Keratinocyte Growth Factor Induces Lipogenesis in Alveolar Type II Cells through a Sterol Regulatory Element Binding Protein-1c-Dependent Pathway

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Keratinocyte growth factor (KGF) stimulates fatty acid and phospholipid synthesis in alveolar type II cells *in vitro*. KGF stimulates lipogenic enzymes, including fatty acid synthase and stearyl-CoA desaturase-1, and transcription factors involved in lipogenesis, such as sterol regulatory element binding protein (SREBP)-1c and CCAAT/enhancer binding protein (C/EBP) α and C/EBP δ . To define the role of SREBP-1c on the induction of lipogenic genes and lipogenesis by KGF in primary cultures of rat type II cells, we used adenoviral vectors to alter levels of SREBP-1c. Overexpression of a dominant-negative form of SREBP-1 decreased lipogenesis and decreased the induction of fatty acid synthase and stearyl coenzyme A desaturase-1 by KGF. Conversely, adenovirus-mediated overexpression of a constitutively active form of SREBP-1c mimicked the effect of KGF on lipogenic enzymes and lipogenesis. These data indicate that SREBP-1c is required for the stimulation of lipogenesis by KGF in the alveolar type II cells and is a key regulator of lung lipid metabolism and that expression of SREBP-1c is sufficient to induce lipogenesis in rat type II cells.

Keywords: adenovirus; fatty acid synthesis; surfactant

Keratinocyte growth factor (KGF) is expressed predominantly by mesenchymal cells and is a potent and relatively specific mitogen for epithelial cells. In the lung, KGF stimulates proliferation, lipogenesis, and surfactant production in alveolar type II cells and is a mitogen for bronchial epithelial cells (1, 2). KGF plays important roles in lung development and lung inflammation and can protect the lung against a variety of insults (3). We are interested in how KGF induces lipogenesis in type II cells *in vitro*. KGF interacts with its specific receptor KGFR, a tyrosine kinase receptor, to initiate a cascade of signaling pathways. Although the signaling pathways are multiple and are not fully defined, it is known that KGF induces rapid changes in the phosphorylation state of some signaling proteins and activates a variety of transcription factors that regulate lipogenic enzymes (4, 5).

Lipogenesis is necessary for the production of surface-active material and for cell proliferation and lung growth. The regulation of lipogenesis is a complex process. In adipocytes, there are at least three classes of transcription factors regulating lipogenesis. These are adipocyte determination differentiation factor/sterol response element binding protein (SREBP) family members, the nuclear hormone receptor peroxisome proliferator-activated receptor γ , and CCAAT/enhancer binding protein (C/EBP) family members (6, 7). Sterol regulatory element-binding proteins, consisting of SREBP-1a, SREBP-1c, and SREBP-2,

have been identified as important transcription factors regulating lipogenesis in multiple tissues (8). SREBP-1 has two isoforms, SREBP-1a and SREBP-1c, derived from a single gene through the use of alternative transcription start sites and splicing. SREBP-1 is synthesized as a 128-kD precursor protein and is bound to the endoplasmic reticulum and the nuclear envelope by SREBP cleavage-activating protein in the presence of sterol. Upon activation, the SREBP/SREBP cleavage-activating protein complex migrates to the Golgi. The active 68-kD mature SREBP protein is released by a two-step cleavage process and translocates into the nucleus where it promotes the transcription of many lipogenic enzymes (9). Genes that have been shown to be regulated at the transcriptional level by SREBPs include genes involved in fatty acid synthesis (fatty acid synthase [FAS], acetyl-CoA carboxylase, stearyl coenzyme A desaturase (SCD), and ATP citrate-lyase) and cholesterol homeostasis (LDL receptor, HMG-CoA synthase, HMG-CoA reductase, farnesyl diphosphate synthase, and squalene synthase). More than 30 genes that can be activated by SREBPs are involved in the synthesis and uptake of cholesterol, fatty acids, triglycerides, and phospholipids (10). Overexpression of SREBP-1c in 3T3-L1 cells results in elevated expression of adipocyte lipogenic enzymes and lipid accumulation, whereas overexpression of dominant-negative SREBP-1c (DN-SREBP-1c) inhibits the ability of preadipocytes to differentiate into adipocytes (7). In general, SREBP-1 regulates fatty acid synthesis, and SREBP-2 is responsible for cholesterol synthesis (11–14).

Lipogenesis is critical for the synthesis of pulmonary surfactant by type II cells. Just before birth, there is a large increase in phospholipid synthesis in type II cells accompanied by an increased expression of SREBP-1c and C/EBP isoforms (15). If we are to develop drugs to increase endogenous surfactant production, we need to identify therapeutic targets. The regulation of fatty acid synthesis would be one of the logical targets. We have shown previously that KGF stimulates fatty acid and phospholipid synthesis in type II cells through the induction of lipogenic enzymes, including FAS, SCD-1, SCD-2, and epidermal fatty acid-binding protein (E-FABP) (1). KGF also stimulates transcription factors, such as SREBP-1c and C/EBP α and C/EBP δ , that are involved in lipogenesis.

The function of SREBPs has been studied extensively in liver, muscle, and adipocytes where they mediate the transcriptional effects of insulin in lipogenic and glycolytic enzymes, including FAS, acetyl-CoA carboxylase, and pyruvate kinase (16–23). The regulation of SREBP-1c expression in the lung has been less extensively studied. We hypothesized that in type II cells, the transcription factor SREBP-1c mediates the transcriptional effects of KGF on lipogenic enzymes. The goals of this study were to determine if SREBP-1c can mimic the positive effect of KGF on lipogenesis in type II cells in the absence of KGF and to demonstrate that SREBP-1c is involved in the regulation of KGF-induced lipogenic genes.

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MATERIALS AND METHODS

Materials

Human recombinant KGF was purchased from R&D Systems Inc. (Minneapolis, MN). Antibodies to actin were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Monoclonal antibody to SREBP-1 was purified from culture supernatants from cell line obtained from the American Type Culture Collection (Manassas, VA). Antibody to FAS was a gift from Stuart Smith (Oakland Children's Medical Center, Oakland, CA). The adenoviruses containing LacZ, DP-SREBP-1, and DN-SREBP-1 were obtained from Jerry Schaack and Jed Friedman, respectively (University of Colorado Health Science Center, Denver, CO). All primers and probes were synthesized at National Jewish Medical and Research Center (Denver, CO). The source of most of the reagents is stated in the description of the individual methods. Dulbecco's modified Eagle's medium (DMEM) and FBS were purchased from GIBCO-BRL and Irvine Scientific (Santa Ana, CA), respectively. Penicillin and streptomycin were purchased from Sigma (St. Louis, MO).

Type II Cell Isolation and Culture

Alveolar type II cells were prepared and cultured as previously described (24). Briefly, type II cells were isolated from adult Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) by dissociation with porcine pancreatic elastase (Roche Molecular Biochemicals, Indianapolis, IN) followed by centrifuge over a discontinuous metrizamide gradient. The cells were plated on Matrigel (Collaborative Biomedical Products, Bedford, MA). Cells were plated in DMEM containing 5% rat serum (RS) (Pel-Freez Biologicals, Rogers, AR), 2 mM glutamine, 2.5 µg/ml amphotericin B, 100 µg/ml streptomycin, 100 units/ml penicillin G, and 10 µg/ml gentamycin (Life Technologies, Rockville, MD). On the next day, the medium was changed to 1% charcoal-stripped FBS (CS-FBS). The medium was changed every 48 h. The day of isolation was considered Day 0 of culture.

Treatment with Recombinant Adenoviruses

The recombinant adenovirus expressing the transcriptionally active amino-terminal fragment (amino acids 1–403) of SREBP-1c was kindly provided by Jed Friedman and was originally constructed by Foretz and colleagues (Ad-DP-SREBP-1c) (18). The recombinant adenovirus expressing dominant-negative rat SREBP-1c (Ad-DN-SREBP-1c) was constructed as previously described (21). For the adenovirus infection experiments, freshly isolated alveolar type II cells were incubated with adenovirus at various titers in DMEM containing 2% heat-inactivated FBS at 37°C for 1 h and were shaken every 15 min. The cells were plated in dishes coated with Matrigel in DMEM containing 5% RS. Cells were washed three times, and fresh DMEM containing 1% CS-FBS was added on Day 1. RNA and protein were harvested on Day 3 of culture.

Quantitative Real-Time PCR

Quantitative and real-time PCR was done as previously described (5). Alveolar type II cells cultured on Matrigel were directly lysed into 4 M guanidinium isothiocyanate, 0.5% N-laurylsarcosine, and 0.1 M β-mercaptoethanol in 25 mM sodium citrate buffer. Total RNA was treated with 4 U of RNase-free DNase I (Promega, Madison, WI) for 30 min at 37°C to remove any genomic DNA. Total RNA (2 mg) was used to synthesize cDNA with TaqMan reverse transcription reagents kit (Applied Biosystems, Branchburg, NJ) in the final volume of 100 µl according to the manufacturer's instructions. Random hexamers were used as primers in the reverse transcription reaction. The reactions were incubated at 25°C for 10 min, at 48°C for 30 min, and at 95°C for 5 min and stored at –20°C until use. Primers and probes for real-time PCR for rat SREBP-1, SCD-1, and FAS were designed using Primer Express software (1.5a; Applied Biosystems). For rat endogenous SREBP-1, 5'-TGC CCT AAG GGT CAA AAC CA-3' and 5'-TGG CGG GCA CTA CTT AGG AA-3' were selected for the forward and reverse primers, respectively. The probe sequence was 5'-CCC AGA GCC TTG CAC TTC TTG ACA CG-3'. For rat total SREBP-1 including endogenous and exogenous form mediated by adenovirus, 5'-GGT GAC ACC TGC ACC CTT GT-3' and 5'-CAC GGA CGG GTA

CAT CTTTAC A-3' were selected for the forward and reverse primers, respectively. The probe sequence is 5'-CCT CCA CCA TCG GCA CCC ACT G-3'. For rat SCD-1, sequence 5'-CCT TAA CCC TGA GAT CCC GTA GA-3' and 5'-AGC CCA TAA AAG ATT TCT GCA AA-3' were used as forward and reverse primers, respectively. The probe sequence was 5'-CTG ATG ATC CTC CAG CCA GCC TCT TG-3'. For rat E-FABP, sequence 5'-GCA ACA ACC TCA CCG TCA AA-3' and 5'-TCT CTC CCA AGG TGC AAG AAA-3' were used as forward and reverse primers, respectively. The probe sequence was 5'-ACG GTC GTC TTC ACC GTG CTC TAC-3'. For rat FAS, 5'-GGA CAT GGT CAC AGA CGA TGA C-3' and 5'-GTC GAA CTT GGA CAG ATC CTT CA-3' were used as forward and reverse primers, respectively. The probe sequence was 5'-CTT AGG CAA CCC ATA GAG CCC AGC CTT-3'. Rat cyclophilin B was used as internal control. Samples were run in triplicate. The reactions were quantitated by selection of the amplification cycle during which the PCR product of interest was detected to be accumulating logarithmically. Data were analyzed with the comparative cycle threshold (CT) method to achieve the results of relative quantitation, as recommended by the manufacturer.

Western Blot Analysis

Cells were lysed using ice-cold radioimmunoprecipitation assay buffer composed of 10 mM Tris-HCl (pH 8), 50 mM NaCl, 0.5% Na deoxycholate, and 0.2% SDS (all from Sigma-Aldrich); 1% Nonidet P-40 (United States Biochemical Corp., Cleveland, OH); 1× protease inhibitor cocktail (catalog no. 214262; PharMingen, San Diego, CA) containing benzamidine-HCl, phenanthroline, aprotinin, leupeptin, pepstatin A, and PMSF; 1× phosphatase inhibitor cocktail 2 (catalog no. P5725; Sigma-Aldrich) containing Na orthovanadate, Na molybdate, Na tartrate, and imidazole, and 25 mg/ml ALLN (N-acetyl-Leu-Leu-Nle-CHO) (Calbiochem-Novabiochem Corp., San Diego, CA). Culture dishes containing the Matrigel were placed on ice, the medium was removed, and the cells were recovered after dissolving the matrix with Cell Recovery Solution (Becton Dickinson Biosciences, San Jose, CA) and washed with cold PBS. The final cell pellet was suspended in 0.2 ml of lysis buffer on ice for 30 min. The DNA was sheared, and insoluble material was removed. One part 4× SDS-PAGE-reducing Laemmli sample buffer was added to three parts lysate. The mixture was boiled for 10 min and stored at –20°C until use. Aliquots of the lysates in reducing sample buffer were layered onto precast 8–16% Tris-glycine polyacrylamide slab gels, and the proteins were separated by electrophoresis in a Novex Xcell MiniCell (Invitrogen Corp., Carlsbad, CA). Nonspecific binding proteins on the nitrocellulose membranes were blocked by incubation of the blots in 5% nonfat dry milk in Tris-Tween buffered saline (20 mM Tris-HCl, 137 mM NaCl, and 0.05% Tween-20 [pH 7.5]) at 4°C overnight. Primary antibodies were diluted in 5% BSA or 5% nonfat dry milk in Tris-Tween buffered saline and incubated overnight at 4°C with rocking. Horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) were applied for 1 h at room temperature. Antigen-antibody complexes were detected by enhanced chemiluminescence (ECL Plus; Amersham Pharmacia Biotech, Piscataway, NJ) and exposure to Hyperfilm (Amersham Pharmacia Biotech).

Lipid Synthesis and Analysis of Newly Synthesized Lipids

Lipid synthesis in type II cells was performed as described previously (1). Briefly, type II cells infected with adenovirus containing (Ad) DP-SREBP-1c and DN-SREBP-1c at various titers were plated on 6-well plates coated with Matrigel in DMEM containing 5% RS on Day 0. On Day 1, the medium was changed to 1% CS-FBS. On Day 4, 10 µCi, 5 µCi/ml [¹⁴C] acetate (MP Biomedicals, Irvine, CA) was added to each well during the last 4 h of culture. Cells were rinsed three times with PBS and scraped off into PBS and extracted with methanol and chloroform. Total extracted lipids were dried under nitrogen, and the radioactivity of each sample was measured in a β counter. To analyze individual phospholipids, total lipids were separated by two-dimensional, thin-layer chromatography on silica gel 60 plates (EMD Chemical Inc., Gibbstown, NJ). The first solvent was 130 ml chloroform, 50 ml methanol, and 20 ml acetic acid; the second solvent was 130 ml chloroform, 50 ml methanol, and 20 ml formic acid. Individual phospholipids and

neutral lipids spots were scraped directly into scintillation vials, and their incorporation radioactivity was measured.

Statistics

For multiple treatments, the analyses were done by ANOVA with a Dunnett's test for multiple comparisons. Statistical significance was set at $P < 0.05$.

RESULTS

Overexpression of DN-SREBP-1c Abolishes the Effect of KGF on FAS and SCD-1

Previously, we demonstrated that KGF stimulates lipogenesis through induction of transcription factors including C/EBP α , C/EBP δ , and SREBP-1c and some lipogenic genes, such as FAS, SCD-1, and E-FABP (1). Because FAS and SCD-1, two key rate-limiting enzymes involved in lipogenesis, were direct target genes of SREBP-1, we hypothesized that KGF stimulates the lipogenic genes through the SREBP-1 pathway. To test the hypothesis, DN-SREBP-1c mediated with adenoviral vector was introduced into type II cells. This form of DN-SREBP-1c consists of the amino-terminal fragment of SREBP-1c (amino acid 1–403) containing an alanine mutation at amino acid 320 (12). This mutation abolishes the binding of SREBP-1c to the sterol regulatory element but dimerizes with endogenous SREBP-1c, thereby sequestering the endogenous active transcription factor and interfering with the availability of SREBP-1c. The DN-SREBP-1c was incorporated into an adenoviral vector to infect type II cells in primary culture efficiently.

We first measured the mRNA level by real-time RT-PCR using primers designed to target endogenous SREBP-1 or total SREBP-1 expression, which includes endogenous and Ad-DN-SREBP-1c. Expression of the endogenous SREBP-1 was not affected by Ad-DN-SREBP-1c or the control virus containing LacZ (Figure 1). However, infection of type II cells with increasing titers of the adenovirus encoding DN-SREBP-1c produced a dose-dependent increase in total SREBP-1 expression, which

was due to an increasing amount of DN-SREBP-1c (Figure 1). KGF stimulated endogenous SREBP-1, confirming previous results (1).

We then used the adenovirus-mediated overexpression of DN-SREBP-1c to test the specific involvement of SREBP-1c in the control of lipogenic genes by KGF in type II cells. We first measured the mRNA levels of SCD-1 and FAS. KGF stimulates the expression of these two genes, but inhibiting endogenous SREBP-1c activity by DN-SREBP-1c abolishes the effect of KGF on these lipogenic genes (Figures 2A and 2B). Infection of type II cells with a control virus containing LacZ has no significant effect, indicating the reduction of these genes was due to the specific effect of DN-SREBP-1c and not to the nonspecific adverse effects related to the viral infection. However, DN-SREBP-1c did not affect the induction of E-FABP by KGF in type II cells, which suggests that KGF stimulates E-FABP through a different pathway (Figure 2C). We then measured the protein level of FAS, and overexpression of DN-SREBP-1c blocked the induction of FAS by KGF in type cells (Figure 2D).

DN-SREBP-1c Inhibits Lipid Synthesis

Because overexpression of the DN-SREBP-1c inhibited the effect of KGF on FAS and SCD-1, we investigated whether the inhibition of SREBP-1c would have functional consequences and inhibit lipid synthesis. To measure lipid synthesis, cells were labeled with [14 C] acetate for 4 h before harvest, after which lipids were extracted and analyzed. In line with our results described previously, KGF stimulated lipogenesis, and overexpression of DN-SREBP-1c blocked the effect of KGF on lipogenesis, whereas infection with Ad-LacZ did not alter the KGF effect (Figure 3).

SREBP-1c Mimics the Effect of KGF on FAS and SCD-1 Gene Expression

If SREBP-1 is a major mediator of KGF stimulation on FAS and SCD-1 expression in type II cells, it should be possible to mimic the effect of KGF by overexpressing SREBP-1c. Therefore, we infected type II cells with an Ad-DP-SREBP-1c. The protein level of the SREBP-1 transgene was increased after infection compared with cells treated with control virus containing LacZ (Figure 4). This constitutively active transgene has a molecular mass of 44 kD.

Overexpression of SREBP-1c induced stimulation of FAS and E-FABP (Figure 4). We also measured the mRNA level of FAS, SCD-1, and E-FABP by real-time RT-PCR. Type II cells infected with Ad-DP-SREBP-1c increased the expression of these lipogenic genes (Figure 5). Hence, the constitutively active form of SREBP-1c mimics the effect of KGF on expression of these lipogenic genes.

SREBP-1c Mimics the Effect of KGF on Lipogenesis in Type II Cells

Because Ad-SREBP-1c stimulated the expression of FAS, SCD-1, and E-FABP, mimicking the effect of KGF, we anticipated that the overexpression of active SREBP-1c would stimulate lipid synthesis in type II cells. Overexpression of SREBP-1c mimics the effect of KGF on lipids synthesis in type II cells as measured by acetate incorporation (Figure 6). KGF and Ad-DP-SREBP-1c increased the percent incorporation into phospholipids, whereas Ad-DN-SREBP-1c inhibited the effect of KGF (Table 1). The radioactive acetate could be incorporated into neutral lipids and cholesterol or into phospholipids. In a previous study, we showed that KGF stimulated acetate incorporation in type II cells cultured with 1% CS-FBS. Although there was a marked increase in total phospholipid synthesis in this

Figure 1. Ad-DN-SREBP-1c is expressed in type II cells. Rat type II cells were isolated and infected with adenoviruses as described in MATERIALS AND METHODS. The cells were plated on Matrigel with DMEM containing 5% RS at a density of 5 million cells/well. On the next day, the medium was replaced by fresh medium containing 1% CS-FBS in the absence or presence of KGF. After 48 h, total RNA was extracted and analyzed by real-time RT-PCR for the expression of total SREBP-1, which includes endogenous SREBP-1 and Ad-DN-SREBP-1c. The primers and probes used to distinguish total and endogenous SREBP-1 are listed in MATERIALS AND METHODS. These figures are the combined results of four separate experiments. Endogenous expression of SREBP-1 mRNA was not altered by infection of type II cells with Ad-DN-SREBP-1c or the control virus encoding LacZ. *Significant difference from the control virus ($P < 0.05$).

Figure 2. Ad-DN-SREBP-1c inhibits induction of FAS and SCD-1 by KGF. (A–C) Type II cells were isolated and infected with adenoviruses as described in MATERIALS AND METHODS. Cells were plated on Matrigel with DMEM containing 5% RS at a density of 5 million cells/well. On the next day, the medium was replaced by fresh medium containing 1% CS-FBS in the presence or absence of KGF. After 48 h, the RNA was extracted and analyzed by real-time RT-PCR for the expression of FAS, SCD-1, and E-FABP. The figures are the combined results of four individual experiments. (D) Type II cells were treated as described previously, and protein was extracted and analyzed by Western blot. A representative Western blot of four independent experiments is shown. *Significant difference from control cultures without KGF ($P < 0.05$).

Figure 3. Ad-DN-SREBP-1c inhibits the KGF-induced acetate incorporation in type II cells. Type II cells were isolated and infected with adenovirus as described in MATERIALS AND METHODS. The cells were plated on Matrigel with DMEM containing 5% RS at a density of 5 million cells/well. On the next day, the medium was replaced by fresh medium containing 1% CS-FBS in the presence or absence of KGF for 3 d. Incorporation of [14 C] acetate was measured during the last 4 h of culture. Each condition was performed in triplicate. The figures demonstrate the combined results of four individual experiments. *Significant difference from control cultures without KGF ($P < 0.05$).

study, there were no large changes in the types of phospholipids synthesized (1). Similarly, although there was a trend toward an increase in the percent incorporated into phosphatidylcholine with KGF, this did not reach statistical significance. If the data in Table 1 are combined with the rates of incorporation in Figures 3 and 6, then Ad-DP-SREBP-1c would greatly increase phosphatidylcholine synthesis, and the stimulation of phosphatidylcholine synthesis by KGF would be inhibited by Ad-DN-SREBP-1c. The dominant effect of SREBP seems to be on fatty acid synthesis and not on the regulation of enzymes that regulate individual phospholipids.

DISCUSSION

Type II cells synthesize and secrete pulmonary surface-active material, which is composed of phospholipids and associated surfactant proteins and reduces surface tension at the air/liquid interface. A reduction or inhibition of pulmonary surface-active material causes atelectasis, labored breathing, and impaired gas exchange in neonatal and adult forms of respiratory distress syndrome. Pharmacologic means of increasing endogenous surfactant have not been achieved. One of the molecular targets for increasing endogenous surfactant would be to increase surfactant lipid synthesis.

We previously reported that KGF stimulated lipogenesis through induction of lipogenic genes such as FAS, SCD-1,

Figure 4. Ad-DP-SREBP-1c induces FAS and E-FABP in cultured type II cells. Type II cells were isolated and treated with adenovirus as described in MATERIALS AND METHODS. The cells were plated on Matrigel with DMEM containing 5% RS at a density of 5 million cells/well. The medium was changed the next day with 1% CS-FBS in the presence or absence of KGF for 2 d. Protein was extracted and analyzed by Western blot. DP-SREBP-1c has a molecular mass of 44 kDa. A representative Western blot of four independent experiments is shown.

E-FABP, and some transcription factors, including C/EBP α , C/EBP δ , and SREBP-1c, but not SREBP-1a or SREBP-2. In general, SREBP-2 is more selective for activating genes involved in cholesterol homeostasis, whereas SREBP-1 regulates fatty acid synthesis and glucose metabolism. FAS and SCD-1 have

Figure 6. Ad-DP-SREBP-1c stimulates acetate incorporation in cultured type II cells. Type II cells were isolated and treated with adenovirus as described in MATERIALS AND METHODS. The cells were plated on Matrigel with DMEM containing 5% RS at a density of 5 million cells/well. On the next day, the medium was changed with 1% CS-FBS in the absence or presence of KGF for 3 d. Incorporation of [14 C] acetate was measured during the last 4 h of culture. The figures demonstrate the combined results of four individual experiments. *Significant difference from control cultures without KGF ($P < 0.05$).

been previously characterized as direct SREBP-1 targets. Despite the importance of KGF on the lipogenesis in type II cells and the cytoprotective effects of KGF, little is known about the transcription factors that mediate KGF stimulation in the lung.

Figure 5. Ad-DP-SREBP-1c induces lipogenic genes in cultured type II cells. Type II cells were isolated and treated with adenovirus as described in MATERIALS AND METHODS. The cells were plated on Matrigel with DMEM containing 5% RS at a density of 5 million cells/well. On the next day, the medium was changed to 1% CS-FBS in the presence or absence of KGF for 2 d. RNA was extracted and analyzed by real-time RT-PCR for the expression of FAS, SCD-1, and E-FABP. The figures demonstrate the combined results of seven individual experiments. *Significant difference from control cultures without KGF ($P < 0.05$).

TABLE 1. PERCENT DISTRIBUTION OF ACETATE INCORPORATION

	1% CS-FBS	KGF	Ad-DP-SREBP-1c	Ad-LacZ	KGF+ Ad-DN-SREBP-1c	KGF+ Ad-LacZ
Pfu/cell			13	13	30	30
Total lipids						
Phospholipids, %	73.3 ± 0.7	90.4 ± 0.5*	82.6 ± 0.5*	75.8 ± 0.3	78.8 ± 0.7*	84.4 ± 0.7*
Neutral lipids, %	26.7 ± 0.7	9.6 ± 0.5*	17.4 ± 0.5*	24.2 ± 0.3	21.2 ± 0.7*	15.6 ± 0.7*
Phospholipids						
LysoPC, %	1.2 ± 0.1	0.6 ± 0.1	1.8 ± 0.3	1.2 ± 0.3	1.2 ± 0.2	0.9 ± 0.1
SM, %	6.0 ± 0.8	4.9 ± 0.3	5.3 ± 0.8	5.2 ± 0.3	5.5 ± 0.3	4.8 ± 0.2
PC, %	70.6 ± 1.1	75.5 ± 2.5	69.3 ± 1.9	70.6 ± 1.5	69.6 ± 1.5	75.9 ± 0.7
PS, %	3.2 ± 0.3	2.7 ± 0.1	4.2 ± 0.6	4.0 ± 0.2	4.3 ± 0.7	2.9 ± 0.2
PI, %	5.2 ± 0.5	4.9 ± 0.1	5.7 ± 0.3	6.6 ± 0.4	5.8 ± 0.2	5.5 ± 0.3
PE, %	9.1 ± 1.1	9.6 ± 2.2	7.9 ± 0.6	8.2 ± 0.5	8.7 ± 0.3	7.0 ± 0.3
PG, %	4.8 ± 0.2	2.0 ± 0.2*	5.4 ± 0.2	5.8 ± 0.3	5.1 ± 0.6	3.0 ± 0.3*

Definitions of abbreviations: Ad-DP-SREBP-1c, adenovirus dominant-positive SREBP-1c; Ad-LacZ, adenovirus LacZ; CS-FBS, charcoal stripped FBS; KGF, keratinocyte growth factor; LysoPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

Alveolar type II cells were treated with adenovirus (Ad) containing dominant-positive (DP) and dominant-negative (DN) SREBP-1c as described in Figures 3 and 6. Incorporation of ^{14}C acetate was measured during the last 4 h. The results are expressed as percentage of total lipids or phospholipids. The data are expressed as the mean $\pm$ SE for four independent experiments.

* Significant difference from control cultures 1%CS-FBS ($P < 0.05$).

Based on these previous studies, we hypothesized the transcription factor SREBP-1c mediates the KGF effect on lipogenesis in type II cells.

The purpose of this study was to define the importance of SREBP-1c in the regulation of fatty acid synthesis in type II cells. Foretz (18) reported that SREBP-1c is the mediator of the effect of insulin on glucokinase in hepatocytes. Overexpression of DN-SREBP-1c mediated by adenovirus in hepatocytes markedly reduced the expression of glucokinase induced by insulin. In adipocytes and muscle cells, SREBP-1c was shown to mediate the insulin effect on hexokinase II and lipogenesis-related genes by infecting the cells with Ad-DN-SREBP-1c (20, 25). We also inhibited the endogenous SREBP-1 activity by using Ad-DN-SREBP-1c. These results indicate that inhibition of SREBP-1c blocks the effect of KGF on induction of FAS and SCD-1 and inhibits lipogenesis induced by KGF. Hence, SREBP-1c is necessary to support the KGF effect on lipogenesis in type II cells. Although these results indicate that SREBP-1c expression is required for the induction of lipogenesis, our findings do not exclude the possibility that other transcription factors, such as C/EBP α and C/EBP δ , might be important.

Overexpression of the mature form of SREBP-1 in cultured preadipocytes and livers of transgenic mice stimulates cell differentiation and the transcriptional activation of genes involved in lipid synthesis (26, 27). Expression of SREBP-1 can mimic the insulin effects on liver lipogenesis and glucose metabolism *in vivo* (28). We used an adenovirus containing this active mature form of SREBP-1c to infect type II cells and demonstrated that this transcription factor can mimic the effect of KGF on some lipogenic genes and lipogenesis in type II cells. That the mature form of SREBP-1c activates FAS and SCD-1 suggests SREBP-1c does not undergo post-translational activation such as phosphorylation (29). We cannot rule out the possibility that KGF also stimulates transcriptional activity of SREBP-1c by an additional post-translational modification. These observations with Ad-SREBP-1c on the ability of SREBP-1 to regulate lipogenesis in type II cells are also supported by our previous observations with the liver X receptor agonist T0901317 (1). This liver X receptor agonist stimulates the expression of SREBP-1, fatty acid synthesis, and acetate incorporation into lipids but not SREBP-2 or C/EBP α .

E-FABP is also an important gene involved in lipogenesis. KGF and DP-SREBP-1c can stimulate its expression in type II

cells, but DN-SREBP-1c did not abolish the induction of E-FABP by KGF, suggesting that E-FABP may be a direct target of SREBP-1 and that other transcription factors are regulators of KGF stimulation of E-FABP in type II cells. Further studies are required to test this hypothesis.

KGF stimulates the expression of SP-B and SP-C in type II cells (1). However, overexpression of SREBP-1c mediated by an adenoviral vector did not change the expression of surfactant protein SP-B or SP-C mRNA or protein levels in type II cells (data not shown). These results suggest that KGF stimulates the surfactant protein expression in type II cells through other pathways.

In summary, this work suggests that SREBP-1c is the master transcription factor for the regulation of fatty acid synthesis in type II cells. KGF stimulates lipogenesis through the induction of SREBP-1c.

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