LEDGF regulation of alcohol and aldehyde dehydrogenases in lens epithelial cells: stimulation of retinoic acid production and protection from ethanol toxicity

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VITAMIN A (retinol) plays an essential role in the growth, development, and maintenance of epithelia (36, 37). The active form of vitamin A is retinoic acid (RA), an oxidative metabolite of retinol, which serves as a ligand for nuclear RA receptors that directly regulate gene transcription. RA signaling requires the conversion of retinol to RA by a two-step process, the first step of which involves catalysis in vitro by class I and class IV alcohol dehydrogenases (ADHs). These enzymes participate in local RA synthesis in target tissues. Several studies have demonstrated in vivo roles for ADH1 and ADH4 in the first step of RA synthesis and aldehyde dehydrogenase (ALDH1) and retinaldehyde 2 (RALDH2) in the second step (6). RA is a key molecule in eye and photoreceptor development (16). It is established that RA administered in the diet replaces most of the functions of vitamin A (expt its role as a visual chromophore) in vitamin A-deficient animals (35). To date, a number of investigations have focused on the function of vitamin A. However, relatively little is known about the regulation of ADH1 and ALDH at present.

Lens epithelium-derived growth factor (LEDGF) is a novel growth and survival factor and a transcriptional activator (10, 11, 32, 33). Cells expressing higher levels of LEDGF survive remarkably well against a wide variety of stress factors (8, 19, 21, 25, 33). Notably, LEDGF mRNA and protein expression is significantly upregulated in conditions of thermal and oxidative stress (28). Our in vitro and in vivo analyses (1, 19, 21, 25, 28, 33) collectively indicate that overexpression of LEDGF provides a selective survival advantage in growing cells by blocking death pathways. In view of the adverse effects of ethanol on cells and tissues and its role in the induction of cataractogenesis (20), we were interested in determining whether LEDGF protects cells from ethanol stress. Because ADH and ALDH are involved in RA synthesis as well as detoxification (5, 9), the levels of these genes were monitored in lens epithelial cells (LECs) overexpressing LEDGF. We additionally assayed the potential of LEDGF to enhance the survival of cells exposed to ethanol and its role in RA production.

A study on the tissue distribution of mouse ADH1 and ADH4 mRNA revealed that ADH1 is primarily expressed in eye, liver, small intestine, kidney, ovary, and uterus (40), whereas class IV mRNA is primarily expressed in eye, stomach, ovary, thymus, and skin. Tissues expressing these two ADH classes possess large numbers of epithelial cells. These enzymes oxidize retinol to RA, and this process is required to regulate epithelial cell differentiation (2). There are five ADH classes in mammals including mice (14). The regulation of these genes is currently poorly understood. However, a number of binding sites and transcriptional regulatory elements have been identified in the promoter region, and a long purine-pyrimidine sequence in mouse ADH1 is suggested to play a role in gene expression (18, 38).

LEDGF, a stress-inducible transcriptional survival factor (28, 34), binds and transactivates heat shock (HSE; nGAAAn) and stress response (A/TGGGGA/T) elements in the promoters of ADH1, ADH4, and retinaldehyde 2 (RALDH2) genes. Electrophoretic mobility and supershift assays disclosed specific binding of LEDGF to nGAAAn and A/TGGGGA/T elements in these gene promoters. Transfection experiments in LECs with promoters linked to a chloramphenicol acetyltransferase (CAT) reporter gene along with LEDGF cDNA revealed higher CAT activity. RT-PCR results confirmed that LECs overexpressing LEDGF contained increased levels of ADH1, ADH4, and RALDH2 mRNA. Notably, LECs displayed higher LEDGF mRNA and protein expression during ethanol stress. Cells overexpressing LEDGF typically exhibited elevated RA levels and survived well during ethanol stress. The present findings indicate that LEDGF is one of the transcriptional activators of these genes that facilitates cellular protection against ethanol stress and plays a role in RA production.

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GST-LEDGF fusion protein was induced with isopropyl β-D-100-mm culture dish. After 10 turer protocol. Cells were plated at a density of 1 ’(GIBCO-BRL, Bethesda, MD) method according to the manufac-

formatic analysis of the 5’-flanking region of mouse ADH and ALDH gene promoters disclosed the presence of potential LEDGF binding sites, STRE, and/or HSE (see Fig. 5). Our present findings additionally confirm that LEDGF is induced by ethanol stress. Moreover, cells overexpressing LEDGF produce higher levels of RA and resist the cytotoxic effects of ethanol.

In this study, we establish that LEDGF regulates adh1, adh4, and raldh2 by binding HSE and STRE sites present in the promoters of these genes. Several reports demonstrate that the levels of ADHs and ALDHs are reliable indicators of RA expression (6, 23, 24). Our data show that LEDGF upregulates these genes. This correlation suggests that LEDGF regulates these genes, participates in the production of RA, and reduces ethanol-induced cytotoxicity.

MATERIALS AND METHODS

Construction of LEDGF in a eukaryotic expression vector. A construct containing an enhanced green fluorescent protein (EGFP) and LEDGF cDNA was generated with the “living color system” (Clontech, Palo Alto, CA) using the pEGFP-C1 vector for eukaryotic expression. This construct was used to produce eukaryotic cells that overexpress LEDGF (pEGFP-LEDGF). Cells transfected with the empty vector (pEGFP-vector) were used as controls.

Plasmid construction, expression, and purification of glutathione S-transferase-LEDGF. A fusion protein of LEDGF and glutathione S-transferase (GST) was generated by inserting the entire coding sequence of LEDGF cDNA into the BamHI and EcoRI sites of a pGEX-2T vector (Pharmacia Biotech, Piscataway, NJ). The construct was used to transform Escherichia coli (BL21) (33). Expression of the GST-LEDGF fusion protein was induced with isopropyl β-D-thiogalactopyranoside (IPTG) (7). Protein was purified with glutathione-Sepharose 4B beads (Pharmacia Biotech) following the manufacturer's protocol. The protein concentration was determined by the Bradford method (3).

Construction of LEDGF antisense. We subcloned LEDGF cDNA into a pcDNA3 vector in the reverse orientation to generate a full-length LEDGF antisense construct.

Cell culture and transfection. Mouse LECs and COS-7 cells were cultured in DMEM with 10% FCS at 37°C in an atmosphere of air-CO2 (19:1). All transfections were performed by the lipofectamine (GIBCO-BRL, Bethesda, MD) method according to the manufacturer’s protocol. Cells were plated at a density of 1 x 106 cells per 100-mm culture dish. After 10–12 h, cells were transfected with pEGFP-LEDGF, pEGFP-vector, ADH, or RALDH2-chloramphenicol acetyltransferase (CAT) constructs.

Real-time PCR and semi-quantitative reverse transcription-PCR. To monitor the level of LEDGF in LECs after ethanol treatment, total RNA was isolated with a single-step guanidium thiocyanate-phenol-chloroform extraction method (TRIZol reagent, Invitrogen) and con-

Table 1. Primers used to detect ADH1, ADH4, and RALDH2 transcripts in mLECs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH1</td>
<td>5’-GTGGAGTCCTGTTCTGGAACCTTCT-3’</td>
</tr>
<tr>
<td>ADH4</td>
<td>5’-GGATTCGATGCTCAGCCTTCCCT-3’</td>
</tr>
<tr>
<td>RALDH2</td>
<td>5’-GTTGTTTACGACAGCAGATG-3’</td>
</tr>
<tr>
<td>LEDGF</td>
<td>5’-AACACACAGGATGATTTACTAC-3’</td>
</tr>
<tr>
<td></td>
<td>5’-TTTCTATGATCAACATTTAGTTAT-3’</td>
</tr>
</tbody>
</table>

ADH, alcohol dehydrogenase; RALDH2, retinaldehyde 2; mLEC, mouse lens epithelial cell; LEDGF, lens epithelium-derived growth factor.

Table 2. Primers used in engineering ADH1, ADH4, and RALDH2-CAT constructs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH1-CAT</td>
<td>SacI site (5’-AAAAACAGGAGGATGCGAATTCT-3’)</td>
</tr>
<tr>
<td>ADH4-CAT</td>
<td>XhoI site (5’-GATGGGTCTGGAGAATCCCTTTCT-3’)</td>
</tr>
<tr>
<td>RALDH2-CAT</td>
<td>NheI site (5’-CGAAGATTTGTGATGAGAATGTTG-3’)</td>
</tr>
<tr>
<td></td>
<td>BglII site (5’-GACTGGGACGAGATGTTGATG-3’)</td>
</tr>
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CAT, chloramphenicol acetyltransferase. Underlined sequences show restriction sites.

To determine the levels of mouse ADH1, ADH4, and RALDH2 in LECs, we synthesized two pairs of primers (Table 1). β-Actin primers were used as control (28). LECs (1 x 106) in 100-mm culture dishes were transiently transfected with pEGFP-LEDGF or pEGFP vector, and mRNA was isolated after 48 h of transfection with the Micro-Fast Track kit (Invitrogen, Carlsbad, CA). cDNA synthesis was performed with a kit (Invitrogen) and used in PCR with specific primers. The following conditions were used: 94°C for 32 min, 15, 25, or 35 cycles of 94°C for 1 min, 55°C for 2 min, 72°C for 3 min, and a final extension step at 72°C for 7 min. The resultant PCR products were electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining.

Electrophoretic mobility shift assay. We selected oligonucleotides containing HSE and STRE from the RALDH2 promoter sequence. Probe I (bp positions 330 to 307), probe 2 (bp positions 429 to 406), and probe 3 (bp positions 860 to 837) were synthesized commercially. Oligonucleotides were annealed and end-labeled with [32P]-ATP by using phage T4 polynucleotide kinase. The binding reaction was performed in 20 μl of binding buffer containing 20 mM Tris-HCl (pH 8.0), 75 mM KCl, 5% glycerol, 50 μg/ml BSA, 0.025% NP-40, 1 mM EDTA, 5 mM DTT, and 1 μg of poly(dl/dC). The end-labeled probe (5 fmol) was incubated on ice for 30 min with 1,000 ng of the GST-LEDGF fusion protein. For the supershift assay, 1 μl of anti-LEDGF antibody was added to the binding reaction for an additional 30 min. In competition assays, a 1,000-fold molar excess of cold probe was added. Samples were loaded on a 5% polyacrylamide gel in 0.5× Tris-Borate-EDTA (TBE) buffer for 2 h at 10 V/cm. The gel was dried and autoradiographed.

Construction of ADH1, ADH4, and RALDH2-CAT reporter vectors. The 5’-flanking regions of mouse ADH1, ADH4, and RALDH2 gene promoters were isolated with a genomic PCR kit (Clontech Genomics) with specific primers containing restriction sites (Table 2, underlined). The resulting promoter fragments were cloned into the EcoRI sites of the TA vector (Invitrogen) and sequenced after amplification. Constructs were digested with the appropriate enzymes and ligated to pcAT BasicVector (Promega, Madison, WI). The resulting plasmid was amplified and used for the CAT assay.

Construction of LEDGF promoter-CAT reporter vector. The genomic human phagmid P1 clone (Genomic System, St. Louis, MO) was used to isolate the 5’-untranslated region (5’-UTR) of the human LEDGF gene as reported previously (31). The genomic P1 clone comprising the LEDGF gene was digested with MluI and Nhel, and converted to cDNA with Superscript II RNase H+ reverse transcriptase (28). Quantitative real-time PCR was performed with TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) in the ABI 7000 sequence detector system (Applied Biosystems). The sequences of LEDGF primers and probe used were as follows: LEDGF primers, 5’-CAGCTGCGATGAGTGAAGTTACT-3’ and 5’GGGTTGCAAAGAAATTTGGATGTT-3’; LEDGF TaqMan MGB probe, 5’-ATGGAGCTGTAAACCA-3’ (FAM dye labeled). The relative quantity of LEDGF mRNA was assessed by the comparative CT method and normalized with predeveloped TaqMan rodent GAPDH as an endogenous control reagent (Applied Biosystems) following the manufacturer’s protocol.

ADH, alcohol dehydrogenase; RALDH2, retinaldehyde 2; mLEC, mouse lens epithelial cell; LEDGF, lens epithelium-derived growth factor.
Fig. 1: A: real-time PCR discloses elevated levels of lens epithelium-derived growth factor (LEDGF) mRNA in lens epithelial cells (LECs) after ethanol exposure. LECs (1 × 10⁶) were exposed to 25, 50, and 100 mM ethanol for 24 h. Total RNA was extracted and reverse-transcribed into cDNA, and real-time PCR was performed. The mRNA level of each sample was adjusted to that of β-actin. Expression of LEDGF was significantly higher in cells treated with 50 or 100 mM ethanol compared with control (0) or 25 mM ethanol-treated cells.

B: expression of LEDGF protein in LECs after ethanol exposure. Cells were exposed to 25, 50, and 100 mM ethanol for 24 h; lane 1, 0; lane 2, 25 mM; lane 3, 50 mM; lane 4, 100 mM. After the recovery period, cell lysates were prepared and protein samples were applied to a sodium dodecyl sulfate (SDS) gel. The LEDGF band was visualized after immunostaining with anti-LEDGF antibody. The relative densities of scanned bands in the Western blot are presented as a bar graph. Gray and filled bars represent untreated (0) and ethanol-treated cells, respectively.

C: transactivation of the LEDGF promoter after ethanol exposure. a: Schematic representation of a construct containing the 5’-proximal regulatory region of the LEDGF promoter and the chloramphenicol acetyltransferase (CAT) reporter gene. A promoter fragment from MluI (−5139) and NheI (+35) was linked to the bacterial reporter gene CAT (a) and used for evaluating LEDGF promoter activity (CAT value, open bar; empty CAT vector, gray bar) in LECs (b). c: CAT values in cells transfected with empty CAT vector (gray bars) and LEDGF-promoter-CAT (filled bars) either left untreated (open bar) or treated with ethanol at different concentrations. Promoter activity of LEDGF increased significantly (P < 0.001) in cells after ethanol exposure (25, 50, or 100 mM). OD, optical density.
and a fragment encompassing −5139 and +35 bp was ligated to basic pCAT vector with the appropriate restriction enzymes. The plasmid was amplified and used in the CAT assay.

**CAT assay.** The CAT assay was performed with a CAT-ELISA kit (Roche Diagnostics). Mouse LECs were transfected with pEGFP-LEDGF, pEGFP-vector, and the ADH1, ADH4, and RALDH2-CAT constructs. After 72 h, cells were harvested and extracts were prepared and normalized to the soluble protein level. The CAT-ELISA procedure was performed according to the manufacturer’s protocol, and absorbance was measured at 405 nm with a microtiter plate ELISA reader. To monitor LEDGF promoter activity, cells were transfected with either the LEDGF-promoter-CAT construct or empty CAT vector and treated with ethanol (25, 50, and 100 mM) for 48 h or left untreated and the CAT assay was performed.

**Cell viability assay (MTS assay).** Cells overexpressing LEDGF were treated with different concentrations of ethanol (25, 50, and 100 mM), and the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt (MTS) assay was performed as described previously (7, 8).

**Statistical analysis.** The unpaired Student’s t-test was used to assess the statistical significance of the differences between the groups.

**Western analysis.** Lysates from ethanol-treated or untreated cells or those transfected with EGFP-LEDGF or EGFP vector were prepared in ice-cold RIPA buffer (1% Igepal CA-630, Sigma, St Louis, MO), 0.5% (wt/vol) sodium deoxycholate, 0.1% (wt/vol) sodium dodecyl sulfate (SDS) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma), and protease inhibitor (Complete-Mini, Roche Diagnostics). The protein concentration was determined by the Bradford method. Protein samples were subjected to SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. After blocking, blots were incubated overnight at 4°C with rabbit polyclonal anti-LEDGF COOH-terminal antibody (1:10,000 dilution) or GFP polyclonal antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were washed with PBS-Tween 20, incubated with anti-rabbit IgG labeled with horseradish peroxidase (Santa Cruz Biotechnology), and visualized by the enhanced chemiluminescence method according to the manufacturer’s protocol.

**Monitoring of endogenous RA levels in cells overexpressing LEDGF.** We used the RA response elements (RARE)-secreted alkaline phosphate (SEAP) reporter system (Clontech) to monitor the levels of RA in cells overexpressing LEDGF. The SEAP vector contains RA response elements. COS-7 cells were transfected with pEGFP-L1DGF, pEGFP-vector, and SEAP expression vector. Cells were additionally transfected with negative controls (SEAP vector lacking enhancer elements) to determine background levels of reporter gene activity. The supernatant was collected after 48 and 72 h of transfection. A fluorescent SEAP assay was performed following the manufacturer’s protocol. Placental alkaline phosphatase was used as a positive control to calculate the values.

**RESULTS**

**Ethanol treatment enhances expression and promoter activity of LEDGF in LECs.** We initially investigated whether ethanol affects the expression of LEDGF in LECs with quantitative real-time PCR, Western analysis, and a CAT reporter assay. Cells were exposed to ethanol (25, 50, or 100 mM) for 24 h. After a recovery period, total RNA was extracted from both treated and untreated groups of cells. After reverse tran-
treated with 50 or 100 mM ethanol for 24 h displayed elevated transcription (RT), cDNA was subjected to real-time PCR. Cells were subjected to RT-PCR. The resulting cDNA was amplified for 25 (lanes 2, 3, 7, and 8) and 35 (lanes 4, 5, 9, 10, 13, and 14) cycles with primers specific for ADH1 (lanes 2–5), ADH4 (lanes 7–10), or β-actin (lanes 13 and 14). Band intensities were markedly higher in LECs overexpressing LEDGF compared with those containing empty vector. Lanes 13 and 14 represent the β-actin bands (because the expression profile of β-actin mRNA remained unaltered in EGFP vector- or EGFP-LEDGF-transfected cells over 25 or 35 PCR cycles, here we incorporate the results of 35 cycles only). Lanes 1, 6, and 12 represent the 100-bp DNA size marker. B: open and filled bars represent cells transfected with pEGFP-LEDGF and empty vector, respectively. Student’s t-test was used to assess the statistical significance between 2 groups (*P < 0.05, **P < 0.001).

LEC's overexpressing LEDGF displayed elevated levels of ADH1, ADH4, and RALDH2 mRNA. Because LEDGF protects cells against stress by transactivating genes (29), we investigated LEDGF-dependent expression of ADH and ALDH mRNA by RT-PCR. Mouse LECs were transfected with pEGFP-LEDGF or empty pEGFP vector, and semiquantitative PCR was conducted with specific primers (Table 1) to quantify the levels of ADH1 and ADH4 (Fig. 3) and RALDH2 (Fig. 4) mRNA. Our data indicate that LECs overexpressing LEDGF contain increased levels of ADH1 and ADH4 (Fig. 3, lanes 5, 8, and 10) and RALDH2 (Fig. 4, lanes 3 and 6) compared with cells transfected with empty vector (Fig. 3, lanes 4, 7, and 9, and Fig. 4, lanes 2 and 5).

LEC's overexpressing LEDGF display elevated levels of ADH1, ADH4, and RALDH2 mRNA in LECs. A: mRNA samples from cells cotransfected with pEGFP-LEDGF (lanes 3 and 6) and empty vector (lanes 2 and 5) were subjected to RT-PCR. The resulting cDNA was amplified for 25 (lanes 2 and 3) and 35 (lanes 5 and 6) cycles with primers specific for RALDH2. Band intensities were markedly higher in LECs overexpressing LEDGF compared with cells containing the empty vector. Lanes 8 and 9 depict the β-actin bands. Filled and open bars represent cells transfected with pEGFP-LEDGF and empty vector, respectively.
or 3) and purified GST-LEDGF were mixed and incubated on ice for 1 h. Electrophoretic mobility shift assay (EMSA) revealed that LEDGF bound to these oligonucleotides to generate complexes (Fig. 6, lanes 1, 4, and 7), and the addition of a 1,000-fold molar excess of unlabeled self-competitor completely eliminated the Cm1 band (Fig. 6, lanes 3, 6, and 9), suggesting that LEDGF binding to oligonucleotides is DNA sequence specific. DNA-LEDGF complexes were further shifted to a higher molecular mass (Ss1 band; Fig. 6, lanes 2, 5, and 8) after incubation with anti-LEDGF antibody. Our results confirm that LEDGF binds to HSE and STRE elements.

**LEDGF activates ADH1, ADH4, and RALDH2 promoters.** To determine whether LEDGF binding to ADH1, ADH4, and RALDH2 promoters is functional, we next performed a CAT reporter assay. For this study, cells were cotransfected with the above-described promoters (Fig. 5) linked to the CAT reporter gene and LEDGF expression vector plasmid. Cells were additionally transfected either with pEGFP-LEDGF or empty vector, and CAT values were analyzed after 72 h. The CAT values were significantly high \( (P < 0.001) \) in cells overexpressing LEDGF compared with empty vector (Fig. 7). Our findings imply that LEDGF transactivates these promoters.

**Cells overexpressing LEDGF contain elevated levels of RA.** RA levels were monitored with the SEAP reporter system. Cells were transfected with pEGFP-LEDGF and pEGFP vector. The cells were then cotransfected with RARE-SEAP vector, and the supernatant was collected after 48 and 72 h of transfection. Alkaline phosphatase was used as a positive control to calculate the SEAP values. Higher SEAP values \( (P < 0.05) \) were evident at 48 and 72 h of transfection in cells overexpressing LEDGF compared with vector-transfected cells (Fig. 8). To determine whether this increase is, in fact, related to LEDGF, cells were transfected with a construct containing LEDGF-antisense cDNA and a SEAP assay was performed. The SEAP values decreased significantly \( (P < 0.001) \) in cells transfected with LEDGF-antisense (Fig. 8).

**DISCUSSION**

We reported previously that LEDGF is a survival factor acting at the transcriptional level that protects various types of...
cells from environmental and physiological stress (29, 30, 33). Notably, this protein is inducible by heat and oxidative stress (28). One of the basic mechanisms of the survival effect(s) of LEDGF involves binding of the protein to HSE and STRE in the promoters of stress-response genes, leading to transcriptional activation (8, 30). Here we report that LEDGF interacts directly with the promoters of adh and aldh genes containing STRE and/or HSE to regulate their transcription (Figs. 6 and 7). In addition, we establish a novel molecular link among LEDGF, ADH, and ALDH by demonstrating that LEDGF is one of the transcriptional regulators of adh1, adh4, and raldh2 genes in LECs. Our data show that LEDGF protects cells against ethanol cytotoxicity (Fig. 2C) and elevates RA levels (Fig. 8). It appears that transactivation of these genes is functionally very important for cells to overcome stress as well as for maintenance of normal cell physiology and function. These genes are well known for their involvement in cellular detoxification and regulation of RA synthesis via converting retinal to its active form, RA (6, 16). Our findings highlight the significance of the regulatory role of LEDGF in cell protection and RA signaling. RA administered in the diet can replace the nonvision functions of vitamin A in vitamin A-deficient animals, indicating that under these circumstances, RA is distributed in tissue. Similarly, we hypothesize that RA produced via ADH/ALDH elevation by LEDGF in cells restores RA deficiency and cellular damage. In this study, we focused on the potential role of LEDGF in regulating ADH and ALDH transcription that directly influences LEC protection against ethanol toxicity and RA productivity (Figs. 7 and 8). RA deficiency or excess has been linked with developmental disorders (15), because the compound is essential for vision and regulates several other processes as well as epithelial cell function. We propose that modulation of these genes is one of the major functions of LEDGF, which in turn facilitates the maintenance of homeostasis in LECs. Using the RARE-SEAP reporter system (Clontech), we investigated the potency of LEDGF in the elevation of RA levels (Fig. 8). However, the increase in RA levels was minor, perhaps because of tight regulation in LECs.

Mouse ALDH1 and RALDH2, which are expressed predominantly in retina, oxidize retinal (15). These enzymes are additionally involved in the oxidation of a wide variety of exogenous and endogenous aldehydes, which are among the most toxic of the metabolites of alcohols (15). In this report, we show that ADH1, ADH4, and RALDH2 (enzymes that function as retinol dehydrogenases in vitro) transcripts are present in significant amounts in mouse LECs overexpressing LEDGF (Figs. 3 and 4). ADH1 and ADH4 are additionally detected in a wide variety of epithelial tissues that convert retinal to RA (5, 6, 17).

Using a transfection system, we showed LEDGF-dependent elevation of RA in cells overexpressing LEDGF compared with control (Fig. 8) and LEDGF antisense-transfected cells as evidenced by the downregulated SEAP values (P < 0.001). These results suggest that LECs generate RA in the presence of LEDGF. It is reported that RA synthesis occurs locally in many
epithelial cell types throughout the body (13). Genetic studies provide evidence that adh1-, adh3-, and adh4-null mice have defects in ethanol clearance, formaldehyde toxicity, and metabolism of retinol to RA (4). A 10-fold decrease in RA production is reported in adh1/-/- knockout and adh1/4/-/- double-knockout mice (23, 24). One of the important findings of the present study is that cells overexpressing LEDGF confer resistance to ethanol stress (Fig. 2C) that is correlated with the elevated expression of adh1, adh4, and raldh2 genes by LEDGF. Similar earlier reports show that these genes are involved in alcohol detoxification and RA production (26).

Recent findings indicate that ethanol has an acute effect on lens cation homeostasis. Clinical and epidemiologic studies over the last 20 years have suggested that moderate to heavy consumption of alcohol is a risk factor for cataract (20). Excess alcohol causes a loss in lens calcium homeostasis, which may be one of the cellular mechanisms that contribute to cataract development. In our experiments, 150 and 200 mM ethanol were cytotoxic to LECs in vitro and resulted in reduced LEDGF expression (data not shown). The diminution of LEDGF expression at a higher concentration of ethanol might be a critical event in destabilizing the homeostasis of cells, resulting in loss of cellular resistance. Other studies have demonstrated that ethanol exposure causes modifications in the membrane permeability of lens that in turn induce increased calcium permeability of lens lipid membranes, resulting in pathological effects (12, 39). The fetal alcohol syndrome involves ethanol inhibition of ADH-catalyzed RA synthesis. RA additionally regulates the fibronectin gene in LECs, thus playing an important role in the functional adhesion of epithelium to the lens capsule (27). Moreover, RA inhibits the formation of mesenchymal cells, which may be activated in pathological transformations, i.e., in anterior capsular cataract from lens epithelium (22). We reported previously (29, 34) that loss of LEDGF from cells is one of the prime events of cell death during stress, whereas cells overexpressing this protein survive LEDGF from cells is one of the prime events of cell death.


RETINOL AND RETINOIC ACID METABOLISM

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