Cytochrome P450 2S1 Depletion Enhances Cell Proliferation and Migration in Bronchial Epithelial Cells, in Part, through Modulation of Prostaglandin E₂ Synthesis

T. W. Madanayake, T. P. Fidler, T. M. Fresquez, N. Bajaj, and A. M. Rowland
Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, New Mexico

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ABSTRACT:
Cytochromes P450 (P450s) contribute to the metabolic activation and inactivation of various endogenous substrates. Despite years of research, the physiological role of CYP2S1 remains unknown. CYP2S1 has demonstrated NADPH P450-reductase-independent metabolism of cyclooxygenase (COX)-derived prostaglandins [e.g., prostaglandin G₂ (PGG₂)] at nanomolar concentrations. Arachidonic acid is converted to prostaglandin precursors [PGG₂ and prostaglandin H₂ (PGH₂)] through COX. These precursors are used to synthesize numerous prostanoids, including PGE₂. Prostaglandin E₂ (PGE₂) promotes cell proliferation and cell migration and inhibits apoptosis. CYP2S1 metabolism of PGG₂ presumably sequesters PGG₂ and PGH₂, making them unavailable for synthesis of prostanoids such as PGE₂. Whether CYP2S1 contributes to prostaglandin metabolism and influences cell physiological remains to be determined. The purpose of this study was to evaluate the physiological role of CYP2S1, if any, in human bronchial epithelial cells [SV40-derived bronchial epithelial cell line (BEAS-2B)]. To do this, we used small interfering RNA to deplete CYP2S1 mRNA and protein by approximately 75% and evaluated the impact of CYP2S1 depletion on cell proliferation and migration. CYP2S1 depletion enhanced both cell proliferation and migration in BEAS-2B cells. Consistent with the proposed role of CYP2S1 in PGE₂ synthesis, the reduction in CYP2S1 expression doubled intracellular PGE₂ levels. Pharmacological administration of PGE₂ enhanced cell proliferation in BEAS-2B cells but failed to promote migration. Our data reveal an important role for CYP2S1 in the regulation of cell proliferation and migration, occurring in part through modulation of prostaglandin synthesis.

Introduction
Cytochromes P450 (P450s) are heme-containing monooxygenase enzymes capable of metabolizing various endogenous or exogenous compounds. CYP2S1 is one of the most recently characterized members of the P450 family (Rylander et al., 2001). Its expression is restricted to extrahepatic epithelial cells (Rivera et al., 2002) and is significantly up-regulated in response to inflammatory disease. CYP2S1 expression is significantly elevated in psoriatic plaques characterized by inflammation and cell proliferation (Smith et al., 2003). Expression data also suggest that CYP2S1 may be linked to carcinogenesis: elevated CYP2S1 immunoreactivity is observed in human epithelial colorectal (Kumarakulasingham et al., 2005), metastatic ovarian (Downie et al., 2005), breast (Murray et al., 2010), and squamous cell carcinomas (Saarikoski et al., 2005) and correlates with poor prognosis in colorectal, ovarian, and breast cancer (Downie et al., 2005; Kumarakulasingham et al., 2005; Murray et al., 2010). Better understanding of how alterations in CYP2S1 expression influence endogenous metabolism and of the cellular consequences associated with this regulation is an essential first step in determining the impact of elevated CYP2S1 expression, if any, in disease.

Despite the identification of potential endogenous substrates (e.g., all-trans-retinoic acid (Bui and Hankinson, 2009) and eicosanoids (Bui et al., 2011), the proposed metabolic mechanism and the relevance of CYP2S1-mediated metabolism remain controversial (Nishida et al., 2010; Xiao et al., 2011). Bui and colleagues identified CYP2S1-mediated metabolism of potential endogenous substrates that include retinoic acid (Bui and Hankinson, 2009) as well as members of the cyclooxygenase (COX) and lipoxygenase (LOX)-derived eicosanoids (Bui et al., 2011), using a codon-optimized synthetic CYP2S1 (Bui and Hankinson, 2009; Bui et al., 2009, 2011). Metabolism of endog-
enous substrates was shown to be independent of NADPH P450 reductase, because it requires peroxide utilization for metabolism (Bui and Hankinson, 2009; Bui et al., 2011). Of the endogenous substrates tested, COX-derived prostaglandins [e.g., prostaglandin G₂ (PGG₂) and prostaglandin H₂ (PGH₂)] were predicted as likely endogenous substrates for CYP2S1 isomerase activity (Bui et al., 2011). Arachidonic acid (AA) is converted to PGG₂ and PGH₂ via the COX enzymes. PGH₂ is further metabolized to bioactive prostanooids, including prostaglandin E₂ (PGE₂). Using peroxide cofactors, CYP2S1 was able to metabolize PGG₂ (Kₘ = 270 nM) and PGH₂ (Kₘ = 11 μM) into numerous metabolites. The authors predicted that CYP2S1 expression may effectively divert synthesis of bioactive prostanooids and demonstrated depletion of PGE₂ and prostaglandin D₂ (PGD₂) in mammalian cells overexpressing CYP2S1 when supplemented with the PGH₂ precursor. Although it suggests that CYP2S1 influences PGE₂ synthesis, whether this modulation is physiologically relevant remains to be determined (Nishida et al., 2010; Xiao et al., 2011) and is the subject of this investigation.

PGE₂ is the most well studied COX-derived prostanooid. The physiological effects of PGE₂ are mediated through PGE₂ activation of its cognate G protein-coupled E prostanooid receptors (EP₁–EP₃) (reviewed in Wang and Dubois, 2006). In epithelial cells, PGE₂ stimulates cell proliferation (Pai et al., 2002) and cell migration (Buchanan et al., 2003) while inhibiting apoptosis (Munkarah et al., 2002).

The purpose of this study was to identify the physiological significance, if any, of CYP2S1 in human bronchial epithelial cells [SV40-derived bronchial epithelial cell line (BEAS-2B)] by selectively depleting its expression and evaluating the cellular consequences. Our study reveals that the CYP2S1 expression and presumably changes in endogenous metabolism alter cell migration and proliferation in human lung cells. Cell migration and proliferation observed in CYP2S1-depleted cells appear to be mediated through disparate actions of distinct intracellular pathways. CYP2S1-depleted cells have twice the level of intracellular PGE₂. Our results suggest that elevated PGE₂ levels may contribute to enhanced cell proliferation, but not migration, in BEAS-2B cells. These data are consistent with the proposed physiological role for CYP2S1-mediated metabolism of PGG₂ (Bui et al., 2011), supporting the idea that PGG₂ may be a physiologically relevant substrate in bronchial epithelial cells. In addition, our data hint that CYP2S1-mediated metabolism influences other, as of yet unidentified, endogenous pathways that influence cell migration.

Materials and Methods

CYP2S1 Depletion Using Short Hairpin RNA. BEAS-2B cells were plated in six-well plates and transfected with pLKO.1 short hairpin RNA (shRNA) plasmids (Sigma MISSION shRNA; Sigma-Aldrich, St. Louis, MO) bearing 21 nucleotide sequences directed against either CYP2S1 or the non-targeting scrambled control (SCSHRAM, referred to as SCRAM). shRNA sequences targeting CYP2S1 were directed toward exon 3 (SCH00984, referred to as SCH00984, and the 3’-untranslated region (UTR) (SCH000759, referred to as SCH000759, the 3’-untranslated region (UTR) (SCH000759, referred to as SCH000759). The non-targeting SCRAM was used as a control. Stable individual colonies expressing the SCRAM, 759, and 984 were identified and used as a control. Stable clone selection was performed using the Femto chemiluminescent detection kit (Millipore, Bil- lerica, MA). Dr. Oliver Hankinson (UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA) kindly provided the CYP2S1 protein loading control. The chemiluminescent signal was visualized using the Chemidoc XRS system (Bio-Rad Laboratories). Protein loading was controlled using the polyclonal anti-rabbit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Sigma G9545; Sigma-Aldrich) antibody.

Wound-Healing Assay. Bronchial epithelial cells were plated in replicates of three in six-well plates at a 300,000 cells/well for 24 h or until confluence was reached. The horizontal scratch was made in the center of the well using a p10 pipette tip. Immediately after the scratch, the cells were washed with PBS, and new media was added. Vertical lines were drawn across the scratch as reference markers for imaging. Images were taken at different time points t = 0 and 24 h using the Zeiss Axioscope II (Carl Zeiss, Inc., Thornwood, NY). Images from each time point were aligned using Photoshop CS3 Professional software suite (Adobe Systems, San Jose, CA), and migrating cells were counted to determine invasive characteristics of cell lines. Cells were counted in replicates of three independent biological experiments.

Cell Proliferation Assays. Cell numbers were calculated using the hemoctometer. BEAS-2B cells were plated in a 96-well plate at 2000 to 4000 cells/well in replicates of at least six per treatment or genotype. Cells were allowed to incubate overnight at 5% CO₂ at 37°C before the addition of alamarBlue (Invitrogen) was added to a final concentration of 10%. Media alone and in the presence of fully reduced alamarBlue (media containing autoclaved alamarBlue) was used to establish the background and 100% reduced alamarBlue. Percentage reduction of alamarBlue was calculated, and these values were used to compare cells. Care was taken to follow only the wells (n = 3 or more) that had similar fluorescent readings between samples at t = 0. These experiments were conducted at least three times in this manner. alamarBlue data were analyzed using a one-way analysis of variance in Prism statistical software (GraphPad Software, Inc., San Diego, CA), analyzing linear growth rates, and differences in growth were assessed at 24 and 48 h by post hoc t tests. All data were normalized to blank media wells (alarmBlue background fluorescence levels). For 3-(4,5-dimethylthiazol-2-yl)-2,5-diphe-nyl-tetrazolium (MTT) assays, Cells were plated as described above (See Cell Proliferation Assays introduction). MTT assays were performed according to the manufacturer’s instructions (Sigma-Aldrich), and the absorbance was monitored at 560 nm using the BioTek FL600 plate reader (BioTek Instruments, Winooski, VT).


Results

CYP2S1 shRNA-Reduced CYP2S1 mRNA and Protein Expression in Bronchial Epithelial BEAS-2B Cells. To determine whether changes in CYP2S1 gene expression influences bronchial epithelial cell physiology, we evaluated the shRNA sequences targeting the CYP2S1 mRNA. Sigma MISSION shRNA plasmids (Sigma-Aldrich) bearing 21 nucleotide sequences directed toward exon 3 (SCH00984, referred to as 984) as well as the 3'-UTR (SCH00759, referred to as 759) of CYP2S1 were used to deplete CYP2S1 expression (Fig. 1A). The nontargeting SCRAM was used as a control. We transfected each of the plasmids into BEAS-2B cells and isolated stable colonies derived from both 759 and 984. Stable colonies demonstrating the most significant difference in CYP2S1 expression relative to SCRAM controls were used for subsequent experiments. CYP2S1 mRNA expression was analyzed using qRT-PCR and normalized to the β-actin (ACTB) housekeeping gene. CYP2S1 mRNA was reduced by approximately 75% in both CYP2S1-depleted 759 (crosshatched bars) and CYP2S1-depleted 984 (horizontal lined bar) compared with the SCRAM (black bar) (Fig. 1B). This data were also normalized to five additional housekeeping genes with similar reductions in CYP2S1 mRNA (see Supplemental Fig. 1). Western analysis was performed using both the human CYP2S1 antibody (provided by Dr. Roland Wolf) and the commercially available antibody (see Materials and Methods). Each CYP2S1 antibody produced similar results (data not shown). BEAS-2B cells displayed a single band at 50 kDa, which was consistent with the positive CYP2S1 protein control (2S1; provided by Dr. Oliver Hankinson). Western (Fig. 1C) and densitometric analysis (Fig. 1D) revealed marked depletion of CYP2S1 protein levels by approximately 75 to 70% in 759 and 984, respectively, compared with the SCRAM. These CYP2S1 mRNA and protein reductions were stable through multiple passages as well as from older (multiple years) frozen stocks.

Effects of CYP2S1 Depletion on Bronchial Epithelial Cell Migration and Proliferation. Because CYP2S1 expression is elevated in epithelial-derived cancers (Downie et al., 2005; Kumarakulasingham et al., 2005; Murray et al., 2010), we tested the possibility that altering CYP2S1 expression in human bronchial epithelial cell would influence cellular pathways that either promote or inhibit cell proliferation and/or migration. To determine whether CYP2S1 expression alters cell proliferation and migration in human lung cells, we compared cell proliferation and migration in stably transformed SCRAM to cells with significantly reduced CYP2S1 mRNA and protein (759 and 984) (Figs. 2 and 3).

To assess the effect of CYP2S1 depletion on bronchial epithelial migration, we performed the wound-healing assay. In brief, the two populations of cells were grown to confluence in six-well plates, and a wound was created using a 10-μl pipette tip. The progression of wound healing was visualized at different time points (0 and 24 h), and the number of cells invading the wound at 24 h was quantified (Fig. 2, A and B). This assay produced striking results. After 24 h, CYP2S1-depleted cells nearly covered the entire wound, whereas SCRAM hardly migrated. Quantification of these results revealed roughly 3-fold increase in cell migration in CYP2S1-depleted (~150 cells) over SCRAM (~50 cells) (Fig. 2B). Our results demonstrate that CYP2S1 depletion promotes cell migration, presumably by reducing CYP2S1-mediated metabolism of currently unknown endogenous substrates.

Differences in cell proliferation were assessed using two independent methods: alamarBlue and MTT cell-viability assays. The alamarBlue assay detects viable cells using the blue nonfluorescent dye (resazurin). Under conditions promoting cell proliferation, resazurin is reduced to its fluorescent form, resorufin, and detected by a fluorescent plate reader. MTT is a comparable cell-viability detection method that measures metabolic activity in viable cells through the reduction of the yellow tetrazole compound to a purple formazan that can be detected though absorbance changes at 562 nm.

CYP2S1-depleted cells (759 and 984) as well as SCRAM controls were plated at equal numbers (~2000 cells) and allowed to proliferate for 24 h (alarmaBlue and MTT) or up to 48 h (alarmaBlue). Each cell type was assessed for increases in viability. Cell viability in CYP2S1-depleted cells was compared with that in SCRAM controls, which are designated as 100% growth (Fig. 3, A and B). CYP2S1-depleted cells exhibited statistically significant increases of 40 (759, crosshatched bars) and 15% (984, horizontal lined bars) in cell viability compared with SCRAM control (SCRAM, closed bars) at 24 h (Fig. 3A). After 48 h, 759 rose an additional 20 to 60% over SCRAM control, whereas 984 increased by roughly 5 to 20% increase over SCRAM control (Fig. 3A). It is interesting that the MTT assay demonstrated a more robust and consistent increase between the CYP2S1-depleted cell lines 759 (crosshatched bars) and 984 (horizontal lined bars). Both CYP2S1-depleted cells (759 and 984) exhibited roughly 300% in-
crease over SCRAM control and were not statistically different from one another. We are uncertain as to what factors may contribute to the differences between these assays. However, these data clearly demonstrate that CYP2S1 depletion can enhance cell proliferation in human bronchial epithelial cells.

Elevated PGE₂ in CYP2S1-Depleted Cells Promotes Cell Proliferation but Not Cell Migration. CYP2S1 was shown to metabolize bioactive lipids derived from the COX and LOX pathways in the absence of P450 reductase. Substrates included prostaglandins PGG₂ and PGH₂ from the COX pathway as well as numerous hydroperoxyeicosatetraenoic acid derivatives from the LOX pathway (Bui et al., 2011). The authors proposed that CYP2S1 metabolism of PGG₂ and PGH₂ could effectively divert the AA-derived production of PGE₂ to CYP2S1-derived metabolites 12(S)-hydroxyheptadeca-5,10,14-trienoic acid (12-HHT) and thromboxane A₂ (TXA₂) (Bui et al., 2011). If true, CYP2S1 depletion should increase PGE₂ levels (see schematic in Fig. 5). PGE₂ enzyme-linked immunosorbent assay was performed to test whether there were differences in PGE₂ levels between CYP2S1-depleted cells (759 and 984) and control (SCRAM). PGE₂ levels in SCRAM were approximately 6 pg/well, or 0.15 pg/μg protein (Fig. 4A). This is similar to PGE₂ concentrations reported previously (Cowan et al., 2006). In contrast, CYP2S1-depleted cells had nearly double the concentration of PGE₂ (12 pg/well, or 0.3 pg/μg protein) in both 759 and 984 cell lines (Fig. 4A). Because PGE₂ is known to promote cell migration and proliferation in a number of different cell types (Sheng et al., 2001), we tested its ability to enhance cell migration (Fig. 4B) and proliferation (Fig. 4C) in BEAS-2B. BEAS-2B cells were exposed to PGE₂ at varying concentrations of PGE₂. However, none of the concentrations of PGE₂ tested was able to promote cellular migration. Thus, the migration observed in CYP2S1-depleted cells (Fig. 2) cannot be attributed to elevated PGE₂ levels.

Next, we tested whether PGE₂ promotes cell proliferation in BEAS-2B. We performed alamarBlue studies, which are similar to the MTT assay because they are both indirect measurements of cell viability. BEAS-2B cells grown in the presence of the lowest concentration of PGE₂ (200 nM) exhibited a significant increase (~25% increase) and, though still significantly different, began approaching dimethyl sulfoxide control levels at 24 h (Fig. 4C). The increase in viability attributed to PGE₂ at 24 h (~25%) was within the range of alamarBlue reduction increases observed in response to CYP2S1 depletion in 759 (~40%) and 984 (~15%) (Fig. 3A). The PGE₂ concentration required to promote cell proliferation was in the low nanomolar range. Once this initial increase in cell viability was attained, increasing PGE₂ levels failed to promote further statistical increases in cell proliferation and at micromolar concentrations appeared to reduce PGE₂’s proliferative effects on normal (nontransformed) BEAS-2B cells. These results are consistent with PGE₂’s biphasic effect on cell proliferation in other cell types (Baylink et al., 1996; Sergeeva et al., 1997), albeit at higher concentrations.
To determine whether the increased levels of PGE₂ observed in CYP2S1-depleted cells were sufficient to promote the cell proliferation observed with PGE₂, we evaluated the effects of PGE₂ on CYP2S1-depleted cells. Because the MTT assay was shown in our studies to be a more robust indicator of differences in cell proliferation after 24 h (Fig. 3), we performed the MTT assay to measure cell viability of both SCRAM controls and CYP2S1-depleted (759 and 984) BEAS-2B cells in response to varying concentrations of PGE₂ (Fig. 4D). Consistent with effects observed in nontransformed normal BEAS-2B (Fig. 4C), the lowest level of PGE₂ tested (1 nM) was sufficient to enhance cell viability by ~60% in SCRAM controls. This increase is MTT reduction was sustained until PGE₂ concentrations reached ~100 μM. In contrast, cell proliferation was not enhanced in CYP2S1-depleted cells in response to exogenous PGE₂ at any concentration. Though not statistically significant, we observe reduced viability of CYP2S1-depleted cells (759 in particular) at 10 μM rather than 100 μM concentrations in SCRAM controls. These data would be consistent with increased concentration of intracellular PGE₂ observed in CYP2S1-depleted cells. Taken together, our data suggest that elevated intracellular levels of PGE₂ contribute to increased cell proliferation observed in CYP2S1-depleted cells. These data are also consistent with the proposed role of CYP2S1 in metabolizing the PGE₂ precursor PGG₂, which would divert the cellular production of PGE₂, and support the hypothesis that PGG₂ is a physiologically relevant substrate for CYP2S1 in human bronchial epithelial cells.

Discussion

In our current study, we reveal an important contribution of the human CYP2S1 enzyme in the regulation of cell growth and migration in BEAS-2B cells, which are used as surrogates for normal bronchial epithelial cells. In particular, by depleting CYP2S1 expression and ultimately CYP2S1-mediated metabolism of endogenous substrates, cell proliferation and migration are enhanced. Proliferation and migration appear to be functionally divergent, suggesting that CYP2S1 depletion promotes each through distinct endogenous substrates, metabolites, or perturbation of downstream bioactive molecules.

Heterologous expression and metabolic studies using a synthetic CYP2S1 enzyme (Bui and Hankinson, 2009) have demonstrated metabolic activity toward potential endogenous substrates of the CYP2S1 enzyme, including the following: lipid products derived from the AA cascade (Bui et al., 2011) as well as all-trans-retinoic acid (Bui and Hankinson, 2009). Free AA is converted to bioactive eicosanoid metabolites via metabolism through either the LOX enzymes or the COX enzymes. Heterologous expression of the synthetic CYP2S1 enzyme has demonstrated P450 reductase-independent metabolic activity toward both LOX- and COX-derived eicosanoids. It is predicted that the most relevant CYP2S1-mediated metabolism may be of the COX-derived prostaglandin intermediate, PGG₂, because of its low Kₘ (270 nM). A summary of the AA cascade as well as the proposed

Fig. 4. CYP2S1 depletion enhances PGE₂ production, which may account for enhanced proliferation but not migration effects observed in CYP2S1-depleted cells. A, PGE₂ enzyme-linked immunosorbent assay detected elevated intracellular PGE₂ in BEAS-2B cells depleted of CYP2S1 (759 and 984, crosshatched and horizontal line bars, respectively) compared with SCRAM (closed bars). PGE₂ levels were normalized to cellular protein content (BCA protein assay kit) within each well. Error bars indicate variability between each well. B, PGE₂ supplementation alone failed to promote migration of nontransformed (normal) BEAS-2B cells into the wound. Confluent BEAS-2B cells were wounded and allowed to migrate for 24 h with the addition of 10 nM and 1 μM PGE₂. C, in contrast, PGE₂ supplementation at nanomolar concentrations was sufficient to enhance cell proliferation in normal BEAS-2B cells. Data from three experiments are represented as percentage increases in alamarBlue reduction 24 h after t = 0 control. Statistical analysis was performed using a standard two-tailed t-test where *** represents p < 0.001. D, MTT assay measuring the metabolic activity in the cells reveals an increase in proliferation at 24 h in response to PGE₂ in SCRAM controls (solid line, closed circles), but not CYP2S1-depleted (759 and 984, dashed lines with open circles and open squares, respectively) BEAS-2B cells. Each experiment was performed at least three times. DMSO, dimethyl sulfoxide. Statistical analysis was performed using one-way ANOVA followed by Tukey Kramer post-hoc analysis. *, **, and *** indicate significant increases from vehicle controls of p < 0.05, p < 0.01, and p < 0.001, respectively.
role for CYP2S1 in prostanoid metabolism is depicted in Fig. 5. The first step in the COX pathway is to convert AA to PGG2 through COX peroxidase activity. PGG2 is then converted via COX enzymes or nonenzymatically to PGH2. PGH2, in turn, is metabolized via thromboxane, prostaglandin, and prostacyclin synthetases to TXA2, prostaglandins (PGD2, prostaglandin F2α, and PGE2), prostacyclin (prostaglandin I2) (reviewed in Kroetz and Zeldin, 2002; Wang and Dubois, 2006; Panigrahy et al., 2010). CYP2S1 is able to convert PGG2 (Km = 270 nM) and PGH2 (Km = 11 μM) to multiple products including 12-HHT, malondialdehyde (MDA), and TXA2 (Bui et al., 2011). If CYP2S1 plays an essential role in modulating prostanoid synthesis in human lung cells, we would predict that CYP2S1 depletion would enable more of the PGG2 precursor to be converted to PGH2 and its subsequent prostanoid products, including PGE2. Consistent with this proposed role for CYP2S1 in prostaglandin metabolism, CYP2S1 depletion may elevate PGG2 and PGH2 precursors, increasing their availability for synthesis of downstream prostanoids, including PGE2. Enzymes are represented by names enclosed in gray boxes. Open boxes contain either CYP2S1 metabolites (12-HHT, MDA, and TXA2) or PGE2.

Four G-protein-coupled EP receptor subtypes have been identified: EP1, EP3, EP4, and EP5. EP1 stimulates increased intracellular calcium through activation of phospholipase C. EP3 and EP4 are linked to Gs, and it activates adenylate cyclase (AC) and increases cAMP synthesis. On the other hand, EP3 is linked to Gi and decreases AC activity and cAMP synthesis. BEAS-2B cells were shown to express each EP receptor mRNA (Tavakoli et al., 2001; N’Guessan et al., 2007) and protein (N’Guessan et al., 2007), suggesting a full complement of activity mediated through PGE2 activation.

PGE2 stimulates proliferation in epithelial cell lines, including non-small-cell lung cancer cell lines. Our data reveal a previously undocumented role for PGE2 in stimulating cell proliferation within BEAS-2B cells (Fig. 4C). PGE2-stimulated proliferation occurs through EP3 (Krysan et al., 2005)—stimulated activation of extracellular signal-regulated kinase (ERK) signaling, EP4-stimulated signaling and transactivation of the epidermal growth factor receptor signaling (Pai et al., 2002), and downstream activation of the integrin-linked kinase (Zheng et al., 2009). Krysan et al. (2005) did not observe rapid stimulation of ERK phosphorylation in BEAS-2B cells, suggesting that the PGE2-mediated proliferation of BEAS-2B is not a consequence of EP3 activation of ERK signaling. Our proliferation data appear to be consistent with EP4 transactivation of epidermal growth factor receptor, which was identified to promote cell cycle progression in gastric epithelial cells, which was arrested in G1/G0 in response to...
Our results suggest that PGE2 alone is not sufficient to promote wound
normal human bronchial epithelial cells (Savla et al., 2001); however,
role for CYP2S1 in regulating prostaglandin (specifically, PGE2)
lung cells. Our data provide further evidence supporting a modulatory
CYP2S1 depletion promotes cell proliferation and migration in human
CYP2S1 depletion.

PGE2 promotes cell migration via EP2 activation of sarcoma (Src)
signaling in human alveolar carcinoma cells (A549) (Kim et al.,
2010). However, we were unable to phenocopy the enhanced migration
phenotype observed in CYP2S1-depleted cells by exogenous application of PGE2,
 even at micromolar concentrations. These data suggest that although PGE2 levels are increased in CYP2S1-depleted
(cells 759 and 984), the cellular migration phenotype observed in
these cells cannot be attributed to PGE2-mediated activation of EP4
and downstream Src signaling. PGE2 promotes cell migration in
normal human bronchial epithelial cells (Savla et al., 2001); however,
our results suggest that PGE2 alone is not sufficient to promote wound
healing in BEAS-2B. This observation is consistent with published results
from Cowan et al. (2006), whereby exogenous application of PGE2
fails to promote cell migration in BEAS-2B. It is possible that
CYP2S1 depletion could influence the production of other eicosanoids
or possibly novel endogenous substrate(s) and/or metabolites
linked to cell migration. Our laboratory is actively pursuing the
mechanism responsible for increased cell migration in response to
CYP2S1 depletion.

In conclusion, this study is the first in vitro demonstration that
CYP2S1 depletion promotes cell proliferation and migration in human
lung cells. Our data provide further evidence supporting a modulatory
role for CYP2S1 in regulating prostaglandin (specifically, PGE2)
synthesis. It also demonstrates a functional role for PGE2 in enhancing
cell proliferation in bronchial epithelial cells. More experiments are
required to elucidate the EP receptors and signaling pathways
responsible for promoting cell proliferation in CYP2S1-depleted cells.
Future studies should reveal other potentially novel CYP2S1 endoge-
nous substrates and/or metabolites responsible for the observed effects of
CYP2S1 depletion on enhanced cell migration. Overall, our data
suggest an important physiological role for CYP2S1 in regulating cell
migration and proliferation and may ultimately have implications in
carcinogenesis, hyperproliferative disease, and inflammatory disease.

Authorship Contributions

Participated in research design: Madanayake, Fidler, and Rowland.
Conducted experiments: Madanayake, Fidler, Fresquez, and Bajaj.
Performed data analysis: Madanayake, Fidler, and Rowland.
Wrote or contributed to the writing of the manuscript: Madanayake, Fidler, and Rowland.

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Address correspondence to: Aaron M. Rowland, Assistant Professor, New
Mexico State University, Department of Chemistry and Biochemistry, 1175 North
Horseshoe Dr., Las Cruces, NM 88003. E-mail: aaronrow@nmsu.edu

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