Arsenic is a well known metalloid human carcinogen, and epidemiological evidence has demonstrated its association with the increased incidence of lung cancer. However, the mechanism involved in its lung carcinogenic effect remains obscure. The current study demonstrated that exposure of human bronchial epithelial cells (Beas-2B) to arsenite can result in a marked induction of cyclooxygenase (COX)-2, an important mediator for inflammation and tumor promotion. Exposure of the Beas-2B cells to arsenite also led to significant transactivation of nuclear factor of activated T-cells (NFAT), but not activator protein-1 (AP-1) and NFκB, suggesting that NFAT, rather than AP-1 or NFκB, is implicated in the responses of Beas-2B cells to arsenite exposure. Furthermore, we found that inhibition of the NFAT pathway by either chemical inhibitors, dominant negative mutants of NFAT, or NFAT3 small interference RNA resulted in the impairment of COX-2 induction and caused cell apoptosis in Beas-2B cells exposed to arsenite. Site-directed mutation of two putative NFAT binding sites between −111 to +65 in the COX-2 promoter region eliminated the COX-2 transcriptional activity induced by arsenite, confirming that those two NFAT binding sites in the COX-2 promoter region are critical for COX-2 induction by arsenite. Moreover, knockdown of COX-2 expression by COX-2-specific small interference RNA also led to an increased cell apoptosis in Beas-2B cells upon arsenite exposure. Together, our results demonstrate that COX-2 induction by arsenite is through the NFAT3-dependent and AP-1- or NFκB-independent pathways and plays a crucial role in antagonizing arsenite-induced cell apoptosis in human bronchial epithelial Beas-2B cells.

Arsenic is an environmental toxin widely distributed in water, food, air, and soil (1). Arsenic, combined with oxygen, chlorine, and sulfur, is called inorganic arsenic, which represents the most common forms of either arsenite or arsenate in the environment (2). Humans are exposed to arsenic mainly by inhalation, ingestion, and skin contact (3, 4). The inhalation route is mainly associated with occupational exposure of ore smelters, insecticide manufacturers, and sheep dip workers. Previous studies have shown that environmental and occupational exposure to arsenic is associated with an increased risk of human cancers, including skin and lung cancers. Although arsenic itself is not a mutagen of DNA, it has some deleterious effects, such as the potential for DNA damage by other agents and inhibition of DNA repair (5–8). More importantly, arsenite resembles many other classic carcinogens in inducing cell tumorigenesis by activating certain genes, especially those involved in tumor promotion. Nonetheless, the mechanism by which arsenite causes human lung cancers remains to be intensively elucidated (9–11).

Cyclooxygenase (COX)3-2, also named prostaglandin endoperoxide synthase 2, is an essential enzyme involved in the inflammation processes and other pathogenesis (12). Previous studies have demonstrated that COX-2 is constitutively overexpressed in a variety of human malignancies, especially in primary lung adenocarcinoma (13, 14). There is a growing amount of evidence both in vitro and in vivo indicating that chronic inflammation and its mediator COX-2 play an important role in cancer development (15, 16). It has also been reported that arsenite exposure can stimulate COX-2 expression through activating the nuclear factor κB (NFκB) pathway in endothelial cells (17). Whether arsenite is able to induce COX-2 expression in human bronchial epithelial cells and, if it does, which signaling pathway mediates its induction, as well as what the role of COX-2 is in cell responses to arsenite exposure, have not yet been investigated. The present study documents that arsenite can markedly induce COX-2 expression through the calcineurin/NFAT-dependent pathway in human bronchial epithelial Beas-2B cells, and we also demonstrate that elevated COX-2 protein expression mediates the protection of Beas-2B cells from apoptosis caused by arsenite.
Materials and Methods

Cell Culture and Reagents—Beas-2B cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Calbiochem) supplemented with 10% fetal bovine serum (FBS), 5% penicillin/streptomycin, and 2 mM L-glutamine (Invitrogen) at 37 °C in a humidified atmosphere with 5% CO2 in air. Sodium metavanadate (vanadate), and sodium arsenite were purchased from Aldrich. Benzo[a]pyrene was from Eagle-Picher Industries, Inc. The substrate for the luciferase assay was purchased from Promega (Madison, WI). NFAT inhibitor peptide was from Calbiochem. Antibodies against COX-2, NFAT, and hemagglutinin tag were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-β-actin antibody was from Sigma. Antibodies for caspase-3 and PARP were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY).

Plasmids and Construction of siRNA Vectors—The COX-2-luciferase reporter plasmid containing the upstream 5’-flanking region of human COX-2 gene promoter linked to a luciferase reporter was previously described (18). The p-AP-1-Luc reporter plasmid was purchased from Stratagene (La Jolla, CA). The NFAT-luciferase reporter plasmid and NFxB-luciferase reporter plasmid were constructed as described previously (19, 20). The dominant negative mutant of NFAT (DN-NFAT) eukaryotic expression construct, kindly provided by Dr. Ching-Wing Chow (Albert Einstein College of Medicine), contains a deletion mutant of NFAT that can inhibit the transcription activity of all NFAT isoforms (21). The specific small interference RNAs targeted human NFAT3 or COX-2, were designed with siRNA converter on the Web site of Ambion Inc. (Austin, TX) according to the gene sequences and the siRNA design guidelines, and synthesized by Invitrogen. The target sequences were 5’-gaactgacctgaggt-3’ (human NFAT3 mRNA) and 5’-agacagataacgaggt-3’ (human COX-2 mRNA). The siRNA sequences were controlled via BLAST search and did not show any homology to other known human genes. The siRNAs were inserted into pSuppressor vector and verified by DNA sequencing. The siRNA constructs for NFAT3 and COX-2 were named as siNFAT3 and siCOX-2, respectively.

Point Mutation of NFAT Binding Site in COX-2 Promoter—Luciferase Reporter—To generate a site-directed mutant of the NFAT binding sequence in the COX-2 promoter region of the COX-2-luciferase reporter plasmid, the QuikChange mutagenesis kit (Stratagene) was used according to the manufacturer’s instruction. The sense primer was 5’-gaggagtgaaatgctgaggtttttttgttgttcaggagaacacag-3’, and the antisense primer was 5’-ctgtgcttcgtgcatgccctccacaataataataaactctc-3’. (The underlined bold characters indicate the mutated nucleotides.) The COX-2 promoter-luciferase reporter plasmid with mutation at both NFAT binding sites was identified and designated as COX-2-lucNFATmut.

Stable Transfection—Beas-2B cells were cultured in a 6-well plate until 90% confluence. The COX-2 reporter construct in combination with either mock control, siNFAT3, or DN-NFAT, as well as the siCOX-2 construct, were co-transfected with a hygromycin-resistant plasmid into Beas-2B cells by the Lipofectamine transfection kit (Invitrogen) according to the manufacturer’s instructions. The stable transfectants, including Beas-2B-COX-2-luc mass1, Beas-2B-COX2-luc NFATmut mass1, Beas-2B/siNFAT3 COX-2-luc mass1, Beas-2B/DN-NFAT COX-2-luc mass1, and Beas-2B/siCOX-2 mass1, were established by selection with 400 μg/ml hygromycin. The established stable transfectants were cultured in hygromycin-free 10% FBS DMEM for at least two passages before each experiment. The stable transfectants for the luciferase reporter were identified by measuring the basal level of luciferase activity, and the stable transfectants of siNFAT3 or siCOX-2 were verified by analyzing their specific gene expression with Western blot. Beas-2B/DN-NFAT COX-2-luc mass1 was identified by analyzing the ectopic expression of Flag tag.

Gene Reporter Assays—Confluent monolayers of stable luciferase reporter transfectants were trypsinized, and 8 × 103 viable cells suspended in 100 μl of 10% FBS DMEM were added to each well of 96-well plates. Plates were incubated at 37 °C in a humidified atmosphere of 5% CO2 in air. After being cultured at 37 °C overnight, the cells were treated with a different concentration of arsenite for various time points as indicated. Cells were then lysed with 50 μl of lysis buffer, and the luciferase activity was finally measured using a Promega luciferase assay reagent with a luminometer (Wallac 1420 Victor2 multiplicable counter system). The results were expressed as transcription factor activation relative to control medium (relative NFAT, NFxB, or AP-1 activation) or COX-2 induction relative to control medium (relative COX-2 induction). Student’s t test was used to determine the significance of the differences, and the differences were considered significant at p ≤ 0.05.

Western Blot Assay—2 × 105 cells of Beas-2B transfectants were cultured in each well of 6-well plates until 70–80% confluence; the culture medium was replaced with 0.1% FBS DMEM. After being cultured for 24 h, the cells were exposed to the indicated amount of arsenite for 12 or 24 h. The cells were then washed once with ice-cold phosphate-buffered saline and extracted with a SDS-sample buffer. The cell extracts were separated on SDS-polyacrylamide gels, transferred, and probed with a rabbit-specific antibody against COX-2. The protein band, specifically bound to the primary antibody, was detected using an anti-rabbit IgG-AP-linked and an ECF Western blotting system (Amersham Biosciences).

Cell Death Assay—The arsenite-treated Beas-2B cells were collected by pooling cells from the culture medium as well as the trypsinized adherent cells. The trypan blue exclusion method was used to determine the dead cells.

Results

Induction of COX-2 by Arsenite in Beas-2B Cells—Previous studies have shown that environmental and occupational exposure to arsenite is associated with an increase in the risk of lung cancer (22), and the carcinogenic effect of arsenite has also been verified in various experimental models (6, 7). It has also been documented that COX-2 is constitutively overexpressed in the human lung cancers (23–25). It is, therefore, interesting to determine whether COX-2 is inducible in human bronchial epithelial cells by arsenite. To address this question, Beas-2B cells were exposed to arsenite. As shown in Fig. 1, a and b, treatment of Beas-2B cells with arsenite resulted in marked COX-2 induction in COX-2-luciferase reporter assay. This
induction appeared to be in both dose- and time-dependent manners (Fig. 1). To further confirm this finding, the levels of COX-2 protein expression induced by arsenite were also determined by Western blot. The results showed that arsenite exposure led to an increase in the COX-2 protein level in Beas-2B cells (Fig. 1c), suggesting that arsenite is a potent carcinogen for COX-2 expression in human bronchial epithelial cells.

Arsenite Exposure Led to Activation of NFAT but Not AP-1 and NFκB in Beas-2B Cells—The COX-2 gene promoter region contains NFκB, AP-1, and NFAT binding sites, which can be recognized by these transcription factors and in turn lead to COX-2 transcription (26–28). Previous studies have indicated that COX-2 regulation appears to involve diversified mechanisms based on cell types and stimuli (26–32). Breyer and co-workers (26) reported that NFκB activation was responsible for COX-2 induction following dehydration or hypertonic stress in renal medullary interstitial cells (26, 27). Whereas in the Jurkat human leukemic T cells, the COX-2 induction was mediated by AP-1 and NFAT (28). It has also been reported that COX-2 can be regulated by NFAT in nonlymphoid tissues (29, 30). Therefore, it is important to know which transcription factors mediate COX-2 induction by arsenite in Beas-2B cells. To test this, stable Beas-2B transfectants with NFκB-, AP-1-, and NFAT-luciferase reporters were exposed to arsenite. As shown in Fig. 2, a–d, arsenite exposure did not show significant effects on activation of AP-1 (Fig. 2, a and b) or NFκB (Fig. 2, c and d). To rule out the deficiency of activation of AP-1 and NFκB in arsenite treatment due to any reason from either luciferase reporter or a defect of signaling pathway leading to AP-1 and NFκB activation in Beas-2B cells, vanadate and benzo[a]pyrene were
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(a) Relative COX-2 induction with Arsenite 20 μM and NFAT inhibitor 10 μM.

(b) HA-tag and β-Actin expression in Beas-2B/Cox-2-luc mass1.

(c) NFAT3 and β-Actin in Beas-2B/DN-NFAT.

(d) Relative COX-2 induction with Medium, Arsenite 20 μM, and Arsenite 10 μM.

(e) Beas-2B/control COX-2-luc mass1 and Beas-2B/siNFAT COX-2-luc mass1.

(f) Sequences: 5'-GACGACGTAGAGTGGTGGCTACGAGCGAAAGAAACAG-3' and 5'-GACGACGTAGAGTGGTGGCTACGAGCGAAAGAAACAG-3'.

(g) Beas-2B/Cox-2-luc mass1 and Beas-2B/DN-NFAT COX-2-luc mass1 with Arsenite (μM) 0, 20.

(h) Beas-2B/Cox-2-luc mass1 and Beas-2B/siNFAT COX-2-luc with Arsenite (μM) 0, 20.
used as positive controls for activation of AP-1 and NFκB, respectively. The results showed that vanadate and benzo(a)pyrene were able to markedly activate AP-1 (Fig. 2, a and b) and NFκB (Fig. 2, c and d), suggesting that AP-1 and NFκB-Luc reporters and signaling pathways leading to their activation are normal. Interestingly, arsenite exposure specifically resulted in NFAT transcriptional activation in both time- and dose-dependent manners (Fig. 2, e and f). It may be noted that NFAT activation by arsenite in the time course studies reached a peak earlier, as compared with that of COX-2 induction (Fig. 1c versus Fig. 2f), and given the factor that there are two NFAT binding sites in the COX-2 promoter region, we anticipate that NFAT may be involved in COX-2 induction by arsenite in Beas-2B cells.

NFAT Activation Is Required for COX-2 Induction by Arsenite—To unravel the role of NFAT in the COX-2 induction in Beas-2B cells by arsenite, NFAT-specific inhibitor, which is a highly selective NFAT peptide inhibitor and is able to inhibit NFAT activation and NFAT-dependent gene expression in T cells, was first used. As shown in Fig. 3a, pretreatment of the cells with NFAT inhibitor impaired the arsenite-associated COX-2 induction, suggesting that NFAT might play a role in the arsenite-induced COX-2 expression in Beas-2B cells. To further address this notion, hemaggulatin-tagged dominant DN-NFAT was stably transfected, and the Beas-2B DN-NFAT COX-2 mass1 was established (Fig. 3c). Overexpression of DN-NFAT resulted in a dramatic inhibition of arsenite-induced COX-2 expression in both the COX-2-luciferase reporter assay and Western blot (Fig. 3, d and g). There are two putative NFAT binding sites in the human COX-2 promoter that were reported to be critical for the COX-2 promoter activity (31). To determine whether NFAT regulated COX-2 expression through direct binding to the COX-2 promoter region, point mutation of the two NFAT binding sites in the promoter region of COX-2-Luc reporter was carried out. The results demonstrated that this mutation resulted in impairment of the COX-2 transcription induced by arsenite exposure (Fig. 3f). All of these data strongly demonstrate that NFAT activation by arsenite is responsible for its COX-2 induction in Beas-2B cells. Previous studies have shown that at least five members of the NFAT family have been identified (32, 33). NFAT1 and NFAT2 are mainly involved in T-cell activation. NFAT4 is mainly expressed in thymus, whereas NFAT5 is crucially involved in cellular response to hypertonic stress, and NFAT3 is primarily expressed in nonlymphoid tissues (33). Since our most recent studies have demonstrated that NFAT3 is a mediator for TNF-α-induced COX-2 expression in mouse CI41 cells (34), we anticipated that NFAT3 might be a major NFAT isoform involved in COX-2 induction by arsenite. To evaluate the potential role of NFAT3 in COX-2 expression by arsenite, human siNFAT3 was constructed and used. Stable transfection of siNFAT3 in Beas-2B cells resulted in a dramatic reduction of NFAT3 protein expression in Beas-2B cells (Fig. 3b). Specific knockdown of NFAT3 expression by siNFAT3 blocked arsenite-induced COX-2 transcription and protein expression (Fig. 3, e and h). These results distinctly demonstrate that NFAT3 is a major mediator for COX-2 induction by arsenite in Beas-2B cells.

Involvement of Ca2+/Calcineurin in COX-2 Induction by Arsenite—Calcineurin signaling has been implicated in a broad spectrum of physiological or pathological conditions in a variety of organ systems (35). There is evidence showing that NFAT activation was mediated by a Ca2+/calcineurin-dependent pathway (33). To evaluate the contribution of calcineurin in arsenite-induced COX-2 expression, cyclosporin A (CsA), a specific inhibitor of calcineurin, was employed. As shown in Fig. 4, a and b, pretreatment of cells with CsA markedly inhibited NFAT activation. Consistent with inhibition of NFAT activation, COX-2 induction by arsenite was also impaired (Fig. 4, c and d). To test the role of Ca2+ in COX-2 induction by arsenite, 2-bis(O-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetra(acetoxyethyl)ester (BAPTA-AM), a specific Ca2+ chelator (36), was used. Pretreatment of cells with BAPTA-AM resulted in a dramatic inhibition of NFAT activation and COX-2 induction (Fig. 4, e and f), whereas it did not show any inhibitory effect on vanadate-induced COX-2 expression (Fig. 4j). These data suggest that arsenite-induced COX-2 transcription may involve calcium signaling and calcineurin activation in Beas-2B cells.

COX-2 Induction by Arsenite Provides Antiapoptotic Signaling in Arsenite-treated Beas-2B Cells—It has been reported that COX-2 may play a role in the regulation of cell proliferation, cell survival, and tumorigenesis (37). Thus, it is of interest to investigate the potential contribution of activated NFAT and elevated COX-2 protein in the arsenite-induced biological effects on Beas-2B cells. As shown in Fig. 5a, inhibition of the calcineurin/NFAT pathway by either pretreatment of cells with CsA or specific knockdown of NFAT3 significantly led to arsenite-associated Beas-2B cell death, whereas either arsenite treatment or CsA pretreat-
ment alone caused only a marginal effect on the cell viabilities (Fig. 5a), suggesting that activation of the calcineurin/NFAT pathway by arsenite provides an inhibitory effect on cell death caused by arsenite. To determine whether calcineurin/NFAT-mediated inhibition of arsenite-induced cell death was due to its antiapoptotic effect, we compared the levels of the arsenite-induced caspase-3 and PARP cleavages among cells from three groups as shown in Fig. 5b. Inhibition of calcineurin by CsA or specific knockdown of NFAT3 significantly increased the levels of cleaved caspase-3 and PARP, suggesting that calcineurin/NFAT provides an inhibitory effect on cell apoptosis caused by arsenite. To further confirm that COX-2 is an NFAT downstream mediator for its antiapoptotic effect, we made a siCOX-2 construct and established a stable Beas-2B-siCOX-2 transfectant. As shown in Fig. 6a, introduction of siCOX-2 was able to knock down COX-2 protein expression in Beas-2B cells and increased the cell death (Fig. 6, b–D) in Beas-2B cells upon arsenite exposure. These data demonstrated that COX-2 appeared to be an NFAT downstream gene product responsible for inhibitory effects on cell death caused by arsenite. Detection of the apoptotic effector by Western blot revealed that the levels of the cleaved caspase-3 and PARP were markedly higher in the siCOX-2 stably transfected cells than those in Beas-2B control cells. Therefore, the data strongly demonstrated that elevated COX-2 protein via a calcineurin/NFAT3-dependent pathway by arsenite treatment rendered Beas-2B cells resistant to the arsenite-induced apoptosis.

DISCUSSION

Although arsenite exposure has been associated with an increase in the risk of human lung cancer development, the mechanism involved in arsenite carcinogenic effects remains unknown (38). The current study demonstrates that arsenite is able to induce COX-2 expression in human Beas-2B cells. The
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activation of NFAT, but not AP-1 or NFκB, is critical for COX-2 induction by arsenite, because NFAT not only could be activated by arsenite exposure but also is required for COX-2 induction demonstrated by either the NFAT-specific peptide inhibitor or the overexpressed DN-NFAT in Beas-2B cells. The NFAT-dependent COX-2 induction has also been confirmed by directly mutating the two NFAT binding sites in the COX-2 promoter region. Further study indicates that NFAT3 is a major NFAT isoform responsible for COX-2 induction, since knockdown of NFAT3 by its specific siRNA could impair COX-2 induction by arsenite. In addition, our results show that COX-2 induction mediated by the NFAT pathway plays a critical role in antagonizing arsenite extracellular stress-triggered apoptosis in Beas-2B cells. This conclusion is based on the findings that inhibition of NFAT activation or COX-2 expression by pretreatment of cells with CsA or knockdown of NFAT3 or COX-2 expression could result in a significant increase of cell apoptosis in Beas-2B cells upon arsenite exposure.

COXs, which catalyze conversion of arachidonic acid to prostaglandin \( \text{H}_2 \), are key enzymes in the metabolic pathway leading to prostaglandin \( \text{E}_2 \), prostaglandin \( \text{I}_2 \), and thromboxane \( \text{A}_2 \) generation (39). Two distinct isoforms of COXs have been discovered. COX-1 is an isoform constitutively expressed in most tissues, whereas COX-2 is an inducible isoform in cells and tissues (40). COX-2 expression is remarkably stimulated by proinflammatory cytokines, bacterial endotoxins, and growth factors (12, 41, 42). The promoter region of human COX-2 contains a canonical TATA-box and multiple regulatory elements, which can be recognized by transcription factors, such as NFκB, AP-1, NFAT, nuclear factor interleukin-6/CCAAT/enhancer-binding protein, and cAMP-response element-binding protein (43–45). Previous studies have suggested that cellular responses to arsenite exposure may be based on cell type and doses used in the experimental system. For example, our previous study has demonstrated that arsenite can induce AP-1 activation both in the mouse JB6 P⁺ cell model and in the skin of AP-1-luciferase transgenic mice, and the induction of AP-1 activity appears to be mediated by the activation of protein kinase C and mitogen-activated protein kinase family members (43, 45). We have also reported that NFκB can be activated by arsenite through a phosphatidylinositol 3-kinase/Akt-dependent pathway, which was responsible for cyclin D1 induction and cell cycle progression in human keratinocyte HaCat cells (43). Our studies have also indicated that a lower concentration of arsenite induces the activation of extracellular signal-regulated kinases that is required for arsenite-associated cell transformation (46), whereas a high concentration of arsenite can turn on the c-Jun N-terminal kinase pathway, which can mediate cellular apoptosis in mouse epidermal Cl41 cells (47). High doses of arsenite also inhibit cellular responses to a variety of stimuli, such as inflammatory cytokines (48), growth factors (49), and lipopolysaccharide (50). In addition, arsenite exposure has also been reported to increase pro-oncogene activities, such as c-fos, c-jun, and AP-1, and decrease p53 activity (51). In addition, Wijeweera et al. (52) have also found that arsenite treatment can result in an enhanced DNA-binding activity of AP-1 and NFκB in both type-II epithelial cells and alveolar macrophages by using precision-cut rat lung slices (52, 53). In the current study, we found that arsenite was able to induce NFAT activation in Beas-2B cells, whereas it did not show any notable inductive effect on AP-1 and NFκB activation. It is known that NFAT activity is tightly regulated by the \( \text{Ca}^{2+} \)/calmodulin-dependent phosphatase calcineurin, a primary target for inhibition by CsA. Calcineurin regulates the NFAT protein translocation from cytoplasm to the nucleus via the dephosphorylation in response to increased intracellular calcium. NFAT activation is also modulated by mitogen-activated protein kinases, such as p38 kinase. In this study, CsA and BAPTA-AM were used to investigate the role of calcineurin in arsenite-induced COX-2 expression. Chelation of intracellular calcium by 2-bis(O-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid or inhibition of calcineurin by CsA both led to a significant reduction of NFAT activation and COX-2 expression induced by arsenite. These data reveal that COX-2 induction by arsenite is specifically mediated by the \( \text{Ca}^{2+} \)/calcineurin/NFAT-dependent pathway.
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in Beas-2B cells. Although the detailed mechanisms involved in the specific NFAT activation by arsenite in Beas-2B cells is unclear, the explanation may be associated with some cross-talk among multiple signaling pathways involved in cell responses to arsenite exposure, which may depend on cell types, so our future studies will focus on cross-talks among different signaling pathways involved in NFAT activation in Beas-2B cells initiated by arsenite exposure. Nonetheless, our current study also demonstrated that NFAT3 is specifically required for arsenite-induced COX-2 expression, since the blockage of NFAT activation by either overexpression of DN-NFAT or knockdown of NFAT3 protein expression by its specific small interference RNA resulted in a dramatic impairment of COX-2 expression induced by arsenite.

The association of COX-2 expression with cancer development has been demonstrated. For example, a high level of COX-2 expression has been found in lung cancer lymph node metastasis as compared with that in primary adenocarcinoma (54). The extensive expressed COX-2 has also been found to correlate with the decrease in the overall survival rate of the non-small lung cancer tumor patient (55). Furthermore, regular use of COX-2 inhibitors is able to reduce the incidence of lung cancer (56). Based on the important role of COX-2 in lung cancer development and the COX-2 induction by arsenite in human bronchial epithelial cells, we anticipate that the COX2 induction by arsenite may contribute to its lung carcinogenic effect.

Increased COX-2 expression is associated with tumor cell growth, tumor promotion, and metastasis (53, 57). The contribution of COX-2 to these processes can be due to COX-2-mediated production of prostaglandin, with subsequent conversion of procarcinogens to carcinogens, inhibition of apoptosis, promotion of angiogenesis, and increased tumor cell invasiveness (48). Li et al. (58) reported that COX-2 and its products may act to protect cells against damage by ionizing radiation. In the current study, CsA, NFAT3 siRNA, and COX-2 siRNA have been used to identify the role of NFAT and its downstream target gene COX-2 in the protection of arsenite-treated Beas-2B cells from apoptosis. The results indicated that inhibition of NFAT activation by either pretreatment of cells with CsA or knockdown of NFAT3 protein expression led to Beas-2B cells more sensitive to arsenite-induced cell apoptosis. Knockdown of COX-2 by its siRNA also led to a markedly increased sensitivity of Beas-2B cells to apoptosis at similar levels. This finding indicates that NFAT-mediated COX-2 expression in cell response to arsenite exposure exerts an antiapoptotic role in Beas-2B cells treated by arsenite by inhibiting the activation of caspase-3 and PARP.

Apoptosis is considered as a mechanism that is important in killing aberrant cells so that they cannot grow to form tumors (59–63). The apoptotic disruption may allow damaged cells to inappropriately escape from apoptosis, potentially proliferate, and further provide initiating events in carcinogenic development (64). Thus, it may lead to an accumulation of genetically damaged cells that have the potential to become malignant (65). Since arsenite-induced COX-2 expression shows an inhibitory effect on arsenite-induced cell apoptosis in Beas-2B cells, this may lead us to anticipate that the elevated COX-2 induced by arsenite may, at least partially, contribute to arsenite-induced human lung carcinogenesis.

In summary, we demonstrate here that arsenite exposure is able to induce COX-2 expression in Beas-2B cells through a Ca$^{2+}$/calcinurin/NFAT-dependent pathway, which mediates an increase in COX-2 expression and subsequently protects arsenite-treated Beas-2B cells from apoptosis. These findings will not only deepen our understanding of the mechanisms of arsenite's carcinogenic effect on the human lung, but also provide some valuable information for the chemoprevention and therapy of lung cancer caused by arsenic exposure.

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