Microarray Analyses Uncover UBE1L as a Candidate Target Gene for Lung Cancer Chemoprevention

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ABSTRACT

Retinoids, natural and synthetic derivatives of vitamin A, are active in cancer therapy and chemoprevention. We reported previously that all-trans-retinoic acid (RA) treatment prevented carcinogen-induced transformation of immortalized human bronchial epithelial (HBE) cells. To identify cancer chemopreventive mechanisms, immortalized (BEAS-2B), carcinogen-transformed (BEAS-2B\(\text{NNK}\)), and RA-chemoprevented (BEAS-2B\(\text{RA}\)) HBE cells were used to conduct microarray analyses independently. Species increased in chemoprevented as compared with immortalized HBE cells (group I) and those augmented in chemoprevented as compared with transformed HBE cells (group II) included known RA-target genes as well as previously unrecognized RA-target genes in HBE cells. Unexpectedly, both groups were also enriched for interferon-stimulated genes. One interferon-stimulated gene of particular interest was UBE1L, the ubiquitin-activating enzyme E1-like protein. UBE1L expression was also induced after prolonged RA-treatment of immortalized HBE cells. UBE1L mRNA was shown previously as repressed in lung cancer cell lines, directly implicating UBE1L in lung carcinogenesis. Notably, UBE1L immunoblot expression was reduced in a subset of malignant as compared with adjacent normal lung tissues that were examined. Immunohistochemical analyses were performed using a new assay developed to detect this species using rabbit polyclonal anti-UBE1L antibodies independently raised against the amino- or carboxyl-termini of UBE1L. Studies done on paraffin-embedded and fixed tissues revealed abundant UBE1L, but low levels of cyclin D1 expression, in the normal human bronchial epithelium, indicating an inverse relationship existed between these species. To study this further, cotransfection into HBE cells of wild-type or mutant UBE1L species was accomplished. In a dose-dependent manner, wild-type but not mutant UBE1L species repressed cyclin D1 expression. This implicated UBE1L in a retinoid chemoprevention mechanism involving cyclin D1 repression described previously. Taken together, these findings directly implicate UBE1L as a candidate-therapeutic target for lung cancer chemoprevention. These findings also provide a mechanistic basis for the tumor suppressive effects of UBE1L through cyclin D1 repression.

INTRODUCTION

Lung cancer is the leading cause of cancer related deaths for men and women in the United States. The prognosis for advanced stage lung cancer patients needs improvement, in addition to those already made in chemotherapy, radiation therapy, and surgery. Cancer chemoprevention, a therapeutic strategy that aims to prevent, inhibit or reverse carcinogenesis (1), is an attractive approach to decrease lung cancer mortality. Carcinogenesis is a multistep process culminating in malignant tumor formation. Previous work identified frequent molecular changes in human bronchial preneoplasia that were likely involved in pulmonary carcinogenesis (2). Among these changes are potential pharmacologic targets or surrogate markers of bronchial preneoplasia such as overexpression of cyclin D1 (2). The mechanisms by which chemopreventive agents such as all-trans-retinoic acid (RA) repress cyclin D1 and prevent or inhibit carcinogenesis remain largely uncharacterized. This study was undertaken to uncover candidate mechanisms engaged during RA-mediated chemoprevention of carcinogen-exposed human bronchial epithelial (HBE) cells.

Retinoids, natural and synthetic derivatives of vitamin A, are useful pharmacologic tools to uncover mechanisms for cancer chemoprevention, as reviewed (2, 3). Retinoids act by binding to the nuclear retinoic acid receptors or heterodimeric retinoid X receptors that, in turn, affect target gene expression (3). Retinoid-induced alterations in gene expression mediate diverse changes in cellular proliferation, differentiation, and apoptosis, among other changes. To understand better the mechanisms responsible for retinoid-mediated chemoprevention, cellular models for this chemoprevention were developed. We reported previously that acute N-nitrosamine-(methyl-nitrosamino)-1-(3-pyridyl)-1-butaneone (NNK) treatment of the immortalized BEAS-2B HBE cell line caused cellular transformation to occur with increased proliferation, anchorage-independent growth, and tumor formation in athymic mice (ref. 4; as summarized Fig. 1). RA-treatment opposed these changes and also established a cellular model to assess in the preclinical setting mechanisms engaged during lung cancer chemoprevention.

When this cellular model was used, candidate chemopreventive targets were previously identified. For instance, the proteasome-dependent degradation of G1 cyclins was one mechanism linked to retinoid or r ixinoid (retinoid X receptor agonist)-based chemoprevention (4–6). This model was also used to highlight the epidermal growth factor receptor as a potential chemopreventive target (7). These in vitro findings were confirmed and extended in our prior work that revealed cyclin D1, cyclin E, and epidermal growth factor receptor were each frequently overexpressed in bronchial preneoplasia and in non–small-cell lung cancers (2, 8–11). These and other findings raised the prospect that these or other differentially overexpressed species in bronchial preneoplasia or malignant lung cancers were chemopreventive targets.

This study examined changes in gene expression that occurred during retinoid chemoprevention of carcinogen-exposed HBE cells. Affymetrix-based microarray analyses of immortalized, carcinogen-transformed, and RA-chemoprevented HBE cells were used to uncover target genes having increased expression during chemoprevention. One of these augmented species was the ubiquitin-activating enzyme E1-like (UBE1L), a potential tumor suppressor in lung cancers (12). Immunohistochemical analyses revealed that UBE1L was abundantly expressed in the histologically normal human bronchial epithelium where low levels of cyclin D1 expression were known to be evident.
This finding raised the prospect that UBE1L was mechanistically involved in the repression of cyclin D1, a target for cancer chemoprevention based on in vitro studies (4–6) as well as an animal model for chemoprevention (13) and clinical observations (8, 14). To examine this possibility further, cotransfection assays were done to determine whether UBE1L expression would trigger cyclin D1 repression. The findings that will be presented directly implicate UBE1L in lung cancer chemoprevention by causing repression of cyclin D1.

MATERIALS AND METHODS

Cell Lines, Culture Conditions, and RA-Treatments. Immortalized (BEAS-2B) and carcinogen-transformed (BEAS-2BNNK) HBE cells were each cultured in serum-free LHC-9 media (Biofluids, Rockville, MD) at 37°C in a humidified atmosphere of 5% CO2, as described previously (15). Chemoprevented HBE cells (BEAS-2BNNK/RA) were also cultured in LHC-9 media but were supplemented with 1 μmol/L RA (Sigma, St. Louis, MO) as described previously (4). For acute (1 day) and prolonged (10 days or longer) RA-treatments, exponentially growing BEAS-2B cells were treated with RA at a 1 μmol/L dosage. The features of these chemoprevented and transformed cells, as compared with the immortalized HBE cell line, were described previously (4, 5).

Gene Expression Profiles. Trizol reagent (Life Technologies, Inc. Gaithersburg, MD) was used to isolate independently total cellular RNA from immortalized (BEAS-2B), transformed (BEAS-2BNNK), and chemoprevented (BEAS-2BNNK/RA) cells, and total cellular RNA was converted to cDNA according to the manufacturer's procedures (Affymetrix, Santa Clara, CA). The Affymetrix Hu6800 and customized 8600 microarrays were described previously (15). Probe arrays were hybridized, washed, and scanned according
to established techniques. Affymetrix Microarray suite 4.0 software was used to process primary data, determine average difference value, and assess signal intensity for each probe set. The global scaling method recommended by Affymetrix was used to normalize microarrays. These data were used to determine fold changes.

**Expression Analyses.** The RNeasy Protect Mini kit (Qiagen, Valencia, CA) was used to isolate total cellular RNA. DNA-free kit (Ambion, Austin, TX) was used to remove contaminating DNA. Random hexamers and SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) were used to synthesize the cDNA from 3 μg total RNA. Tag polymerase (Invitrogen) was used for PCR assays, and products were resolved on a 1% agarose gel stained with Gelstar nucleic acid stain (BioWhittaker Molecular Applications, Rockville, MD).

The primers for the initiator of DNA binding 1 (Id1), insulin-like growth factor-binding protein 6 (IGFBP6), tumor necrosis factor, α-induced protein 2 (TNFAIP2), and E74-like factor 3 (ELF3) have been described (15) as have primers for UBE1L (16). The remaining primers used were as follows: 2'-5'- oligo(dT)20 (GAPDH) forward primer, 5'-CTGCAAGAAAGGGGGCTGATT-3', and OAS1 reverse primer, 5'-TTCCAGGACCGTGCGATCTC-3'; retinoic acid-induced gene, G protein (RIG-G) forward primer, 5'-CAGAAAGGCCAGCTACCTG-3', and RIG-G reverse primer, 5'-ATAGGCAGAGATCGCATTACCC-3'; interferon (IFN), α-inducible protein 27 (IFI27) forward primer, 5'-TGGCCAGAGATTGCTAGTTG, and IFI27 reverse primer, 5'-CCAGTGTCCCGACTGACT-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward primer, 5'-GGCTGATCCCTACAAACA-3', and GAPDH reverse primer, 5'-GAAGGTGAAAGTCGAGCTCA-3'; malind (MK) forward primer, 5'-CCTGCAACTGGAAGAAGGAG-3', and (MK) reverse primer, 5'-CTTCCCTCCCTTCTTCTG-3'; murine virus resistance 1 (MXA) forward primer, 5'-GAGGACGGACCGTGCACTCTT-3', and MXA reverse primer, 5'-GTGATGAGCTCGCTGGTAA-3'; and IFN-stimulated protein, 15 kDa (ISG15) forward primer, 5'-GGTGGACAAATGCGACGAAC-3', and ISG15 reverse primer, 5'-ATCGTGTCTGAGCCTCTAG-3'.

Established techniques were used to do Northern and Western analyses (15). For Northern analyses, the radiolabeled cDNA probes used were as follows: a 1 kb Ncol/VegOrf fragment of UBE1L cDNA and a 497 bp fragment containing the coding region of ISG15 isolated from pCDNA-ISG15 by BamH1 restriction endonuclease digests. For Western analyses, the antibodies were described previously for UBE1L (16) and IFGBP6 (16). A polyclonal antibody that recognized IRF7 (Santa Cruz Biotechnology). The symbol * indicated that this was not previously reported. For established techniques, the symbol † indicated the most stringent selection criteria of a 2.5-fold change in gene expression in the Hu6800 array, we found only 73 of 6,095 (1.1%) regulated species in the transformed versus the immortalized HBE cell line and 166 of 6,095 (2.7%) regulated species in the transformed versus immortalized HBE cell line. Similar results were found for 2.5-fold augmented species in the 8,600 arrays (data not shown).

**Table 1** Summary of prominently increased species in chemoprevented versus immortalized human bronchial epithelial cells

<table>
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<tr>
<th>Gene*</th>
<th>Access number†</th>
<th>8600 Array</th>
<th>6800 Array</th>
<th>Validation‡</th>
<th>RA target§</th>
<th>ISG§</th>
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<td>-</td>
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<td>7.1</td>
<td>Two arrays</td>
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<td>+</td>
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<td>X77956</td>
<td>7.9</td>
<td>6</td>
<td>W and two arrays</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
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<td>M92357</td>
<td>7.2</td>
<td>4.1</td>
<td>W and two arrays</td>
<td>+</td>
<td>-</td>
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<tr>
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<td>5.3</td>
<td>Two arrays</td>
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<td>+</td>
</tr>
<tr>
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<td>M69054</td>
<td>6.1</td>
<td>7.7</td>
<td>Two arrays</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
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‡ Validation was performed by Western analysis (W), Northern analysis (N), reverse transcription polymerase chain reaction (RT-PCR) assay, real time RT-PCR (real time) assay, and multiple array analyses (two arrays).

§ The symbol ‘+’ indicated prior published evidence for all-trans-retinoic acid (RA) or interferon (IFN)-stimulated gene (ISG). The symbol ‘−’ indicated that this was not previously reported.
shown). Notably, these analyses showed that the expression profile of the immortalized HBE cell line more closely resembled that of the chemoprevented than the carcinogen-transformed HBE cell line (data not shown).

Highlighted species included known RA target genes as well as previously unrecognized RA-regulated species in HBE cells, as depicted in Tables 1 and 2. To determine whether these species were RA-regulated, reverse transcription (RT)-PCR assays were performed for representative species after RA-treatment (1 μmol/L) of BEAS-2B HBE cells. As shown in Fig. 2, some species were rapidly induced within 24 hours of RA-treatment. Species that were acutely induced after RA-treatment of these cells had been uncovered previously as regulated by RA-treatment of BEAS-2B cells (15), as shown in Tables 1 and 2. In contrast, other species displayed in Fig. 2 were induced only after 10 days of RA-treatment and had not been highlighted previously as RA-regulated species in HBE cells, as displayed in Tables 1 and 2. These species included OAS1, MXA, RIG-G, UBE1L, MK, and ISG15. Notably, these species included IFN-stimulated genes (ISGs).

One of the highlighted species that was of particular interest for lung carcinogenesis and perhaps for chemoprevention of HBE cells was UBE1L. UBE1L mRNA was shown previously as repressed in lung cancer cell lines (12). This result was extended in Fig. 3 that shows reduced UBE1L immunoblot expression in subsets of non–small-cell lung cancers as compared with adjacent normal lung tissues. To examine further the expression profile of UBE1L, an immunohistochemical assay was developed to detect UBE1L expression in human tissues. Attention was focused on UBE1L expression in the normal human bronchial epithelium, because UBE1L expression was expected to be evident in this tissue. Independent polyclonal antibodies that recognized the amino- or carboxyl-terminal epitope of UBE1L (16), respectively, were used to examine the immunohistochemical expression of UBE1L.

The amino-terminus immunoreactive antibody was used to detect abundant UBE1L immunohistochemical expression. This was evident in a representative specimen containing histologically normal human bronchial epithelium with pulmonary macrophages, as shown in Fig. 4A. We detected a similar immunostaining profile using the polyclonal antibody that recognized the carboxyl-terminus of UBE1L (data not shown). Preincubation of each of these anti-UBE1L antibodies with the relevant blocking peptide markedly inhibited UBE1L immunohistochemical staining and immunoblot detection (Fig. 4B and data not shown). This result confirmed the specificity of these antibodies. Higher magnification revealed cytoplasmic, nuclear, and apical membrane staining in the normal human bronchial epithelium, as shown in Fig. 4C. Preimmune sera served as an additional negative control, as displayed in Fig. 4D.

With this immunohistochemical assay available, the relationship between UBE1L expression and that of a known retinoid chemopreventive target, cyclin D1, was next examined. Cyclin D1 was recognized previously as a target for lung cancer chemoprevention based on in vitro studies of normal, immortalized, and transformed HBE cells (5, 6), an experimental animal model for lung cancer chemoprevention (13), and also from clinical findings from a chemopreventive trial (14). Cyclin D1 was proposed as a surrogate marker for chemoprevention in the lung because its expression was increased in preneoplastic and neoplastic human lung tissues, as compared with the normal human bronchial epithelium (8). Reduced cyclin D1 protein expression in the normal human bronchial epithelium was confirmed in Fig. 4F. An inverse relationship was found to exist in Fig. 4E and F between UBE1L and cyclin D1 immunohistochemical expression. This raised the prospect that UBE1L might directly cause cyclin D1 repression.

This possibility was explored through a cotransfection assay that was developed in BEAS-2B HBE cells. Cotransfection of HA-tagged cyclin D1 with UBE1L repressed cyclin D1 expression in a manner that depended on the dosage of transfected UBE1L, as displayed in Fig. 5A. In marked contrast, cotransfection of a truncated UBE1L (UBE1L-T) that was known to inactivate UBE1L (16) was no longer able to repress cyclin D1 expression. A reporter plasmid containing the β-galactosidase gene was cotransfected with UBE1L as a control to confirm similar transfection efficiencies in each experiment. This transfected species was unaffected by UBE1L transfection and β-actin expression was also unaffected (data not shown). These results directly linked UBE1L expression to repression of cyclin D1, a chemopreventive target in the lung (2, 17).

**DISCUSSION**

Lung cancer is the leading cause of cancer-related mortality in the United States. Cancer chemoprevention is an appealing strategy to combat this major public health problem (2). Smoking cessation and prevention will ultimately result in a reduction of lung carcinogenesis. However, the large number of former smokers and many individuals exposed to second-hand smoke mean that consequences of lung carcinogenesis will remain a substantial societal problem for years to come. The increased prevalence of smoking and the rising incidence of lung cancer in the United States exemplify this public health problem.
Chemoprevention of lung carcinogenesis in high-risk individuals promises to reduce lung cancer-related mortality. However, effective pharmacologic measures for lung cancer chemoprevention do not currently exist. Therefore, there is a need to understand better how to prevent lung cancers in those at high-risk. This study was undertaken to identify target genes directly linked to lung cancer chemoprevention. Studies were done to confirm that at least one of these targets, UBE1L, was directly involved in a lung cancer chemoprevention mechanism linked to repression of cyclin D1.

This study used an established in vitro model of lung cancer chemoprevention. Our prior work revealed that acute NNK-treatment of HBE cells resulted in malignant transformation with increased proliferation, anchorage-independent growth, and ability to form tumors in athymic mice (4). Chronic RA-treatment resulted in chemoprevention and inhibited this transformation, as summarized in Fig. 1A. In this study, microarray analyses of the immortalized (BEAS-2B), transformed (BEAS-2BNNK), and chemoprevented (BEAS-2BNNK/RA) HBE cell lines showed that RA-treatment inhibited gene expression changes associated with transformation (data not shown). These results underscore that chronic RA-treatment inhibited changes associated with NNK-mediated cellular transformation.

This model has already proven useful to identify degradation of G1 cyclins as a target for retinoid- or rexinoid-mediated chemoprevention (4–6, 18) and to validate the epidermal growth factor receptor as a target for lung cancer chemoprevention (7). In this study, further analyses were done to identify species augmented during this chemoprevention. Among the highlighted species were several identified previously as RA-target genes in HBE cells (15) and others that had not been highlighted in prior work, as summarized in Tables 1 and 2. To investigate whether these species were regulated in the BEAS-2B

![Fig. 2. Increased expression of specific ISGs occurred after prolonged all-RA-treatment of BEAS-2B HBE cells. A, these cells were treated with RA (+) (1 μmol/L) or DMSO (--) as a vehicle control for 1 day or 10 days, respectively, before semi-quantitative RT-PCR assays were done. RT-PCR assay expression of the representative regulated RA target genes or ISGs were compared with total RNA levels as well as to GAPDH expression that served as independent controls for loading in each Lane. Some RA-target genes in HBE cells (TNFAIP2, ELF3/ESX, ID1, and IGFBP6) implicated previously were prominently induced after only 1 day of RA-treatment. In contrast, induction of several ISGs (RIG-G, MK, MXA, UBE1L, ISG15, and OAS1) was not readily evident until 10 days of RA-treatment. B, densitometric analyses of these results were displayed. To determine fold changes, the relative intensities for these expression patterns were measured and compared with GAPDH expression in vehicle-treated BEAS-2B HBE cells. “ND” indicated that fold change could not be assessed because basal expression was undetected.]

![Fig. 3. Immunoblot expression of UBE1L in some representative non–small-cell lung cancer cases is reduced as compared with adjacent normal lung tissues. UBE1L immunoblot expression was reduced in some malignant (T) as compared with adjacent normal (N) lung tissues from five non–small-cell lung cancer cases. Expression of α-tubulin was used to normalize for total protein loading in each Lane. The histopathology of these cases was as follows: large cell carcinoma (case 1), squamous cell carcinoma (cases 2 and 3), and adenocarcinoma (cases 4 and 5). In this study, an anti-UBE1L amino-terminal antibody was used to detect UBE1L protein, and similar results were obtained with the anti-UBE1L antibody that recognized the carboxyl-terminal region of UBE1L (data not shown).]
cell line, expression of representative species after acute (1 day) and prolonged (10 days) RA-treatment was assessed by RT-PCR analyses. Figure 2 shows that some species were increased acutely with RA-treatment whereas other species required longer RA-treatment for efficient induction. Interestingly, some species increased only after prolonged RA exposure included those identified previously as ISGs. The requirement for prolonged RA-treatment for induction of these species suggested that additional signaling mechanisms preceded this induction. Future work should explore the nature of these signals as well as potential cross-talk with the IFN signaling pathway.

One of these species, UBE1L, had been highlighted as a candidate tumor suppressor gene in lung carcinogenesis (12) and was shown in this study as having repressed immunoblot expression in some examined lung cancers as compared with adjacent normal lung tissue, as shown in Fig. 3. Immunohistochemical analyses revealed abundant UBE1L immunohistochemical expression in pulmonary macropathies (long arrows) as well as in the human bronchial epithelium (short arrows). B, specificity for this expression pattern was confirmed by use of blocking peptide. C, higher magnification reveals cytoplasmic, nuclear, and apical membrane immunostaining for UBE1L in the bronchial epithelium. D, absence of this immunohistochemical detection of UBE1L using preimmune sera that served as a negative control. An inverse relationship was found for immunohistochemical detection of UBE1L (panel E) and expression of cyclin D1 (arrows) in the histologically normal human bronchial epithelium (panel F). Magnifications were either 10× (panel A and B), 40× (panels C and D), or 20× (panels E and F). An anti-UBE1L amino-terminal immunoreactive antibody was used in this study with similar patterns for UBE1L immunohistochemical detection observed with the antibody that recognized the carboxyl-terminus of UBE1L (data not shown).

The current study extends prior work by showing that RA-treatment activated expression of ISGs during chemoprevention. It is perhaps not a surprise that IFN target genes are activated during cancer chemoprevention because IFNs can trigger growth suppression and cell cycle arrest (22). This would permit repair of carcinogenic damage in a manner similar to that proposed previously for retinoids, as reviewed (21). Notably, UBE1L expression has been affected by both retinoid and IFN treatments (23). Prior work revealed that RA degraded the oncogenic translocation product, promyelocytic leukemia/retinoic acid receptor α, and UBE1L, a retinoid target gene, substituted for RA in triggering this effect (16). This study extended that transfection of wild-type UBE1L because a mutant UBE1L species was unable to cause this repression. How UBE1L confers this repression of cyclin D1 is the subject of future work.

UBE1L is the activating enzyme for the IFN stimulated gene 15 kDa (ISG15; 19). ISG15 belongs to the family of ubiquitin-like proteins and is conjugated to different substrates after IFN treatment, microbial challenge, and genotoxic stress, among other stimuli, as reviewed (20). Work conducted with the ISG15 deconjugating enzyme UBP43/USP18, has linked ISG15 conjugation to increased IFN response (20). UBE1L was associated with these effects because restoration of UBE1L expression in IFN-resistant K562 cells could restore IFN-induced ISG15 conjugation and IFN-signaling, as reviewed (20). RA- and IFN-treatments are each tightly associated with reduced cellular proliferation as well as the frequent induction of apoptosis (21, 22). The proposed involvement of UBE1L expression and ISG15 conjugation in these biological effects is an attractive hypothesis to explore in future work.

A role for UBE1L in cancer chemoprevention has not been established previously. This study revealed that UBE1L and ISG15 were each induced by 10 days of RA-treatment, as shown in Fig. 2. This coordinate induction occurred along with the induction of other ISGs. Prior work has confirmed an interaction between UBE1L and ISG15 during retinoid-induced differentiation (23). A potential role for UBE1L in lung cancer chemoprevention was consistent with the inverse relationship found between immunohistochemical expression of UBE1L and cyclin D1 in the human bronchial epithelium, as shown in Fig. 4E and F. A coincidental relationship between these regulated species was excluded by the results from engineered expression of UBE1L in HBE cells where exogenous cyclin D1 was repressed in these cells. Whether UBE1L, ISG15, or the ISG15 deconjugase, UBP43, are themselves pharmacologic targets remains to be shown.

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The current study extends prior work by showing that RA-treatment activated expression of ISGs during chemoprevention. It is perhaps not a surprise that IFN target genes are activated during cancer chemoprevention because IFNs can trigger growth suppression and cell cycle arrest (22). This would permit repair of carcinogenic damage in a manner similar to that proposed previously for retinoids, as reviewed (21). Notably, UBE1L expression has been affected by both retinoid and IFN treatments (23). Prior work revealed that RA degraded the oncogenic translocation product, promyelocytic leukemia/retinoic acid receptor α, and UBE1L, a retinoid target gene, substituted for RA in triggering this effect (16). This study extended that transfection of wild-type UBE1L because a mutant UBE1L species was unable to cause this repression. How UBE1L confers this repression of cyclin D1 is the subject of future work.

UBE1L is the activating enzyme for the IFN stimulated gene 15 kDa (ISG15; 19). ISG15 belongs to the family of ubiquitin-like proteins and is conjugated to different substrates after IFN treatment, microbial challenge, and genotoxic stress, among other stimuli, as reviewed (20). Work conducted with the ISG15 deconjugating enzyme UBP43/USP18, has linked ISG15 conjugation to increased IFN response (20). UBE1L was associated with these effects because restoration of UBE1L expression in IFN-resistant K562 cells could restore IFN-induced ISG15 conjugation and IFN-signaling, as reviewed (20). RA- and IFN-treatments are each tightly associated with reduced cellular proliferation as well as the frequent induction of apoptosis (21, 22). The proposed involvement of UBE1L expression and ISG15 conjugation in these biological effects is an attractive hypothesis to explore in future work.
work by showing a cell cycle regulator was also susceptible to UBE1L-mediated repression.

In summary, retinoids are useful tools to uncover therapeutic and chemopreventive pathways. This study used an in vitro model and gene profiling experiments to discover target genes activated during HBE cell chemoprevention. Few studies have used a genomic approach to identify retinoid target genes (21). The application of gene expression arrays to highlight chemopreventive targets represents a new application for this powerful technology. The challenge for future work is to learn whether species highlighted through these gene profiling experiments are engaged during clinical cancer chemoprevention.

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Microarray Analyses Uncover UBE1L as a Candidate Target Gene for Lung Cancer Chemoprevention

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