Changes in human bladder epithelial cell gene expression associated with interstitial cystitis or antiproliferative factor treatment

Susan Keay,1,2 Francoise Seillier-Moiseiwitsch,3 Chen-Ou Zhang,1 Toby C. Chai,4 and Jialu Zhang3

1Division of Infectious Diseases, Department of Medicine, University of Maryland School of Medicine, and 2Research Service, Veterans Affairs Maryland Health Care System, Baltimore 21201; 3Department of Mathematics and Statistics, Bioinformatics Research Center, University of Maryland, Baltimore County, Baltimore 21250; and 4Division of Urology, Department of Surgery, University of Maryland School of Medicine, Baltimore, Maryland 21201

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INTERSTITIAL CYSTITIS (IC) is a debilitating chronic bladder disorder for which the etiology remains unknown. IC affects several hundred thousand people in the United States (7, 16, 37, 41), and is characterized by cystoscopic abnormalities including petechial hemorrhages called “glomerulations” and ulcers that extend into the lamina propria (21, 46). The most consistent histological abnormalities in IC include denudation or tears in the bladder epithelium, as well as thinning of the bladder epithelium to 1–2 cell layers (16, 38, 45), suggesting that the pathogenesis of IC involves abnormal epithelial proliferation.

We previously reported the presence of a factor (“antiproliferative factor” or “APF”) in the urine of IC patients that is made by explanted bladder epithelial cells from these patients and inhibits the proliferation of primary normal bladder epithelial cells in vitro (22–24). The same cells from IC patients that produce this factor also exhibit abnormally low rates of cell proliferation compared with bladder epithelial cells explanted from normal (asymptomatic) controls (26). This intrinsically low growth rate may be mediated by a complex regulation of epithelial cell growth factor production shown to occur both in IC cells and in APF-treated normal bladder cells, including decreased production of heparin-binding epidermal growth factor type I (IGF-I), and insulin-like growth factor binding protein 3 (IGFBP3) (22, 25, 27).

However, the extent to which epithelial cell growth factor production and other gene expression is altered in bladder epithelial cells from IC patients and/or in APF-treated normal bladder epithelial cells and the mechanism by which gene expression is regulated in these cells are unknown.

Microarray technology allows quantitative profiling of gene expression patterns for a large number of genes simultaneously. For our studies, it had the potential to provide insight into the following: 1) the extent to which gene expression alterations are present in IC bladder epithelial cells compared with normal cells, 2) whether specific gene expression alterations in IC cells are caused by the APF (i.e., are also seen in normal cells following APF treatment), and 3) the possible mechanism(s) by which gene expression is altered in IC bladder; growth factors; microarray analysis.

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Address for reprint requests and other correspondence: S. Keay, Veterans Administration Medical Center, Rm. 3E-184, 10 N. Greene St., Baltimore, MD 21201 (E-mail: skey@medicine.umd.edu).

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or APF-treated cells. The following studies were therefore performed using GeneFilter human “named genes” microarray filters which contain cDNAs for over 3,900 human genes including many genes for proteins related to cell proliferation (such as certain epithelial growth factors, their binding proteins, and cell cycle-related proteins).

**MATERIALS AND METHODS**

**Patients**

IC patients had previously undergone cystoscopy and fulfilled the National Institute of Diabetes and Digestive and Kidney Diseases diagnostic criteria for IC (10); age- and gender-matched controls were asymptomatic for urinary tract disease. All participants were at least 18 yr old and were enrolled in accordance with guidelines of the Institutional Review Board of the University of Maryland School of Medicine.

**Cell Culture**

Cystoscopy was performed under general anesthesia, and 4-mm² pieces of transitional epithelium with submucosal bladder tissue were obtained using rigid cold cup biopsy forceps from five IC patients and four controls for the growth of primary bladder epithelial cells, as previously described (24); in addition, epithelial cells were grown from bladder tissue obtained at autopsy from one patient who had no history of bladder disorder. The epithelial cells were propagated in DMEM-F12 (Media Tech, Herndon, VA) with 10% tissue-culture solution, and 1% L-glutamine for experiments was minimal essential medium (MEM, GIBCO-BRL; Life Technologies, Grand Island, NY). Once the cultured cells adhered to the bottom of the tissue culture flask, they were rinsed with phosphate-buffered saline (PBS), and RNA was extracted as described below. For the microarray experiments, cells were plated in Corn- ing T75 tissue culture flasks (VWR Scientific Products, Bridgeport, NJ) at a density of 1 × 10⁶ cells/ml. Once the explanted epithelial cells were confluent, serum-free Eagle’s minimal essential medium (MEM, GIBCO-BRL; Life Technologies, Grand Island, NY) containing 1% antibiotic/antimycotic solution, 1% l-glutamine, 1.0 U/ml insulin (all from Sigma, St. Louis, MO), and 5 μg/ml hEGF (R & D Systems, Minneapolis, MN) at 37°C in a 5% CO₂ atmosphere and characterized by binding of AE-1/AE-3 pancytokeratin antibodies (Signet, Dedham, MA), as previously described (24). To minimize differences in gene expression resulting from senescence in vitro, only cells with low passage numbers (passages 2–4) were used for these experiments.

For the microarray experiments, cells were plated in Corn- ing T75 tissue culture flasks (VWR Scientific Products, Bridgeport, NJ) at a density of 1 × 10⁶ cells/ml. Once the explanted epithelial cells were confluent, serum-free Eagle’s minimal essential medium (MEM, GIBCO-BRL; Life Technologies, Grand Island, NY) containing 1% antibiotic/antimycotic solution, 1% l-glutamine for experiments was added to the flasks, and cells were incubated at 37°C in a 5% CO₂ atmosphere overnight. For experiments involving HPLC-purified APF or mock APF these were added to the medium the next day, and cells further incubated by 37°C in a 5% CO₂ atmosphere. Two days later cells were rinsed with Tris-EDTA buffer pH 7.4, wrapped in plastic wrap, and harvested by scraping into PBS, and RNA was extracted as described below.

**APF Purification**

APF was harvested from the supernatant of bladder epithelial cells explanted from one of the IC patients and purified using molecular weight fractionation, ion-exchange chromatography, and reversed-phase HPLC, as previously described (22). Mock APF was prepared using supernatant of bladder epithelial cells from a normal control and the same purification procedure.

**Immunofluorescence Assay**

Cells from IC patients or normal controls (2 × 10⁶ cells/well) were plated on 8-well LabTek chamber slides (Nalge Nunc International, Naperville, IL) and grown to confluence in DMEM-F12 containing supplements as listed above. Cells were then fixed using ethanol/acetone (1:1) for 15 min at room temperature, washed three times with PBS, and incubated with the appropriate primary antibody diluted in PBS [anti-E-cadherin 1:100 (Zymed, San Francisco, CA), anti-α-catenin 1:2,000 (Sigma), anti-vimentin 1:100 (Sigma), or anti-α-2-integrin 1:100 (Sigma)] for 2 h at 37°C. After three washes with PBS, the cells were further incubated with the appropriate fluorescein-conjugated secondary antibody diluted in PBS [goat anti-mouse IgG 1:50 (Zymed), goat anti-rabbit Ig 1:100 (Southern Biotechnology Associates, Birmingham, AL), or rabbit anti-goat IgG (heavy and light chains) 1:50 (Zymed)] for 2 h at 37°C, washed five times with PBS, and examined using a Zeiss LSM510 confocal laser-scanning microscope. Negative controls for the method included cells incubated without primary or secondary antibodies, as well as cells incubated with each secondary (fluorescein-labeled) antibody alone.

**Microarray Procedure**

GeneFilters Human “Named Genes” Microarrays Release I filters spotted with 3,964 cDNAs for cellular genes (including 84 putative housekeeping genes) plus 192 control points (containing a mixture of all cDNAs, for membrane alignment) (Research Genetics, Huntsville, AL) were used for these experiments. The cDNA clones were selected from the Integrated Molecular Analysis of Genomes and their Expression (IMAGE) cDNA collection. The spots were arranged in two fields (top and bottom) containing 8 grids each, with 12 columns and 30 rows per grid. The housekeeping genes were spotted in duplicate (one in each field at the same location) and were present at various points throughout each grid except for the right-most grid. The names, identification numbers, and accession numbers for all genes are provided in the GEO web site (http://www.ncbi.nlm.nih.gov/geo/) under platform accession no. GPL262.

Membranes were prehybridized with MicroHyb solution (Research Genetics) containing 5.0 μg denatured human Cot-1 DNA and 5.0 μg poly-dA at 42°C for 2 h. RNA was isolated from the pelleted cells using RNA Trizol (GIBCO-BRL) with chloroform/isopropanol extraction, and suitability was confirmed by obtaining a gel image of the RNA as well as performing spectrophotometry at 260 and 280 nm, according to the microarray membrane manufacturer’s instructions. RNA was then primed with oligo-dT (GIBCO-BRL) (2 μg oligo-dT/μg total RNA) at 70°C for 10 min, and primed RNA was then elongated using DTT, a dNTP mixture (containing dATP, dGTP, and dTTP) (Pharmacia, Piscataway, NJ), SuperScript II reverse transcriptase (Life Technologies, Invitrogen, Carlsbad, CA), and [³²P]dCTP (New England Nuclear, Boston, MA) incubated in First Strand Buffer (GIBCO-BRL) at 37°C for 90 min. Probe was then purified by passage through a Quick Spin G50 column (Pharmacia) and boiled for 3 min prior to being diluted in fresh MicroHyb solution (containing Cot-1 DNA and poly-dA) and hybridized with the GeneFilter membrane overnight at 42°C. Following hybridization, membranes were washed with sodium chloride/sodium citrate buffer pH 7.0, placed on filter paper moistened with Tris-EDTA buffer pH 7.4, wrapped in plastic wrap, and exposed to the phosphor imaging screen using a Molecular Dynamics Storm 860 system (Molecular Dynamics, Amer- sham, Piscataway, NJ).
Table 1. Experiment 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Run</th>
<th>Membrane</th>
<th>Cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>APF</td>
<td>1</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>MockAPF</td>
<td>1</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

APF, antiproliferative factor.

Experimental Design

To assess the contribution of different potential sources of variability, such as runs, membranes, cell lines, and patients, to the observed variation in the data, three different experimental approaches were used:

Experiment 1. A normal bladder epithelial cell explant from a single normal control was treated with purified APF from a single IC patient or mock APF from an age-, race-, and gender-matched control on four separate occasions.

The comparison of the two treatments is based on duplicate membranes and a crossover design, as shown in Table 1.

Experiment 2. Normal bladder epithelial cell explants from two different normal controls were treated with purified APF from two different IC patients or mock APF from two different matched controls, as shown in Table 2.

Experiment 3. Cells from six different IC patients were run in parallel with cells from their six different age- and gender-matched controls, as shown in Table 3.

Data Acquisition

Raw images were acquired using the ImageQuant software (Molecular Dynamics), then imported and further processed using the ImaGene software (Molecular Dynamics) which subtracted background from the intensity of each spot. The background-corrected data were then normalized by dividing the intensity for each spot by the total intensity. Because the resulting values did not have a constant variance, the normalized data then underwent cube-root transformation.

For each experiment, the standard deviation was determined for each gene in each treatment group. Genes with low levels of expression (more than 2 observations with a value less than 1 standard deviation above zero) were eliminated from further analysis. Any remaining small negative values were set at zero. The final number of genes considered were experiment 1 = 3,462, experiment 2 = 3,045, and experiment 3 = 3,105.

Data Analysis

We utilized the analysis of variance (ANOVA) approach to analyze the data from the different experiments (28). Because of their duplicate spotting, housekeeping genes allowed us to estimate interactions between fields and other factors. We therefore first estimated all the possible main effects and interactions from housekeeping genes grid by grid and then used these estimates to adjust the values of the non-housekeeping genes in the same grid for the statistically significant interactions. Because housekeeping genes were located in the left seven grids but not the eighth grid, the estimates for the last grid were obtained by pooling all the housekeeping genes together. The adjusted non-housekeeping data were then analyzed using the different ANOVA models for the three experiments.

Following the ANOVA, we used a bootstrap resampling method on the model residuals to obtain the reference distribution and standard error for the estimates of the interactions between genes and treatments (gene=mock-apf) in experiments 1 and 2 and for the estimates of the interactions between genes and groups (gene=nbc-ic) in experiment 3. In the bootstrap procedure, we resampled, with replacement, the residuals from the ANOVA model that was fitted to the non-housekeeping genes. We ended up with the same number of residuals as there are data points in each experiment. Then each resampled residual was added to the sum of all estimated factors (membrane, gene, . . . ) to generate the bootstrap observations. The same ANOVA model was fitted on the bootstrap observations, and the gene+treatment interactions were reestimated. These resampling and reestimating steps were repeated 500 times to generate a reference distribution for each gene+treatment estimate. We performed separate bootstraps for each of the three datasets. Only non-housekeeping genes were involved.

We considered those estimates that were more than 2 standard errors above 0 to be significantly upregulated in IC vs. normal bladder cells or in APF-treated vs. mock APF-treated normal cells, and we considered those that were more than 2 standard errors below 0 to be significantly downregulated in the same groups.

RESULTS

Variability and Estimation of Effects

The analysis of data from experiment 1 indicated that factors such as runs, membranes, and even different fields on the same membrane contributed significantly to the variation observed in the gene expression data and that statistically significant interactions between these factors also existed. For the housekeeping genes, the treatment effect was confounded with the
field effect, which precludes us from estimating the treatment-by-field as well as the treatment-by-membrane interactions. For those genes, the field-effect estimate therefore encompasses possible treatment effects. In adjusting the non-housekeeping values for the field effect (as estimated from the housekeeping genes), we may be removing a portion of the treatment effect. Our subsequent significance analysis of the gene-by-treatment and gene-by-group interactions therefore err on the conservative side.

Let $I$ denote the spot intensity, $R$ the run effect, $G$ the gene effect, $M$ the membrane effect, $T$ the treatment effect (APF vs. mock APF), $D$ the disease effects (IC vs. normal controls), $C$ the cell-line effect, and $F$ the field effect.

Experiment 1. For the housekeeping gene model in experiment 1

$$I = R + G + M + F + M*G + R*M + R*F + G*R + F*G + F*M$$

The factors adjusted for in non-housekeeping genes were $F, M*R, F*R, F*M$.

For the non-housekeeping gene model in experiment 1

$$I = R + G + T + M + G*T + G*R + M*G$$

Experiment 2. For the housekeeping gene model in experiment 2

$$I = C + G + M + F + C(R) + C*G + C*M + M*C(R) + M*G + C*F + F*G + F*C(R) + G*C(R) + M*F$$

(Note that this is a nested model: the factor “patients” is nested within the factor “runs”.) The factors adjusted for in non-housekeeping genes were $C(R), F, M, M*C(R), F*C(R), F*M, F*C, M*C$.

For the non-housekeeping gene model in experiment 2

$$I = C + G + T + G*T + C*G + C*T$$

Experiment 3. For the housekeeping gene model in experiment 3

$$I = G + F + R + R(P) + F*R + G*R(P) + F*R(R)$$

(Note that this is a nested model: the factor “patients” is nested within the factor “runs”.) The factors adjusted for in non-housekeeping genes were $F, R, R(P), F*R(R)$.

For the non-housekeeping gene model in experiment 3

$$I = R + G + D + D*G + R*G + R*D$$

Changes in Gene Expression in IC Cells and APF-Treated Normal Bladder Epithelial Cells by Microarray Analysis

The expression of four genes that function in cell proliferation or differentiation appeared to be significantly increased in IC cells vs. normal bladder cells, as well as APF-treated vs. mock APF-treated normal bladder cells, using data from all three data sets (E-cadherin, arylsulfatase A, phosphoribosylpyrophosphate synthetase (PRPPS)-associated protein 39, and SWI/SNF complex 170-kDa subunit) (Table 4). In comparison, the expression of seven genes that function in cell proliferation or differentiation appeared to be significantly decreased in cells from IC patients or APF-treated normal cells in all three data sets (Table 5).

Table 4. Genes significantly upregulated in explanted IC vs. normal bladder epithelial cells and in APF-treated vs. mock-treated normal bladder epithelial cells

<table>
<thead>
<tr>
<th>Cluster ID</th>
<th>Accession</th>
<th>Name</th>
<th>SE from Null Hypothesis</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exp 1</td>
<td>Exp 2</td>
</tr>
<tr>
<td>Hs.82004</td>
<td>H97778</td>
<td>E-cadherin</td>
<td>3.21</td>
<td>5.59</td>
</tr>
<tr>
<td>Hs.88351</td>
<td>H65449</td>
<td>arylsulfatase A</td>
<td>2.29</td>
<td>2.27</td>
</tr>
<tr>
<td>Hs.77498</td>
<td>R44822</td>
<td>phosphoribosylpyrophosphate synthetase-associated protein 39</td>
<td>2.16</td>
<td>2.49</td>
</tr>
<tr>
<td>Hs.76116</td>
<td>H24688</td>
<td>SWI/SNF complex 1.70-kDa subunit</td>
<td>2.44</td>
<td>5.84</td>
</tr>
</tbody>
</table>

SE, standard error; IC, interstitial cystitis; APF, antiproliferative factor; Exp, experiment.
including the structural genes vimentin, α1-catenin, and α2-integrin. In addition, the expression of two cell cycle-related genes (stress-activated protein kinase JNK1 and cyclin D1) was significantly downregulated in APF-treated cells (experiments 1 and 2); although the expression of these genes also tended to be decreased in IC patients vs. controls (experiment 3), it did not reach statistical significance at greater than 2 standard errors from the mean.

Confirmation of Changes in Structural Gene Expression by Immunohistochemistry

Alterations in the expression of genes that encode structural proteins were confirmed by immunohistochemistry using primary antibodies against human E-cadherin, vimentin, α1-catenin, and α2-integrin. As shown in Fig. 1, IC patient cells (Fig. 1A) and APF-treated normal bladder epithelial cells (Fig. 1C) expressed increased E-cadherin compared with untreated (Fig. 1B) or mock APF-treated (Fig. 1D) normal cells. In comparison, IC patient cells (Fig. 1A) and APF-treated normal bladder epithelial cells (Fig. 1C) expressed decreased vimentin, α1-catenin, and α2-integrin, compared with untreated (Fig. 1B) or mock APF-treated (Fig. 1D) normal cells (Figs. 2–4, respectively). The observed fluorescence was determined to result from specific binding of the primary antibodies rather than nonspecific binding of the secondary antibodies; control cells incubated with PBS alone, or secondary antibody diluted in PBS alone, did not have any fluorescent signal.

DISCUSSION

The reported data present evidence for alterations in specific gene expression in explanted bladder epithelial cells from IC patients in vitro which are also caused by treatment with purified APF in normal bladder epithelial cells. Although IC cells and APF-treated normal cells have previously been shown to have increased or decreased production of certain growth factors, these data present the first evidence that APF can effect changes in gene expression of other cell proteins at the mRNA level. By focusing our attention on those genes whose mRNA was significantly increased or decreased in both IC cells and APF-treated normal cells, we were able to determine a pattern of gene regulation caused by the APF from a more proliferative, less differentiated and adherent phenotype, to a less proliferative, more adherent one.

The analysis of microarray data requires determining the contribution of the treatment or disease to the observed overall variation. This involves disentangling biological and treatment variation from experimental variation (caused by runs, membranes, fields, and interactions between each of these factors). One approach to this investigation is the popular “significance analysis for microarrays” (SAM) (51); however, because SAM allows adjustment for no more than one factor, it was not appropriate for our experimental designs. Instead, we chose the more classic ANOVA methods. This approach identified several genes that achieved statistically significant increases or decreases in transcription in IC cells or APF-treated normal cells compared with their controls; confirmation of increased or decreased transcription was achievable at the protein level for all four structural proteins identified, indicating that the microarray technology and the inference procedure for the microarray data were appropriate. We also considered an analysis based on “classification and regression trees” (CART) (4) for experiment 3 to identify pathways being stimulated or repressed. However, the limited number of samples available for CART analysis and the large variability in the data resulted in generation of a tree with two terminal nodes in a single step without providing much information on possible gene pathways.

Some information regarding possible pathways involved in APF’s inhibition of bladder cell proliferation...
was provided by the specific gene products that were up- or downregulated. Three of the four upregulated genes in IC cells or APF-treated normal cells (E-cadherin, PRPPS-associated protein 39, and SWI/SNF complex subunit protein) are known to be associated with inhibition of cell proliferation. E-cadherin is a transmembrane protein that functions in calcium-dependent epithelial cell-cell adhesion (3, 53). Its cytoplasmic domain is linked to actin via α- or β-catenins located in the adherens junction between cells in normal epithelium. E-cadherin is expressed throughout the normal bladder epithelium (34). Increased E-cadherin is associated with decreased cell proliferation as a result of both G1 and G2/M phase cell cycle block, and decreased E-cadherin expression and/or disruption of the catenin-cadherin complex, which often occurs in various tumor cells including bladder carcinoma, is associated with dedifferentiation and infiltration (3, 14, 34, 49, 53). PRPPS-associated protein 39 negatively regulates PRPPS activity required for cell proliferation. PRPPS functions in purine synthesis, and protein 39 binds to the catalytic subunits of this enzyme and inhibits its activity (19). Human SWI/SNF complex 170-kDa subunit is a member of a family of transcription factors involved in chromatin remodeling and suppression of c-fos oncogene factor transcription (35). SWI/SNF complex interacts with the tumor suppressor gene p53 and activates p53-mediated transcription (31). Increased SWI/SNF activity is therefore also associated with decreased cell proliferation as well as increased differentiation (9).

The fourth gene upregulated in IC cells or APF-treated normal cells, arylsulfatase A, is a lysosomal sulfotransferase whose deficiency is associated with metachromatic leukodystrophy (40), a neurological disease with decreased removal of sulfate groups from glycolipids, abnormal mucopolysaccharide storage, and altered glycosaminoglycan composition (13). Increased levels of arylsulfatase A have been seen in the urine of patients with urothelial malignancies (2), although the functional significance of this finding is unclear. Although the effect of increased arylsulfatase A on bladder epithelial cell proliferation or differentiation is also unclear, its production is of potential interest in IC in which alterations in cellular sulfated glycosaminoglycans may exist (18).

Of the four upregulated genes, three are known to have binding sites for transcription factor SP-1 (E-cadherin, arylsulfatase A, and PRPPS-associated protein 39), and the fourth is from a family of factors, some of which are also known to be positively regulated by SP-1 (the SWI/SNF transcription factor complex) (12, 20, 30, 36). Studies are therefore underway to determine whether APF stimulates SP-1-regulated gene transcription in bladder epithelial cells.

Of the downregulated genes, two of the structural genes (α2-integrin and vimentin) are particularly interesting because of their association with decreased cell differentiation and/or increased cell proliferation. Integrins are cell protein collagen/laminin receptors that link the cell to its substratum. They are made up of specific α- and β-subunits. Urothelial cells typically express α2β1 and α3β1-integrins in all layers at intercellular junctions, and in the cytoplasm of cells in the basal layer, whereas α6β4-integrin is expressed exclusively by basal cells (47). Increased expression of α2-integrin is seen following epithelial wounding, and the cytoplasmic domain appears to function in cell cycling via an MAPK pathway with stimulation of cyclin E and cyclin-dependent kinase (cdk) 2 (29, 43); increased α2 expression is therefore associated with increased cell scattering (motility) of bladder carcinoma cells (52) and is seen in undifferentiated prostate epithelial stem cells (6). Downregulation of α2-integrin in IC cells or by APF would therefore result in a less proliferative, more differentiated cell phenotype.

Vimentin is an intermediate filament subunit protein that is expressed by basal but not terminally...
differentiated bladder epithelial cells (8) and is considered to be a marker of decreased differentiation in monolayer cultures of human urothelial cells (5). Decreased expression of vimentin often correlates with increased expression of E-cadherin and vice versa, possibly via PAX-2 transcription factor regulation (50). Downregulation of vimentin would therefore also result in a more differentiated bladder epithelial cell phenotype.

The E-cadherin/catenin complex is required for tethering E-cadherin to cell actin microfilaments. β-Catenin and γ-catenin bind to the cytoplasmic domain of E-cadherin, and α-catenin then links the bound β- or γ-catenin to actin (1). However, α- and β-catenins can also be found in the cell nucleus where they have been shown to function in gene regulation in various types of epithelial cells. For example, β-catenins interact with the TCF/LEF-1 family of transcription factors, which results in increased cell proliferation and decreased cell differentiation in bowel epithelial cells (15). α-Catenins are thought to either sequester β-catenin in the cytoplasm and/or bind to β-catenin/TCF complexes and regulate their activity in the nucleus (15). But whether α-catenin plays a role in bladder cell gene regulation, and the type of effect it might have on bladder cell growth or differentiation, are unknown. It is therefore difficult to appreciate the role that α1-catenin downregulation may be playing in IC. However, the profound decrease in α-catenin, which is required for cell-cell adhesion, in IC cells or following APF treatment of normal bladder cells, may help explain the leakiness of the bladder epithelium in IC (11).

Expression of three of the four downregulated genes associated with cell proliferation (vimentin, α2-integrin, and cyclin D1) is under control of activating protein promoters (AP-1) (33, 44, 55), suggesting that the AP-1 site may also be involved in APF regulation of cell proliferation. The transcription factors that bind to the AP-1 promoters are homo- or heterodimers of members of the Jun, Fos, and ATF factor families. Stimulation of AP-1-regulated transcription is usually mediated by positive growth factor stimulation via several different signal transduction pathways, leading to phosphorylation of JNK1. JNK1 which has been activated by phosphorylation translocates to the nucleus where it phosphorylates c-Jun and increases its binding to AP-1 sites and therefore its transcriptional activity (54), resulting in increased cell proliferation. AP-1-regulated gene transcription is typically downregulated in association with cell differentiation (42).

Although both proHB-EGF and stromelysin (an enzyme that cleaves proHB-EGF to form the active growth factor) are also under control of the AP-1 promoter site (17, 39), proHB-EGF transcription was not downregulated in IC cells or following APF treatment of normal cells by microarray analysis, indicating post-transcriptional regulation of HB-EGF production by APF. A trend toward downregulation of stromelysin transcription (which was present in all three data sets but achieved significance only for experiment 3, data not shown), indicates a possible decrease in processing of the mature growth factor by stromelysin in response to APF. However, additional aspects of HB-EGF post-transcriptional processing may also be inhibited by APF to account for the profound decrease in mature HB-EGF levels in IC cells or following APF treatment of normal bladder cells.

Conclusions

Explanted bladder epithelial cells from IC patients have an altered pattern of gene expression, indicating a less proliferative phenotype than cells from normal controls. Normal bladder cells treated with purified APF have similar changes in gene expression patterns, suggesting that this factor may play an important role in the pathogenesis of this disorder. These results are compatible with the previously noted decreased proliferation rate of IC and APF-treated normal cells and indicate that the mechanism whereby APF inhibits cell proliferation may involve both downregulation of genes that stimulate cell proliferation along with upregulation of genes that inhibit cell growth.

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DISCLOSURES

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