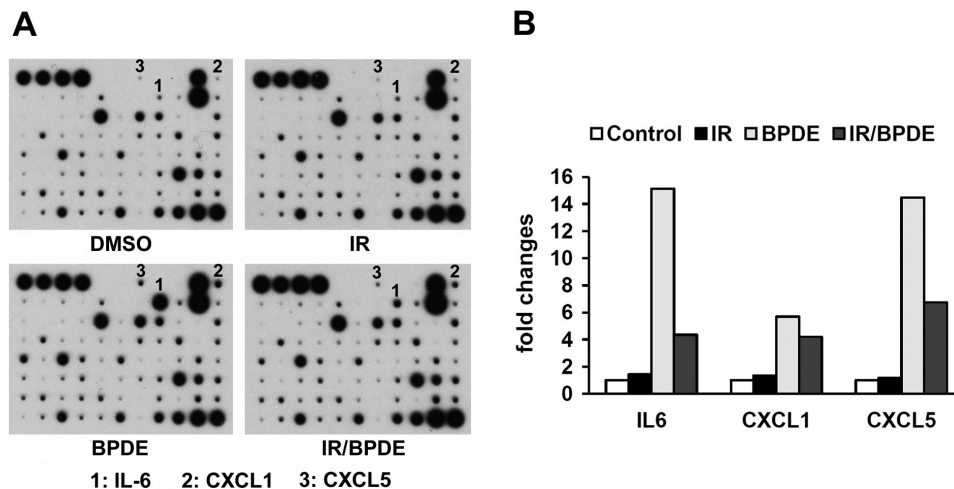
Fig. 1. Identification of BPDE-induced cytokine and chemokine secretion from human lung fibroblasts that can be inhibited by LDR with antibody array. (**A**) HFL1 cells were left untreated or received 90 mGy of gamma irradiation followed by incubation with BPDE (0.4 µM) or DMSO as control. Cell culture supernatants were collected 24 h later for human cytokine antibody array assay (RayBiotech). The same factor on different membranes was labeled by 1, 2 and 3. The left four dots showing strong signals from the first row on each membrane are positive controls for that membrane, followed by two negative controls that have no signals. (**B**) The relative density of the selected cytokines to the average of the positive controls. Fold changes are shown with the level in control media taken as 1.
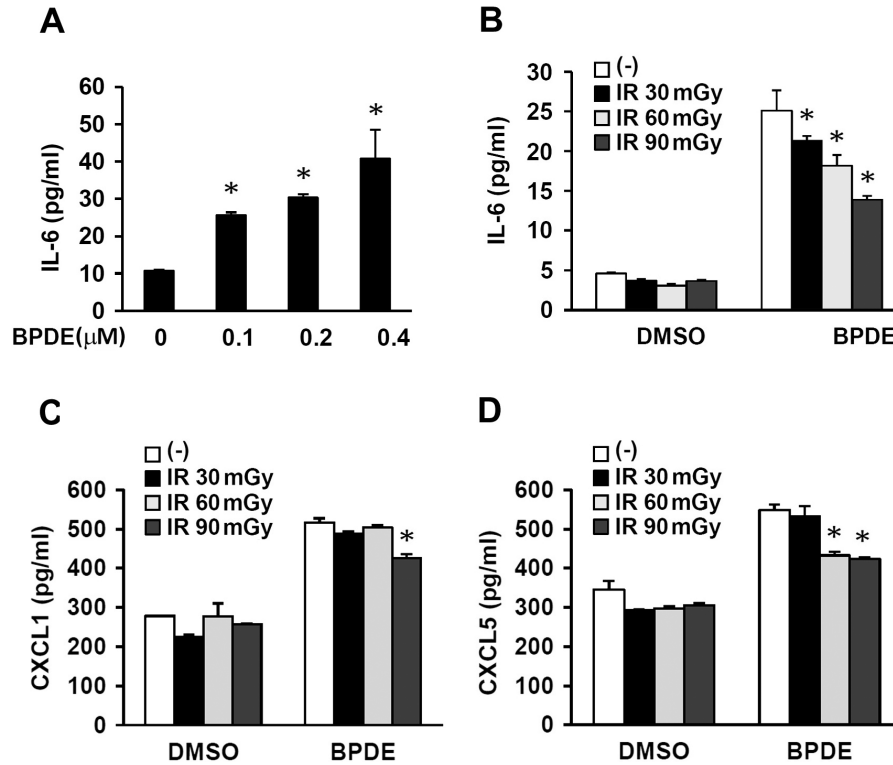
Fig. 2. LDR inhibited BPDE-induced IL-6, CXCL1 and CXCL5 production in HFL1. (**A**) HFL1 cells were left untreated or treated with various concentrations of BPDE for 24 h. IL-6 concentration in culture supernatant was determined with ELISA. Experiment was done in duplicate. *$P < 0.05$ compared with untreated control. (**B**) HFL1 cells were irradiated with various doses of γ-ray, and then were incubated with 0.4 µM of BPDE or DMSO as control for 24 h. IL-6 concentration in culture supernatant was quantified with ELISA. Experiment was done in triplicate. *$P < 0.05$. (**C**) and (**D**) HFL1 cells were treated as in B, and CXCL1 and CXCL5 concentrations in culture supernatants were measured with ELISA. Experiment was done in duplicate. *$P < 0.05$.
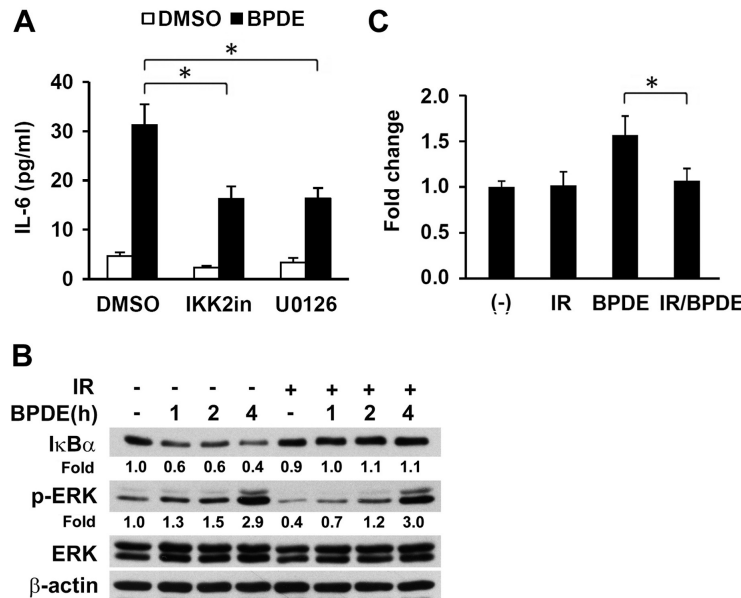
Fig. 3. LDR inhibited BPDE-induced activation of NF-κB but not ERK in fibroblasts. (**A**) HFL1 cells were pretreated with IκB Kinase 2 inhibitor (50 µM) or U0126 (10 µM) for 30 min followed by BPDE (0.4 µM) incubation for 24 h. IL-6 in culture supernatants was detected with ELISA. Experiment was done in triplicate. *$P < 0.05$. (**B**) HFL1 cells were left untreated or irradiated at 60 mGy, and then cells were incubated with 0.4 µM of BPDE or DMSO as control for the indicated times. Whole-cell lysate was analyzed for IκBα degradation, ERK phosphorylation and total ERK expression. β-actin was probed as a loading control. (**C**) HFL1 cells were transfected with NF-κB–driven luciferase construct and β-gal expressing vector to monitor transfection efficiency. Twenty-four hours posttransfection, cells were left untreated or irradiated at 60 mGy, followed by 0.2 µM of BPDE treatment for 6 h. Luciferase activity was measured and normalized to β-gal reading as described in Materials and Methods and expressed as fold change against control as 1. Experiment was done in triplicate.

Inhibition of BPDE-induced IL-6 secretion by LDR was also confirmed by ELISA. A dose-dependent inhibition by LDR was detected with 16–44% inhibition with the irradiation doses from 30 to 90 mGy (Figure 2B). Similarly, secretion of CXCL1 and CXCL5 from HFL1 was also suppressed by LDR to a lesser extent (Figure 2C and D). These results confirmed that LDR suppresses BPDE-induced secretion of IL-6, CXCL1 and CXCL5 from fibroblasts.

Macrophages are also a key component of tumor microenvironment. Thus, we used the same antibody arrays to profile culture media from the macrophage cell line THP-1 after similar LDR and BPDE treatment as done in HFL1 cells. Although secretion of some cytokines including IL-6 from THP-1 was stimulated by BPDE, no effect of LDR on the stimulation of IL-6 secretion was observed, which was confirmed by ELISA (Supplementary Figure 1 is available at *Carcinogenesis* Online, data not shown). Because human bronchial epithelial cells (HBECs) are a possible source for IL-6 production in the lung, two HBEC lines, Beas-2B and HBEC-2, were also examined. The results showed that the basal IL-6 secretion from HBECs was marginal and BPDE had little effect on IL-6 secretion from Beas-2B and HBEC-2 cells (Supplementary Figure 2 is available at *Carcinogenesis* Online), which is consistent with literature (25). Therefore, we focused on the effect of LDR on induction of IL-6 from fibroblasts.

BPDE induces IL-6 secretion from human lung fibroblasts through the NF-κB and ERK pathways

Because IL-6 plays an important role in the development of different types of cancer including lung cancer and LDR showed the strongest effect on IL-6 secretion from HFL1, we then focused on elucidating the mechanisms by which LDR inhibits BPDE-induced IL-6 production. HFL1 was pretreated with NF-κB and mitogen activated protein kinase pathway inhibitors prior to BPDE stimulation, and their effects were quantified on BPDE-induced IL-6 expression. The IκB Kinase 2 inhibitor, which inhibits IκB Kinase activity required for NF-κB activation, and the ERK inhibitor U0126 abrogated BPDE-induced IL-6 secretion (Figure 3A), suggesting that NF-κB and ERK are two pathways contributing to LDR's inhibitory effect on BPDE-induced IL-6 secretion from fibroblasts.

LDR inhibits BPDE-induced activation of NF-κB, which is involved in IL-6 secretion in fibroblasts

We then examined the effect of LDR on BPDE-induced NF-κB and ERK activation in HFL1 cells. BPDE caused a gradual degradation of IκBα, the hallmark of NF-κB activation, which was blocked by LDR (Figure 3B). In addition, in a reporter assay, BPDE-induced NF-κB-driven luciferase expression in HFL1 was suppressed by LDR (Figure 3C). Although LDR slightly reduced the basal ERK activity, it had little effect on the BPDE-induced ERK activation that was detected at 4 h post-BPDE exposure (Figure 3B). Therefore, ERK is unlikely to play a significant role in LDR's inhibition on IL-6 secretion from fibroblasts. These results indicate that NF-κB but not ERK contributes to LDR's inhibitory effect on BPDE-induced IL-6 secretion from fibroblasts.

IL-6 activates STAT3 that potentiates proliferation in HBECs

We examined if IL-6 in the CM from BPDE-treated HFL1 cells activates STAT3 in bronchial epithelial cells. IL-6 potently induces activation of STAT3 in the immortalized bronchial epithelial cell line Beas-2B as shown by the phosphorylation of tyrosine 705 (Y705) and serine 727 (S727) (Figure 4A). Similarly, the CM from BPDE-treated HFL1 cells strongly induced phosphorylation of STAT3 at these sites, which was effectively blocked by pretreatment of the cells with an IL-6 receptor neutralizing antibody (Figure 4B and C). Importantly, cells treated with the BPDE-CM from LDR pretreated HFL1, in which IL-6 concentration was suppressed (Figures 1 and B), showed reduced STAT3 activation (Figure 4C). These results strongly suggest that IL-6 secretion from fibroblasts stimulates cell signaling pathways such as STAT3, and LDR suppresses this pathway. Additionally, the STAT3 inhibitor potentiated proliferation suppression by BPDE (Figure 4D and E), suggesting that IL-6–induced STAT3

activation alleviates BPDE's proliferation suppression and cytotoxicity in Beas-2B cells.

IL-6 facilitates BPDE-induced transformation of Beas-2B cells

We further examined the role of IL-6 in BPDE-induced bronchial epithelial cell transformation, an early step in lung carcinogenesis. Beas-2B cells were chosen because this cell line is sensitive to transformation. Cell transformation was measured by colony formation in soft agar. IL-6 or LDR alone had marginal effects on colony formation. However, IL-6 markedly enhanced colony growth of BPDE-transformed cells, as evinced by increase in both cell colony number and size (Figure 5A and B). Exposing Beas-2B cells to LDR before BPDE and IL-6 treatment resulted in similar transformation efficiency (Figure 5C and D). These clearly show that IL-6 promotes BPDE-induced bronchial epithelial cell transformation.

Discussion

This study demonstrates that LDR effectively inhibits cigarette-smoke carcinogen BPDE-induced secretion of proinflammatory cytokines such as IL-6, CXCL1 and CXCL5 from human lung fibroblasts. The inhibitory effect on IL-6 was achieved at least partially by blocking the BPDE-activated NF-κB pathway. Further, we show that IL-6 enhances BPDE-induced bronchial epithelial cell transformation, presumably through activation of STAT3 in the cells. Notably, CM from BPDE-treated fibroblasts activated STAT3, which was inhibited by priming the fibroblasts with LDR. Our data reveal that LDR modulates lung-tumor microenvironment and inflammatory response to cigarette-smoke carcinogen. Given that accumulating evidence showing IL-6 and STAT3 as tumorigenic factors in lung cancer (12,13,15) and the importance of fibroblasts as a source of IL-6 (26,27), it is plausible to speculate that LDR is able to inhibit the development of cigarette smoke–associated lung carcinogenesis.

Cigarette smoke elicits a chronic inflammation in the lung, which is assumed to be involved in lung cancer development. As one of the main proinflammatory cytokines, IL-6 is secreted from stromal cells including fibroblasts to facilitate cancer initiation, promotion and progression (27). IL-6 expression is controlled by several pathways, the most important ones being NF-κB and mitogen activated protein kinases (c-Jun N-terminal kinase, ERK and p38) (14). Benzo[a]pyrene or BPDE has been shown to activate NF-κB and mitogen activated protein kinases in different cell types (28–31). We show that BPDE strongly stimulates the production of IL-6 in lung fibroblasts by activating the NF-κB and ERK pathways. Consistent with our observation, a recent report showed that BPDE induces a variety of cytokines and chemokines including IL-6 production in the human lung fibroblast cell line WI-38 depending on p53 and c-Jun N-terminal kinase (26). We found NF-κB and ERK but not c-Jun N-terminal kinase are important for BPDE-induced IL-6 secretion from HFL1. The discrepancy is possibly due to different cell lines and different concentrations of BPDE used. Whereas BPDE activates both NF-κB and ERK pathways for IL-6 secretion, LDR only suppresses BPDE-induced NF-κB activation in fibroblasts. Thus, the suppression of IL-6 secretion from fibroblasts by LDR is likely dependent on NF-κB.

NF-κB is an important cellular signaling pathway regulated by a complex signaling network in the cell (32). How BPDE activates NF-κB in fibroblasts is not well understood, and the LDR's effect on NF-κB activation is not clear. Previous studies showed moderate or transient NF-κB activation by 100 mGy of γ-rays in human lymphoblastoid cells (33) and in mouse bone marrow cells *in vivo* (34) and by 100 mGy X-ray in the mouse epidermal cell line JB6P+ (35). Whereas 75 mGy of whole-body X-ray irradiation appeared to induce prolonged NF-κB activation in mouse peritoneal macrophages (36), no activation was found in mouse bone marrow cells with 50 mGy of whole-body γ-ray irradiation (34). In our settings, 60 mGy of γ-ray irradiation alone had no detectable effect on NF-κB in fibroblasts. The reason for the discrepancy between our study and previous reports is currently unknown, but it may be related to different cell types, radiation sources and doses employed.
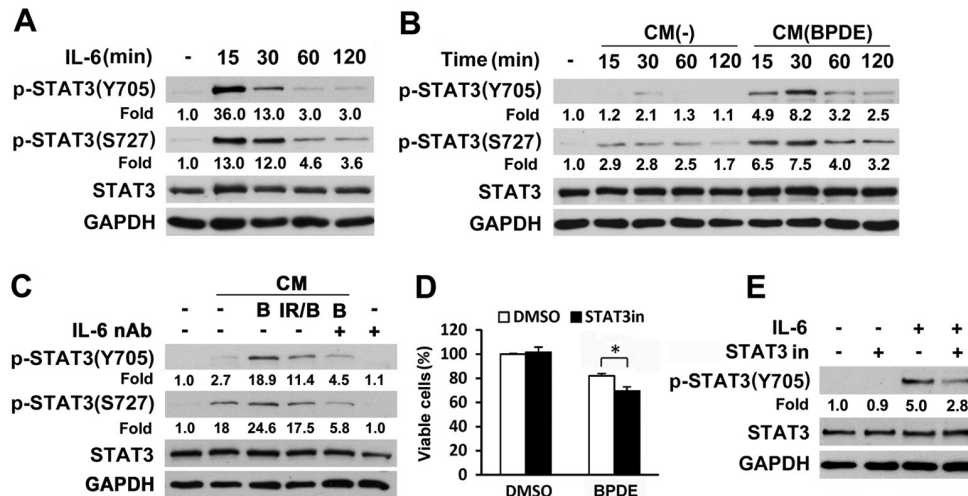
Fig. 4. Diminished capacity of CM from LDR primed and BPDE-treated fibroblasts in activation of STAT3 signaling in Beas-2B cells was associated with reduction of IL-6. (**A**) Beas-2B cells were treated with IL-6 (10 ng/ml) for various times. Cell lysate was prepared and subjected to western blot with antibodies specific to phospho-Stat 3 (Y705 and S727) and total STAT3. GADPH was probed as a loading control. (**B**) Beas-2B cells were left untreated or incubated with CM from untreated or BPDE (0.2 µM) treated fibroblasts for various times. Western blot was performed as in (**A**). (**C**) Beas-2B cells were left untreated or incubated with different CM from fibroblasts (control, treated with BPDE or irradiated with 90 mGy and then treated with BPDE) for 30 min; some cells were incubated with IL-6 receptor neutralizing antibody (2 µg/ml) for 30 min prior to addition of BPDE-treated CM. Western blot was performed as in (**A**). nAb, neutralizing antibody; B, BPDE. (**D**) Beas-2B cells were treated with 0.5 µM of STAT3 inhibitor X for 30 min followed by 0.1 µM of BPDE for another 1 h. Cells were then incubated in fresh medium, and MTT assay was done 24 h later. STAT3 inhibitor X was used at low concentration due to strong growth inhibition of the cells at higher doses. Experiments were done in triplicate. (**E**) STAT3 inhibitor X partially inhibited IL-6–induced activation of STAT3. Beas-2B cells were treated with 0.5 µM of STAT3 inhibitor X for 30 min followed by 10 ng/ml of IL-6 for 15 min. Phosphorylation of STAT3 was detected by western blot. GADPH was used as a loading control. $*P < 0.05$.
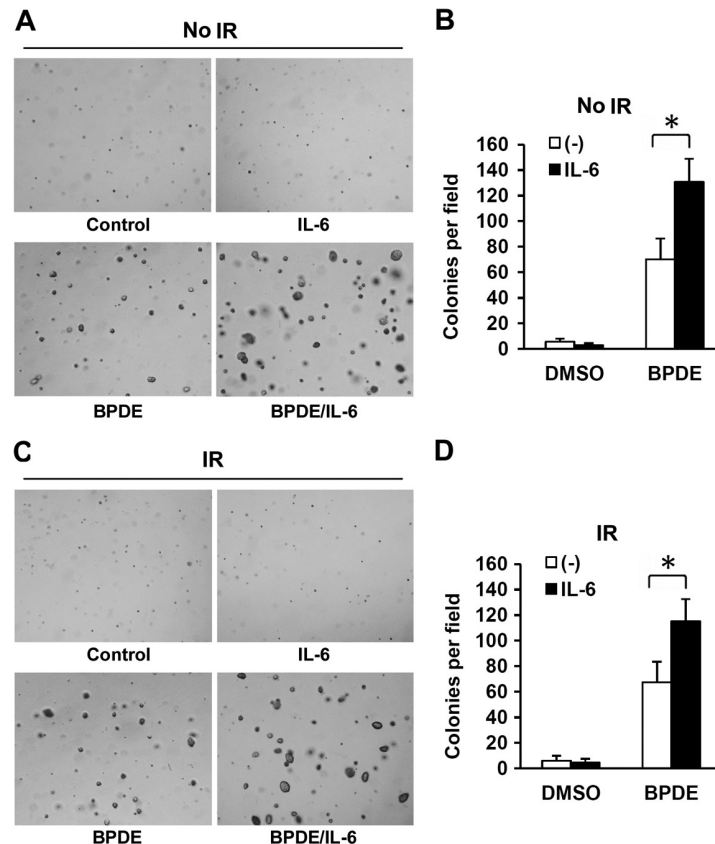
Fig. 5. IL-6 enhanced BPDE-induced Beas-2B cell transformation. Beas-2B cells were treated once with 0.2 µM of BPDE or DMSO for 1 h. Twenty-four hours later, IL-6 (10 ng/ml) was added, and the cells were cultured in the presence of IL-6 for 1 week. The cells were then seeded in soft agar in 12-well plate (1 × 10^4 cells/well) with 0.5 ml medium containing IL-6 (10 ng/ml) over the agar. Cell colonies were photographed in three random fields per well and counted after 10 days. Experiments were done in triplicate. Shown are representative images and quantification of colonies of cells without irradiation prior to the treatments (**A**, **B**) or of cells irradiated at 60 mGy prior to the treatments (**C**, **D**). $*P < 0.05$.

Although we did not observe direct effect of LDR on NF-κB activity in fibroblasts, we show that LDR suppressed BPDE-induced NF-κB activation in the cells. The underlying mechanism is unknown. There is a report showing that radiation inhibits proteasome activity that is required for IκBα degradation, which in turn inhibits NF-κB activation (37). Indeed, BPDE-induced IκBα degradation in fibroblasts was compromised when the cells were pretreated with LDR (Figure 3B). Whether LDR functions by interfering with the activity of proteasome or other proteins upstream of IκBα in fibroblasts deserves further investigation. Nonetheless, our results clearly demonstrate that NF-κB is an important target for LDR in human fibroblasts for suppressing IL-6 production.

Of note, we did not detect any inhibitory effect of LDR on BPDE-induced IL-6 secretion from the macrophage cell line THP-1 (Supplementary Figure 1 is available at *Carcinogenesis* Online), suggesting that the IL-6–suppressing role of LDR could be cell-type specific. We found that in fibroblasts both the NF-κB and ERK pathways are required, whereas in macrophages the ERK pathway is more dominant, for BPDE-induced IL-6 secretion (Supplementary Figure 3 is available at *Carcinogenesis* Online). LDR had very little effect on BPDE-induced ERK activation in fibroblasts (Figure 3B). Therefore, although the exact mechanism for the differential effects of LDR on BPDE-induced IL-6 secretion in fibroblasts and macrophages remains unclear, the different dependence on cellular signaling pathways such as ERK may contribute to the distinct responses to LDR. The IL-6 secretion from HBECs is very low and BPDE had little effect on IL-6 secretion from HBECs (Supplementary Figure 2 is available at *Carcinogenesis* Online), which is consistent with the literature (25).Thus, it is plausible that fibroblast is one of the main cell types for LDR in modulating microenvironment through regulation of IL-6.

Several questions remain to be answered: (i) whether cigarette-smoke carcinogens induce IL-6 expression in lung fibroblasts *in vivo*; (ii) what is the contribution of fibroblast-derived IL-6 in promoting lung cancer and (iii) whether the inhibitory effects of LDR on carcinogen-induced IL-6 expression in lung fibroblasts occur *in vivo*. Some supportive evidence for this scenario is provided by the findings that cigarette smoke induces IL-6 production and activation of STAT3 signaling in mouse lungs (38). The effect of LDR on BPDE-induced lung cancer development in A/J mice is currently underway in our group and should help further address these questions. Of note, for humans there is evidence that chronic exposure at a low rate to LDR suppresses occurrence of smoking-related lung cancer (39,40).

In summary, we provide *in vitro* evidence that LDR inhibits cigarette-smoke carcinogen-induced IL-6 secretion from lung fibroblasts, and IL-6 enhances carcinogen-induced bronchial epithelial cell transformation. These results favor the hormetic model, where LDR inhibits cigarette smoke–induced lung carcinogenesis. It remains to be determined if our observations can be reproduced *in vivo*.

Supplementary material

Supplementary Figures 1–3 can be found at http://carcin.oxfordjournals.org/.

Funding

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