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Protective Effect of Quercetin against Arsenite-Induced COX-2 Expression by Targeting PI3K in Rat Liver Epithelial Cells

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Abnormal expression of cyclooxygenase-2 (COX-2) and prostaglandin (PG)E_2 is an important mediator in inflammation and tumor promotion. Arsenite is a well-known metalloid carcinogen that is strongly associated with increased risk of liver cancer, but the underlying mechanism remains to be clarified. The present study demonstrates that COX-2 expression and PGE_2 secretion are up-regulated by arsenite in rat liver epithelial (RLE) cells. The possible inhibitory effect of quercetin, a naturally occurring dietary flavonol, on arsenite-induced COX-2 expression and PGE_2 production was investigated. Pretreatment with quercetin resulted in the reduction of arsenite-induced expression of COX-2 and production of PGE_2. The arsenite-induced phosphorylation of Akt, p70S6K, and extracellular signal-regulated protein kinases (ERKs), but not p38, was inhibited by quercetin treatment. An ex vivo kinase assay revealed that quercetin suppressed arsenite-induced phosphoinositide 3-kinase (PI3K) activity upstream of Akt in RLE cell lysates. Ex vivo pull-down assays demonstrated that quercetin directly bound with PI3K to inhibit PI3K activity. Moreover, LY294002 (a PI3K inhibitor) significantly attenuated COX-2 expression and PGE_2 production in arsenite-treated RLE cells. These results suggest that quercetin suppresses arsenite-induced COX-2 expression mainly by blocking the activation of the PI3K signaling pathway, which may contribute to its chemopreventive potential.

KEYWORDS: Arsenite; cyclooxygenase-2; phosphatidylinositol 3-kinase; quercetin

INTRODUCTION

Inorganic arsenic is a well-documented carcinogen in human beings. Millions of people in many countries consume drinking water that is contaminated by arsenic, which is present primarily as arsenite (**Figure 1A**). Exposure occurs mainly through the ingestion of contaminated water and food and to some extent from the inhalation of arsenic in the ambient air (*1*). Exposure to environmental arsenic in drinking water is associated with various internal cancers, including liver, bladder, kidney, and lung cancer (*2*). Epidemiologic studies based on high arsenic exposure in Taiwan suggest an increased risk of liver cancer (*3*, *4*). Arsenic exposure in pregnant mice from contaminated drinking water results in an elevated frequency of hepatocellular carcinomas in the offspring when they attain adulthood (*5*). Therefore, the liver is a target for arsenic carcinogenesis in both humans and nonhuman species.

Cyclooxygenase (COX) catalyzes the biosynthesis of various prostaglandins (PGs). COX-1 is expressed constitutively in many tissues (*6*), whereas COX-2 accounts for the elevated production of PGs in response to various inflammatory cytokines and other cellular stresses (*7*). Previous studies have shown a high expression of COX-2 in patients with various liver diseases, suggesting its possible role in chronic liver disease and during hepatocarcinogenesis (*8*, *9*). Recent experimental evidence has suggested that increased PG production may contribute to the development of hepatocellular carcinoma (*10*). Thus, blocking the COX-2-mediated PG pathway presents a promising strategy for reducing liver cancer.

COX-2 expression is regulated by multiple signaling pathways, such as phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) and mitogen-activated protein kinases (MAPKs). Many studies have demonstrated that PI3K/Akt pathways are involved in carcinogenesis (*11*, *12*). PI3K is a dimer composed of a regulatory subunit (p85) and a catalytic subunit (p110), the signaling of which is frequently deregulated in carcinogenesis (*13*, *14*). When stimulated, PI3K also can use phosphorylated forms of phosphatidylinositol as substrates, resulting in the generation of PI-3,4-P_2 and PI-3,4,5-triphosphate (*15*). Akt, which is located downstream of PI3K, has a well-established role in promoting cell survival. However, arsenite-induced COX-2 expression and its underlying mechanisms have not been demonstrated in liver cells, which are one of the primary targets for the induction of cancer by arsenite.

Flavonoids are polyphenolic compounds found in fruits, vegetables, and beverages (*16*). Numerous epidemiological studies

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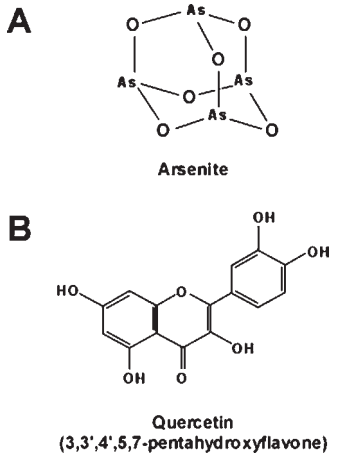

Figure 1. Chemical structures of arsenite (**A**) and quercetin (**B**).

have indicated that various flavonoids influence important cellular and molecular mechanisms related to carcinogenesis (*17*). Quercetin (3,3′,4′,5,7-pentahydroxyflavone, **Figure 1B**), an important member of the flavonoid family, was reported to exhibit various pharmacological properties, including antioxidant, anti-inflammatory, and anticarcinogenic activities (*18*). The present study is the first to demonstrate the arsenite-induced COX-2 expression and PGE_2 production in rat liver epithelial (RLE) cells and that quercetin significantly inhibited arsenite-induced COX-2 expression, which was associated with a decrease in PI3K activity.

MATERIALS AND METHODS

Materials. Quercetin, sodium arsenite, and antibodies against β-actin were obtained from Sigma-Aldrich (St. Louis, MO). LY294002 was obtained from Calbiochem (San Diego, CA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine, and penicillin/streptomycin were purchased from GIBCO BRL (Grand Island, NY). COX-2 antibodies were purchased from Cayman Chemical (Ann Arbor, MI). Protein A/G Sepharose beads and antibodies against phosphorylated ERK1/2 (Thr202/Tyr204) and total ERK1/2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against phosphorylated p38 (Tyr180/Tyr182), total p38, phosphorylated Akt (Ser473), total Akt, phosphorylated p70S6K (Thr389), and total p70S6K were obtained from Cell Signal Biotechnology (Beverly, MA). Antibodies against PI3K p85 were obtained from Upstate Biotechnology (Lake Placid, NY). CNBr-Sepharose 4B and [γ-^{32}P]ATP were purchased from Amersham Pharmacia Biotech (Piscataway, NJ), and the protein assay kit was from Bio-Rad Laboratories (Hercules, CA).

Cell and Culture Conditions. RLE cells were kindly provided by Dr. J. E. Trosko (Michigan State University) and cultured in DMEM supplemented with 10% FBS and penicillin/streptomycin in a 37 °C humidified incubator containing 5% CO_2 and 95% air.

Determination of Cell Viability. Cell viability was evaluated by an MTT (3-[4,5-diethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, which measures the mitochondrial reduction of MTT to formazan, according to the manufacturer's protocol (Roche Diagnostics, Mannheim, Germany). RLE cells were incubated at 37 °C with sample for 24 h. After incubation, cells were treated with the MTT solution (final concentration = 1 mg/mL) for 2 h. The dark blue formazan crystals formed in intact cells were dissolved in dimethyl sulfoxide (DMSO), and the absorbance at 570 nm was measured with a microplate reader. Results are expressed here as the percentage MTT reduction, assuming that the absorbance of control cells was 100%.

Western Blotting Analysis. After the cells were cultured for 48 h, they were starved in DMEM containing 0.1% FBS for another 24 h. The cells were then treated with each chemical for 30 min before being exposed to 30 μM arsenite for different time periods. Cell lysates were scraped and treated with lysis buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1 mM DTT, 0.1 mM PMSF, 10% glycerol, and a protease inhibitor cocktail tablet) for 40 min on ice followed by centrifugation at 14000 rpm for 10 min. The protein concentration of the supernatant was determined using a dye-binding protein assay kit (Bio-Rad Laboratories) as described in the manufacturer's manual. Lysate protein (20 μg) was subjected to 10% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to a PVDF membrane (Millipore Corp., Bedford, MA). After blotting, the membrane was blocked in 5% fat-free dry milk for 1 h and then incubated with the specific primary antibody for 2 h at room temperature. Protein bands were detected using an enhanced chemiluminescence (ECL) detection kit (Amersham) after hybridization with the HRP-conjugated secondary antibody.

PGE_2 Assay. RLE cells were plated in 12-well dishes and grown to 80% confluence in 500 μL of growth medium. The cells were then treated with 30 μM arsenite in the absence or presence of quercetin and LY294002. Following treatment, culture medium was collected, centrifuged at 14000 rpm for 5 min to remove cell debris, and frozen at −80 °C prior to analysis. The amount of PGE_2 released into the medium was measured using the PGE_2 enzyme immunoassay kit (Cayman Chemical). Experiments were performed in triplicate.

Ex Vivo PI3K Immunoprecipitation and Kinase Assay. PI3K activity was assayed as previously described (*19*). Briefly, the cells were treated with 30 μM arsenite alone or together with quercetin (10 and 20 μM), for 30 min, and then lysed in 400 μL of lysis buffer (20 mM Tris-HCl, pH 7.4, 137 mM NaCl, 1 mM $MgCl_2$, 10% glycerol, 1% NP-40, 1 mM DTT, 1 mM sodium orthovanadate, 1 mM phenylmethanesulfonyl fluoride, 10 μg/mL aprotinin, and 10 μg/mL leupeptin). Five hundred micrograms of protein from cell lysates was immunoprecipitated with 2 μg of a monoclonal antiphosphotyrosine antibody (pY99) followed by incubation with protein A/G Sepharose beads. The immunoprecipitates were washed twice with each of the following buffers: (1) PBS containing 1% NP-40, 1 mM DTT, and 0.1 mM sodium orthovanadate; (2) 100 mM Tris-HCl, pH 7.6, 0.5 M LiCl, 1 mM DTT, and 0.1 mM sodium orthovanadate; (3) 10 mM Tris-HCl, pH 7.6, 0.1 M NaCl, 1 mM DTT, and 0.1 mM sodium orthovanadate. After the last wash, the immunoprecipitates were resuspended in 20 μL of buffer 3 on ice for 5 min, and then 20 μL of 0.5 mg/mL phosphatidylinositol (Avanti Polar Lipids) was added. After 5 min at room temperature, the immunoprecipitates were incubated with reaction buffer (100 mM HEPES, pH 7.6, 50 mM $MgCl_2$, and 250 μM ATP containing 10 μCi [γ-^{32}P]ATP) for an additional 10 min at 30 °C. The reaction was stopped by adding 15 μL of 4 N HCl and 130 μL of chloroform/methanol (1:1). After vortexing, 30 μL of the lower chloroform phase was spotted onto a 1% potassium oxalate-coated silica gel plate, which was previously activated for 1 h at 110 °C. The resulting ^{32}P-labeled phosphatidylinositol 3-phosphate (PI3P) was separated by thin layer chromatography (TLC), and radiolabeled spots were visualized by autoradiography.

Ex Vivo Pull-down Assays. An RLE cellular supernatant fraction (500 μg) was incubated with the quercetin−Sepharose 4B (or Sepharose 4B as control) beads (100 μL, 50% slurry) in reaction buffer (50 mM Tris, pH 7.5, 5 mM EDTA, 150 mM NaCl, 1 mM DTT, 0.01% Nonidet P-40, 2 μg/mL bovine serum albumin, 0.02 mM PMSF, and 1× protease inhibitor mixture). After incubation with gentle rocking overnight at 4 °C, the beads were washed five times with buffer (50 mM Tris, pH 7.5, 5 mM EDTA, 150 mM NaCl, 1 mM DTT, 0.01% Nonidet P-40, and 0.02 mM PMSF), and proteins bound to the beads were analyzed by immunoblotting.

Statistical Analysis. When necessary, data were expressed as means ± SD, and Student's t test was used to perform statistical analysis for single comparison. A probability value of $p < 0.05$ was used as the criteria for statistical significance.

RESULTS

Arsenite Induces COX-2 Expression and PGE_2 Production in RLE Cells. Much evidence has been documented to support the hypothesis that the RLE cell system is well-developed cell culture model for molecular events of tumor promotion and chemopreventive agents (*20*). To examine whether arsenite was able to induce COX-2 expression, RLE cells were incubated with

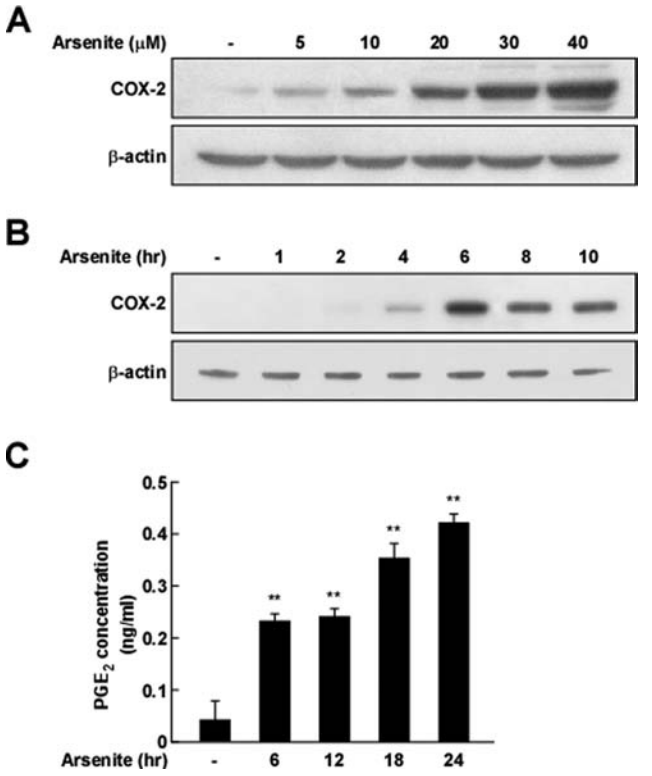

Figure 2. Dose- and time-dependent effects of arsenite on COX-2 expression and PGE_2 production in RLE cells. (**A**) Arsenite elevates COX-2 expression in a dose-dependent manner. Cells were treated with 0, 5, 10, 20, 30, or 40 μM arsenite for 6 h, extracted, and then analyzed using the Western blot assay as described under Materials and Methods. (**B**) Arsenite elevates COX-2 expression in a time-dependent manner. Cells were exposed to 30 μM arsenite and harvested at different time intervals, from 1 to 10 h, as indicated. The levels of COX-2 and β-actin proteins were determined by Western blot analysis, as described under Materials and Methods, using specific antibodies. (**C**) Arsenite stimulates PGE_2 secretion in a time-dependent manner. RLE cells were treated with 30 μM arsenite for the indicated time. PGE_2 generation was determined by the PGE_2 assay kit as described under Materials and Methods. The asterisks (**) indicate a significant difference from controls ($p < 0.01$).

various concentrations of arsenite. Treatment of RLE cells with arsenite for 6 h induced COX-2 protein expression in a dose-dependent manner (**Figure 2A**). We next examined the time-dependent effects of arsenite on COX-2 expression in RLE cells. COX-2 protein expression gradually increased, peaked at 6 h, and remained elevated for up to 10 h (**Figure 2B**). These data show that COX-2 expression is up-regulated by arsenite in RLE cells. COX-2 catalyzes the synthesis and release of prostaglandins that are proposed to be involved in cancer. We then examined whether arsenite might increase PGE_2 release from RLE cells and found that arsenite increased PGE_2 release in a time-dependent manner (**Figure 2C**). Collectively, these results indicate that arsenite can induce COX-2 expression and PGE_2 secretion in RLE cells.

Quercetin Inhibits Arsenite-Induced COX-2 Expression and PGE_2 Production. To reveal that the concentration we used did not affect the cell viability, we performed the MTT assay. We found that quercetin did not significantly inhibit cell proliferation up to the concentration of 40 μM (**Figure 3A**). To evaluate the inhibitory effects of quercetin on arsenite-induced COX-2 expression and PGE_2 production, cells were pretreated with or without quercetin at concentrations of 10 or 20 μM for 30 min. Compared with arsenite-induced COX-2 expression, treatment

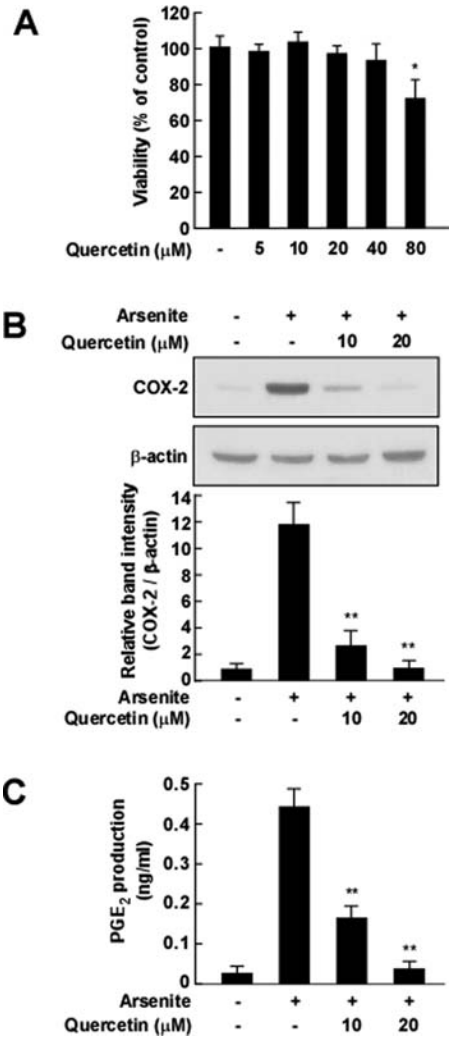

Figure 3. Effects of quercetin on arsenite-induced COX-2 expression and PGE_2 generation in RLE cells. (**A**) Effect of the quercetin on cell viability. RLE cells were treated with quercetin alone at the indicated concentration for 24 h. Cell viability was determined using the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described under Materials and Methods. Data are mean and SD values for three independent experiments. (**B**) Quercetin inhibited arsenite-induced COX-2 expression. RLE cells were pretreated with quercetin (10 or 20 μM) for 30 min before incubation with 30 μM arsenite for 6 h. The levels of COX-2 and β-actin proteins were determined by Western blot analysis, as described under Materials and Methods, using specific antibodies. The data represent the mean $\pm$ SD of three separate experiments, and the picture is a representative of those experiments, each with similar results. Quantification of COX-2 expression was normalized to β-actin using the Image J program. (**C**) Arsenite-induced PGE_2 synthesis is inhibited by quercetin. RLE cells were pretreated with quercetin at the indicated concentrations (10 and 20 μM) for 30 min before incubation with 30 μM arsenite for 24 h. PGE_2 generation was determined by the PGE_2 assay kit as described under Materials and Methods. Experiments were performed in triplicate. The asterisks (**) indicate a significant difference between groups treated with arsenite and quercetin and the group treated with arsenite alone ($p < 0.01$).

with 10 or 20 μM quercetin strongly inhibited arsenite-induced COX-2 expression (**Figure 3B**). Because quercetin treatment inhibited arsenite-induced COX-2 protein levels, we next determined the effect of quercetin treatment on PGE_2, a downstream product of COX-2. Experiments were performed in triplicate. Quercetin at 10 or 20 μM inhibited arsenite-induced PGE_2

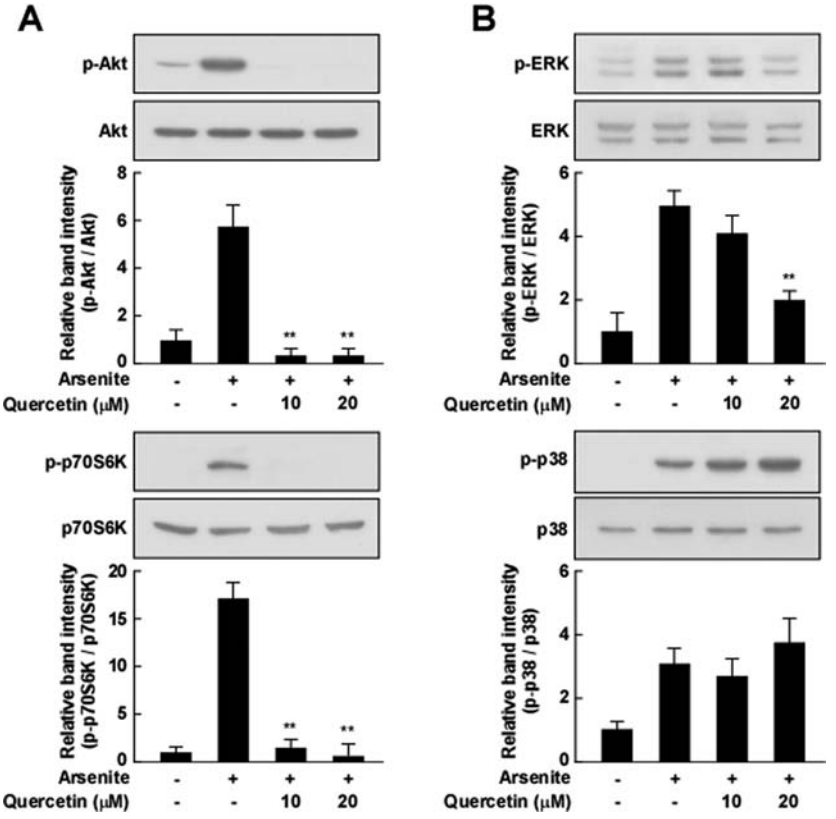

Figure 4. Effects of quercetin on arsenite-induced phosphorylation of Akt, p70S6K, ERK, and p38 in RLE cells. (**A**) Quercetin blocked arsenite-induced Akt and p70S6K phosphorylation. (**B**) Quercetin blocked arsenite-induced ERK1/2 phosphorylation, but not p38 phosphorylation. Cells were treated with quercetin at the indicated concentrations (10 or 20 μM) for 30 min before incubation with 30 μM arsenite for 1 h. Lysates were prepared and Western blotting was performed to probe phosphorylated and total protein levels of Akt, p70S6K, ERK1/2, and p38 as mentioned under Materials and Methods. The data represent the mean ± SD of three separate experiments, and the picture is a representative of those experiments, each with similar results. Quantification was performed using Image J software.

production by 46 and 94%, respectively (**Figure 3C**). Thus, quercetin regulates arsenite-induced COX-2 expression and PGE_2 production in RLE cells.

Quercetin Attenuates the Arsenite-Induced Activation of Akt, p70S6K, and ERK. Because the activation of Akt and MAPKs is related to the induction of COX-2 in several cell types, we next investigated the influence of quercetin on arsenite-induced activation of Akt, p70S6K, ERK, and p38. Quercetin resulted in a complete inhibition of arsenite-induced phosphorylation of Akt and p70S6K at concentrations of 10 or 20 μM (**Figure 4A**), suggesting that quercetin may suppress arsenite-induced COX-2 expression, mainly by blocking the activation of the Akt and p70S6K signaling pathways. Quercetin also blocked the arsenite-induced phosphorylation of ERK, but not of p38, in RLE cells (**Figure 4B**). These results suggest that quercetin regulates Akt and ERK phosphorylation in RLE cells. Previously, we found that quercetin acts as an inhibitor of MEK signaling (*21*). Thus, this paper focuses on the role of quercetin on arsenite-induced Akt signaling.

Quercetin Directly Suppresses Arsenite-Induced PI3K Activity by Binding with PI3K. Because previous studies indicated that Akt and p70S6K may serve as downstream targets of PI3K (*22*), we examined the effects of quercetin on arsenite-induced PI3K activity in RLE cells. The increased activity of PI3K caused by arsenite treatment for 30 min was decreased by quercetin treatment (**Figure 5A**). These results suggested that PI3K might be a direct molecular target of quercetin in arsenite-induced COX-2 expression in RLE cells and that quercetin may inhibit downstream signals by inhibiting PI3K activity. To further confirm the mechanism of the inhibitory effect of quercetin against PI3K activity, we investigated whether quercetin can directly bind with PI3K in RLE cell lysates by performing ex vivo pull-down assays. After being treated with arsenite for 30 min, cell lysates were collected and loaded onto SDS-PAGE. PI3K p85 was observed in quercetin−Sepharose 4B beads (**Figure 5B**, lane 3), but not in Sepharose 4B-only beads (**Figure 5B**, lane 2). As indicated in the input lane (lane 1) in **Figure 5B**, only cell lysates were loaded as a marker to ensure that the band detects the p85 protein itself. These results indicate that quercetin can directly bind with the p85 subunit of PI3K and subsequently inhibit the activation of PI3K and downstream signals.

PI3K Inhibitor Suppresses Arsenite-Induced COX-2 Expression. To confirm that PI3K was required for COX-2 induction by arsenite, RLE cells were pretreated with kinase inhibitors 30 min before treatment with arsenite for 6 h. We found that 20 or 40 μM LY294002, a pharmacological inhibitor of PI3K, blocked arsenite-induced COX-2 expression (**Figure 6A**). Cells pretreated with LY294002 showed an inhibition of PGE_2 release, suggesting a role of PI3K in PGE_2 release (**Figure 6B**). These findings indicate that PI3K may mediate COX-2 expression and PGE_2 secretion induced by arsenite.

DISCUSSION

Quercetin, a polyphenolic flavonoid compound, has been shown to have therapeutic potential against many diseases. However, the molecular mechanism by which quercetin inhibits arsenite-induced tumorigenesis is still not known. There is compelling evidence that mediators of inflammation, such as PGs, are involved in hepatic carcinogenesis. For example, increased COX-2 expression and PGE_2 production are found in human and

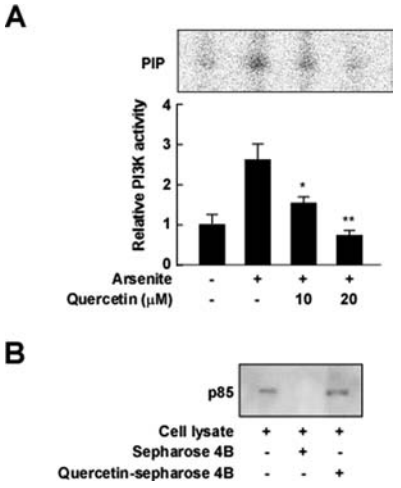

Figure 5. Effects of quercetin on arsenite-induced PI3K activity by binding with PI3K. (**A**) Quercetin inhibited arsenite-induced PI3K activity. RLE cells were pretreated with quercetin at the indicated concentrations (10 or 20 μM) for 30 min and stimulated by 30 μM arsenite for an additional 30 min. The cells were lysed, and then the activity of PI3K was determined as described under Materials and Methods. The data represent the mean $\pm$ SD of three separate experiments, and the picture is a representative of those experiments, each with similar results. Quantification was performed using Image J software. (**B**) Quercetin specifically bound with the p85 subunit of PI3K. PI3K−quercetin binding in RLE cells was confirmed by immunoblotting using an antibody against the p85 subunit of PI3K: lane 1 (input control), whole cell lysates from RLE cells; lane 2 (control), lysates of RLE cells precipitated with Sepharose 4B beads as described under Materials and Methods; lane 3, whole-cell lysates from RLE cells precipitated by quercetin−Sepharose 4B affinity beads as described under Materials and Methods.

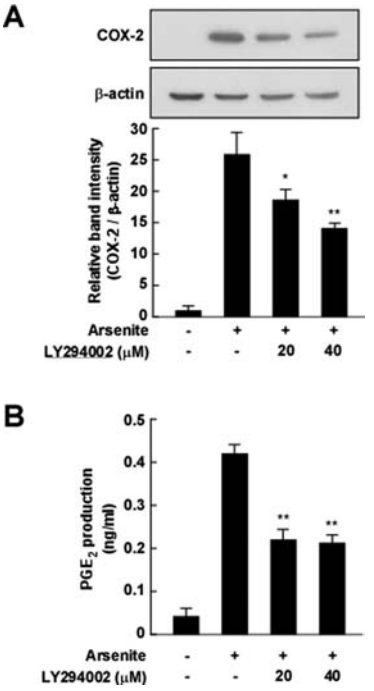

Figure 6. Effects of LY294002 on arsenite-induced COX-2 expression and PGE_2 production. (**A**) LY294002 suppressed arsenite-induced COX-2 expression in RLE cells. RLE cells were pretreated with LY294002 (20 or 40 μM) for 30 min before incubation with 30 μM arsenite for 6 h. The levels of COX-2 and β-actin proteins were determined by Western blot analysis, as described under Materials and Methods, using specific antibodies. The data represent the mean $\pm$ SD of three separate experiments, and the picture is a representative of those experiments, each with similar results. Quantification was performed using Image J software. (**B**) LY294002 reduced the arsenite-induced generation of PGE_2. RLE cells were pretreated with LY294002 at the indicated concentrations (20 or 40 μM) for 30 min before incubation with 30 μM arsenite for 24 h. PGE_2 generation was determined by the PGE_2 assay kit as described underMaterials and Methods. Asterisks ($**$) indicate a significant difference between groups treated with arsenite and LY294002 and the group treated with arsenite alone ($p < 0.01$).

animal hepatocellular carcinomas (*23*, *24*). In this study, we showed that arsenite stimulated COX-2 expression and increased PGE_2 release in RLE cells. Previous studies demonstrated that selective COX-2 inhibition exerts antitumorigenic effects in hepatocarcinoma cells in vitro as well as in vivo (*25*, *26*). Thus, targeting COX-2-derived PG signaling represents a promising strategy to reduce tumors. We found that quercetin inhibits arsenite-induced COX-2 expression. Numerous studies demonstrate that the induction of COX-2 synthesis results in increased PGE_2 production, whereas inhibitors of COX-2 reduce PGE_2 levels (*27*). We determined that quercetin significantly inhibited the production of PGE_2, most likely by blocking the expression of COX-2. These data support the hypothesis that the antitumor activity of quercetin in arsenite carcinogenesis may be due in part to its ability to block COX-2 production.

The expression of COX-2 caused by arsenite exposure was correlated with its effect on signal pathway activation. The PI3K/Akt signaling pathway was demonstrated to play a role in tumorigenesis (*28*). Previous studies demonstrated that inhibition of Akt activation by PI3K inhibitor LY294002 decreases hepatocellular carcinoma cell growth (*29*) and LY294002 blocked the arsenic-induced COX-2 expression (*30*). Here, quercetin significantly decreased the arsenite-induced phosphorylated levels of both Akt and p70S6K and acted as a key downstream effector of Akt in RLE cells. Also, quercetin inhibited arsenite-induced phosphorylation of ERK, but not p38, without any effect on their total protein levels. Because quercetin suppressed the activation of Akt and ERK signaling, we hypothesized that the molecular target of quercetin on arsenite-induced COX-2 expression might be the upstream kinase of Akt and ERK. In our previous study, we demonstrated that MEK1, the upstream kinase of ERK, is a molecular target of quercetin for suppressing neoplastic transformation (*21*). Therefore, we focused on the critical role of PI3K in arsenite-induced COX-2 expression by regulating Akt. Our results clearly showed that quercetin significantly inhibited PI3K activity through direct binding with PI3K.

Furthermore, our results provide evidence that Akt activation plays an important role in the arsenite-induced expression of COX-2. We found that inhibition of PI3K with LY294002 resulted in arsenite-stimulated COX-2 expression. In our study, PD098059 (a MEK inhibitor) and SB203580 (a p38 inhibitor) inhibited arsenite-induced COX-2 expression (data not shown). Even though quercetin suppressed arsenite-induced ERK phosphorylation, its inhibitory effect is insignificant compared to Akt phosphorylation, suggesting that quercetin suppresses arsenite-induced COX-2 expression, mainly by blocking the activation of the Akt signaling pathway. LY294002 also reduces the increase in PGE_2 production induced by arsenite. Taken together, these results suggest that PI3K activation participates in the arsenite-mediated full induction of COX-2 protein expression and PGE_2 secretion in normal RLE cells.

In conclusion, we demonstrated that quercetin effectively inhibits arsenite-induced COX-2 expression and PGE_2 production by targeting PI3K in RLE cells. This suggests that quercetin

is a potential chemopreventive agent against arsenite-induced liver cancer.

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