The regulation of stearoyl-CoA desaturase (SCD), a rate-limiting enzyme in the synthesis of unsaturated fatty acids, is physiologically important because the ratio of saturated to unsaturated fatty acids is thought to control cellular functions by modulating the structural integrity and fluidity of cell membranes. Transforming growth factor-β (TGF-β), a multifunctional cytokine, increased SCD mRNA expression in cultured human retinal pigment epithelial cells. This response was elicited by all three TGF-β isoforms, β1, β2, and β3. However, SCD mRNA expression was not increased either by other members of the TGF-β family or by other growth factors or cytokines. TGF-β also increased SCD mRNA expression in several other cell lines tested. The increase in SCD mRNA expression was preceded by a marked increase in Smad2 phosphorylation in TGF-β-treated human retinal pigment epithelial cells. TGF-β did not induce SCD mRNA expression in a Smad4-deficient cell line. However, Smad4 overexpression restored the TGF-β effect in this cell line. Moreover, TGF-β-induced SCD mRNA expression was effectively blocked by the overexpression of Smad7, an inhibitory Smad. Thus, a TGF-β signal transduction pathway involving Smad proteins appears to regulate the cellular expression of the SCD gene, and this regulation may play an important role in lipid metabolism.

Stearyl-CoA desaturase (SCD, EC 1.14.99.5) is a rate-limiting enzyme in the biosynthesis of unsaturated fatty acids, catalyzes the desaturation of stearic acid and palmitic acid into oleic acid and palmitoleic acid, respectively (1). The oxidative reaction catalyzed by this microsomal protein requires the participation of O₂, NADPH, cytochrome b₅₆, and cytochrome b₅₇ reductase. Oleic and palmitoleic acids are the predominant unsaturated fatty acids present in fat depots and membrane phospholipids (2). The ratio of saturated to unsaturated fatty acids is thought to control the structural integrity and fluidity of membranes, which in turn modulate cell growth and differentiation (3, 4). The regulation of SCD is of physiological importance because any changes in its activity can modulate the membrane fluidity and thereby alter cellular functions. Stearoyl-CoA desaturase has been shown to be regulated by dietary factors, hormones, metals, and peroxisomal proliferators (2, 5–9). In the mouse and rat, there are two Scd genes, Scd1 and Scd2 (10, 11). Recently, a third Scd gene, Scd3, has been identified in mouse (12). Although the proteins encoded by these three genes show >87% sequence homology, they exhibit tissue-specific expression patterns, and transcriptional regulation, likely because of marked difference in their promoter sequences. However, in the human, SCD is expressed as a single gene that yields two transcripts, 3.9 and 5.2 kb in size, resulting from the alternative usage of polyadenylation sites present in the 3′-untranslated region (13). Both transcripts encode the same functionally active 359-amino acid protein with a molecular mass of 41.5 kDa. The human SCD promoter region has recently been characterized, and this gene is transcriptionally regulated by sterol regulatory binding protein, mono- and polyunsaturated fatty acids, and cholesterol (14, 15). Recently, we have shown that the expression of SCD in human retinal pigment epithelial cells is regulated by retinoic acid (16).

The retinal pigment epithelium (RPE) is a polarized multilayer of highly differentiated epithelial cells, situated between the neurosensory retina and the vascularized choroid (17). It forms part of the blood-retinal barrier and is important for homeostasis of the outer retina. RPE cells provide nutrients to the photoreceptor cells and carry out phagocytosis and degradation of rod outer segment tips undergoing circadian shedding. These cells are also able to produce a variety of cytokines, which may play a role not only in the development, differentiation, and survival of retinal cells but also in several intracellular pathological conditions (18, 19). Among these cytokines is transforming growth factor-β (TGF-β), an anti-inflammatory growth factor that plays a pivotal role in ocular diseases such as proliferative vitreoretinopathy and age-related macular degeneration (20, 21). It is thus of interest to know whether this cytokine regulates SCD expression.

The TGF-β family comprises a large group of multifunctional cytokines with widespread distribution. They have a broad range of biological effects including cell growth, differentiation, wound healing, apoptosis, immunomodulation, and stimulation of extracellular matrix formation (22, 23). There are three mammalian TGF-β-isoforms, TGF-β1, TGF-β2 and TGF-β3,
that are encoded by different genes located on different chromosomes. They propagate signals generally through two related transmembrane proteins called TGF-β receptors type I and type II (24). Signals from these cell-surface receptors, members of the serine-threonine kinase family, to the nucleus are transduced via structurally related cytoplasmic proteins called Smads (25, 26). There are three Smad subfamilies: receptor-regulated Smads (R-Smads), common mediator Smads, and inhibitory or antagonistic Smads (anti-Smads). The receptor-regulated Smads, Smad2 and Smad3, are transiently and specifically regulated by phosphorylation mediated through TGF-β receptors (27). Following phosphorylation, R-Smads form a complex with the common mediator Smad, Smad4, and then translocate into the nucleus to regulate the transcription of target genes (28–30). The antagonistic Smads, Smad6 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β1–β3, activin A, inhibin A, bone morphogenetic protein 4 (BMP-4), and anti-human TGF-β1 polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-human Smad2 and anti-human phospho-Smad2 (Ser-465/467) were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-human Smad4 and Smad7, on the other hand, inhibit the phosphorylation of R-Smads by the TGF-β receptors and prevent the association of the R-Smads with Smad4 (31, 32).

In this study we have demonstrated for the first time that SCD expression is induced by TGF-β and that this induction is mediated through a signal transduction pathway involving Smad proteins.
Enzyme activity was measured in HRPE cells in culture. Cells into a corresponding increase in SCD enzyme activity. SCD treatment with 5 ng/ml TGF-β increased to 216% after TGF-β. The increase in SCD expression was observed at the optimal TGF-β concentration of 5 ng/ml. The increase in SCD mRNA expression by TGF-β1 is completely blocked by an anti-TGF-β1 neutralizing antibody. The cells were treated in the presence or absence of the indicated concentration of the antibody preparations. TGF-β1 (5 ng/ml) was preincubated with the neutralizing antibody (2.5 and 5 μg/μl) for 30 min before addition to the cell culture medium.

Effect of TGF-β isofoms and its family members on SCD mRNA expression in HRPE cells. Cells were treated with the indicated TGF-β isoform (β1, β2, or β3), BMP-4, activin A, or inhibin A at a concentration of 5 ng/ml for 18 h. Total RNA preparations were analyzed for SCD mRNA expression by Northern blotting. Amounts of RNA loaded are comparable, as shown by the ethidium bromide staining of 28 and 18 S ribosomal RNAs. Panel A, all three TGF-β isofoms tested were equally effective in inducing SCD mRNA expression. Panel B, BMP-4, activin A, and inhibin A were not effective inducers of SCD expression compared with TGF-β.

mRNA expression were analyzed (Fig. 3). The expression of both SCD transcripts increased with increasing concentrations of TGF-β1. More than a 3-fold increase over the base line in SCD expression was observed at the optimal TGF-β1 concentration of 5 ng/ml. The increase in SCD mRNA expression induced by TGF-β1 was also time-dependent. Expression was increased within 2 h, and the maximum was observed at 18 h following treatment with TGF-β1. SCD expression was not changed in untreated cells used as controls for the various time points (data not shown).

It was of interest to see whether the TGF-β1-induced increase in SCD mRNA expression in HRPE cells is translated into a corresponding increase in SCD enzyme activity. SCD enzyme activity was measured in HRPE cells in culture. Cells were incubated with [1-14C]oleic acid for 6 h following a 12-h treatment with 5 ng/ml TGF-β1. The lipids extracted from the cells were analyzed for oleic acid formation using HPLC. As shown in Fig. 4, the oleic acid formation in HRPE cells was increased to 216% after TGF-β1 treatment. As expected, the treatment also resulted in a 4-fold increase in SCD mRNA expression. This result shows that the increased SCD mRNA expression induced by TGF-β1 in HRPE cells is associated with an increase in SCD enzyme activity.

TGF-β1 Induces SCD mRNA Expression in Several Types of Cultured Human Cells—The effect of TGF-β1 on SCD mRNA expression was studied in various types of human cells in culture (Fig. 5). Apart from HRPE cells, the increased SCD mRNA expression induced by TGF-β1 was also observed in ARPE-19, a well characterized human RPE cell line (37). We then tested cells derived from sources other than RPE. The TGF-β1-induced elevation in SCD mRNA expression was also noted in corneal epithelial cells, corneal fibroblasts, choroidal fibroblasts, and lung fibroblasts. However, there was no appreciable induction of SCD mRNA expression in human amniotic epithelial cell line (WISH), where the basal expression level was already high. Thus, the regulation of SCD mRNA expression by TGF-β1 is not limited to RPE cells but rather is present in multiple cell types.

Inhibition of TGF-β1-induced SCD mRNA Expression in HRPE Cells by Actinomycin D—The effect of actinomycin D, an intercalating transcriptional inhibitor, on TGF-β1-induced SCD mRNA expression was studied (Fig. 6). HRPE cells were treated with TGF-β1 in the presence of indicated concentrations of actinomycin D. Essentially complete inhibition of TGF-β1-induced increase in SCD mRNA expression is found even at 1 ng/ml, the lowest concentration of actinomycin D. Thus, the regulation of SCD mRNA expression by TGF-β appears to occur at the level of transcription rather than through stabilization of the message.

Increase in Smad2 Phosphorylation in HRPE Cells by TGF-β—TGF-β signals from the cell surface to the nucleus are transduced by Smads 2, 3, and 4, and phosphorylation of Smad2 is often crucial for this downstream signaling cascade (27, 28). Cell extracts from HRPE cells, treated with TGF-β (5 ng/ml) at various time points, were analyzed by Western blotting for phosphorylated Smad2 (upper panel) and total Smad2 (lower panel) using an anti-phospho-Smad2 antibody and an anti-Smad2 antibody, respectively (Fig. 7). The ~58-kDa Smad2 protein was present in both control and TGF-β-treated HRPE cells, and an increase in phosphorylated Smad2 was noticed within 20 min of TGF-β treatment. This increase in Smad2 phosphorylation was transient and lasted for up to 60 min. Thus, Smad2 phosphorylation in HRPE cells is increased shortly after TGF-β treatment, indicating that these cells can respond to the ligand through a Smad-dependent pathway.

Smad7 Overexpression Inhibits TGF-β-induced SCD mRNA Expression—Overexpression of Smad7, an inhibitory Smad,
has been shown to block TGF-β-induced cellular responses (38, 39). To demonstrate further that the increase in SCD expression occurs through a TGF-β receptor-mediated mechanism, overexpression of Smad7 was used to abrogate the induction of this message. As shown in Fig. 8A, both SCD transcripts were present in HepG2 cells, and their expression was up-regulated by TGF-β in a time-dependent manner. Smad7 overexpression was attained in these cells using an adenoviral construct. The overexpression of Smad7 was determined by Western blot using an anti-FLAG monoclonal antibody to the FLAG epitope
Regulation of Stearoyl-CoA Desaturase by TGF-β

Effect of TGF-β1 on SCD mRNA expression in various human cell cultures. HRPE cells, ARPE-19 human RPE cells, human amniotic epithelial cells (WISH), human corneal epithelial cells (HCE-T), human corneal fibroblast cells (HCRF), human choroidal fibroblast cells (MRC-5), or human lung fibroblast cells (MRC-5) were treated with TGF-β1 (5 ng/ml) for 18 h. Total RNA preparations from control (−) or treated (+) cells were analyzed by Northern blotting. TGF-β1 treatment greatly increased the expression of both SCD transcripts (5.2 and 3.9 kb) in all the cell types tested except WISH. Ethidium bromide-stained 28 and 18 S RNAs are shown to indicate that similar amounts of RNA were loaded in different lanes.

Fig. 5. Effect of TGF-β1 on SCD mRNA expression in various human cell cultures. HRPE cells, ARPE-19 human RPE cells, human amniotic epithelial cells (WISH), human corneal epithelial cells (HCE-T), human corneal fibroblast cells (HCRF), human choroidal fibroblast cells (MRC-5), or human lung fibroblast cells (MRC-5) were treated with TGF-β1 (5 ng/ml) for 18 h. Total RNA preparations from control (−) or treated (+) cells were analyzed by Northern blotting. TGF-β1 treatment greatly increased the expression of both SCD transcripts (5.2 and 3.9 kb) in all the cell types tested except WISH. Ethidium bromide-stained 28 and 18 S RNAs are shown to indicate that similar amounts of RNA were loaded in different lanes.

In the present study, we have shown for the first time that the expression of the SCD gene is regulated by TGF-β. SCD mRNA expression in cultured human RPE cells is induced by all three TGF-β isoforms found in mammals. Other TGF-β family members, however, and several other unrelated growth factors and cytokines failed to elicit this response. We further determined that the regulation of SCD mRNA expression by TGF-β is not limited to RPE cells. Several cell culture systems tested responded to TGF-β treatment by increasing the expression of SCD mRNA. The increase in SCD mRNA expression in TGF-β-treated HRPE cells was associated with an increase in SCD enzyme activity, as evidenced by the increased formation of oleic acid from stearic acid in treated cells. However, the increase in the SCD enzyme activity was much smaller than that expected from the increase in the SCD mRNA expression.

The physiological role of the regulation of SCD expression by TGF-β remains to be elucidated. TGF-β is known as a potent regulator of many biological functions including cell growth, differentiation, and apoptosis (22, 23). Interestingly, the regulation of SCD expression could also affect cellular functions. An increase in the activity of SCD, an important regulatory enzyme in the synthesis of unsaturated fatty acids, could lead to increased formation of unsaturated fatty acids, oleic acid, and palmitoleic acid (1). This in turn modulates the ratio of saturated to unsaturated fatty acids in the cell membrane.

Thus, TGF-β may play an important role in cell growth, differentiation, and apoptosis. It appears that the regulation of SCD gene expression by TGF-β is mediated through a Smad signaling pathway. Increased phosphorylation of Smad2 preceded the induction of SCD mRNA expression by TGF-β (Fig. 9A). This indicates that a signal transduction pathway involving Smad2 is likely to be necessary for the TGF-β1-induced SCD mRNA expression. If so, the overexpression of Smad4 should restore the TGF-β1 effect on SCD mRNA expression in the Smad4-deficient cell line. SCD mRNA expression was analyzed in MDA-MB-468 after overexpression of Smad4 using an adenoviral construct. Expression of exogenous Smad4 was demonstrated in infected cells by Western blot analysis using anti-FLAG antibody (data not shown). SCD mRNA expression was increased in a time-dependent manner by TGF-β in Smad4 overexpressed MDA-MB-468 cells (Fig. 9B).

DISCUSSION

In the present study, we have shown for the first time that the expression of the SCD gene is regulated by TGF-β. SCD mRNA expression in cultured human RPE cells is induced by all three TGF-β isoforms found in mammals. Other TGF-β family members, however, and several other unrelated growth factors and cytokines failed to elicit this response. We further determined that the regulation of SCD mRNA expression by TGF-β is not limited to RPE cells. Several cell culture systems tested responded to TGF-β treatment by increasing the expression of SCD mRNA. The increase in SCD mRNA expression in TGF-β-treated HRPE cells was associated with an increase in SCD enzyme activity, as evidenced by the increased formation of oleic acid from stearic acid in treated cells. However, the increase in the SCD enzyme activity was much smaller than that expected from the increase in the SCD mRNA expression.

It is interesting to note that a large increase in SCD mRNA expression observed in all-trans-retinoic acid-treated ARPE-19 cells, another human RPE cell culture system, was also not accompanied by a corresponding increase in SCD enzyme activity (16). This could be because of the translational deficiency associated with certain genes in cultured RPE cells. It has been reported that these cells under certain conditions express a large amount of RPE65 mRNA with no detectable amount of RPE65 protein (41).

The physiological role of the regulation of SCD expression by TGF-β remains to be elucidated. TGF-β is known as a potent regulator of many biological functions including cell growth, differentiation, and apoptosis (22, 23). Interestingly, the regulation of SCD expression could also affect cellular functions. An increase in the activity of SCD, an important regulatory enzyme in the synthesis of unsaturated fatty acids, could lead to increased formation of unsaturated fatty acids, oleic acid, and palmitoleic acid (1). This in turn modulates the ratio of saturated to unsaturated fatty acids in the cell membrane.

This ratio is a key regulator of cell membrane fluidity and structural integrity (3, 42). Alteration in the unsaturated fatty acid content in the membrane caused by changes in the SCD enzyme activity has been shown to control membrane fluidity (43). Cell membrane fluidity is thought to play an important role in cell growth, differentiation, and apoptosis (4, 44). Change in Scd1 expression is associated with the differentiation of mouse preadipocytes to adipocytes (45). Increased oleic acid content is thought to be responsible for the accelerated cell growth, metabolism, and cell division associated with cancer cells (46). Thus, it is possible that the effect of TGF-β on cell growth, differentiation, and apoptosis could be partly mediated through the regulation of SCD expression.

It appears that the regulation of SCD gene expression by TGF-β is mediated through a Smad signaling pathway. Increased phosphorylation of Smad2 preceded the induction of SCD mRNA expression in TGF-β-treated human RPE cells. Normally, Smad2 is predominantly localized in the cytoplasm of the unstimulated cells (47). TGF-β induces the phosphorylation of Smad2, which then forms a complex with Smad4 and subsequently translocates to the nucleus to regulate gene transcription (27, 28). Transcriptional regulation of several genes such as those for heme oxygenase-1, plasminogen activator inhibitor-1, JunB, human type VII collagen, platelet-derived growth factor-B chain, and human α2(I) procollagen by TGF-β has been shown to be mediated through a signal transduction pathway involving Smad proteins (38, 48–52).

SCD mRNA expression in human breast cancer cell line MDA-MB-468 was not induced by TGF-β treatment. The TGF-β signal transduction pathway involving Smad proteins is not functional in this Smad4-null cell line (40). It has been reported that the overexpression of Smad4 can restore the TGF-β signaling in this cell line (40, 53). The MDA-MB-468 cells overexpressing Smad4 responded to TGF-β treatment by increasing SCD mRNA expression. Several studies have demonstrated that Smad4 is necessary for the TGF-β signaling (30, 54). Thus, Smad4 appears to mediate the induction of SCD gene expression by TGF-β.

SCD expression induced by TGF-β1 in human hepatocellular carcinoma cell line, HepG2, was completely suppressed by the overexpression of Smad7. Smad7, an inhibitory Smad, has been shown to attenuate TGF-β signal transduction through an in-
tracellular negative feedback loop (32, 38, 39). Smad7 is thought to preferentially inhibit TGF-β signaling by preventing the phosphorylation of Smad2 and/or Smad3 by type I receptor (32, 55). Thus, it appears that overexpression of Smad7 suppresses TGF-β-induced SCD mRNA expression by interfering with the TGF-β signal transduction pathway involving Smad proteins.

Apart from the TGF-β-specific Smad pathway, a parallel pathway involving mitogen-activated protein kinase (MAPK) has been reported for the regulation of certain genes by TGF-β (56). However, it appears that an MAPK pathway may not mediate the regulation of SCD gene expression by TGF-β.

PD98059 and SB203580 are known inhibitors of different phosphorylation cascades in the MAPK pathway (57). Both these compounds failed to inhibit the TGF-β-induced SCD mRNA expression (data not shown).

The regulation of SCD mRNA expression by TGF-β appears to be at the level of transcription. Actinomycin D, a known transcriptional inhibitor, effectively blocked the TGF-β-induced SCD mRNA expression. The transcriptional regulation

**FIG. 6.** Inhibition of TGF-β-induced SCD mRNA expression by actinomycin D. HRPE cells were treated with 5 ng/ml TGF-β1 for 18 h in the presence or absence of actinomycin D. Total RNA preparations were analyzed by Northern blot analysis using a SCD cDNA-specific probe. Northern blot analysis of SCD mRNA expression is shown in the upper panel. Ethidium bromide-stained 28S and 18S rRNA bands are shown to indicate that similar amounts of RNA were loaded in different lanes. The chart in the lower panel shows the intensities of bands on the Northern blot as determined using a PhosphorImager.

**FIG. 7.** Phosphorylation of Smad2 by TGF-β in HRPE cells. HRPE cells were treated with 5 ng/ml TGF-β1 for indicated time periods. Cell lysates run in parallel were analyzed by Western blotting with anti-phosphorylated Smad2 antibody (upper panel) for Smad2 phosphorylation or anti-Smad2 antibody (lower panel) for protein expression. Smad2 protein (~58 kDa) was detected in both control and TGF-β1-treated cells. A marked increase in phosphorylation of Smad2 was observed from 20–60 min following TGF-β1 treatment.
Regulation of Stearoyl-CoA Desaturase by TGF-β

FIG. 8. TGF-β1-induced SCD mRNA expression in HepG2 cells is inhibited by Smad7 overexpression. Total RNA preparations from the human hepatocellular carcinoma cell line HepG2 treated with 5 ng/ml TGF-β1 for indicated time intervals were analyzed by Northern blotting using a SCD cDNA-specific probe. Ethidium bromide-stained 28 and 18 S rRNA bands are shown to indicate that similar amounts of RNA were loaded in different lanes. Panel A, induction of SCD mRNA expression in HepG2 cells by TGF-β1. Panel B, inhibition of TGF-β1-induced SCD mRNA expression in HepG2 cells overexpressing Smad7. The cells were transiently transfected with a Smad7 adenoviral construct prior to the TGF-β1 treatment as described under “Experimental Procedures.”

FIG. 9. Smad4 is involved in TGF-β1-induced SCD mRNA expression. Panel A, SCD mRNA expression in a Smad4-deficient cell line was not induced by TGF-β1 treatment. Human MDA-MB-468 cells, a breast cancer cell line deficient in Smad4, were treated with 5 ng/ml TGF-β1. Total RNA prepared at indicated time periods was analyzed by Northern blotting using a SCD cDNA-specific probe. Ethidium bromide-stained 28 and 18 S rRNA bands are shown to indicate that similar amounts of RNA were loaded in different lanes. Panel B, SCD mRNA expression in this Smad4-deficient cell line was induced by TGF-β1 following Smad4 overexpression. MDA-MB-468 cells were transiently infected with an adenoviral construct expressing Smad4 as described under “Experimental Procedures.” Total RNA preparations from cells treated with 5 ng/ml TGF-β1 for indicated time intervals were analyzed by Northern blot for SCD mRNA expression.

by TGF-β is mediated through the binding of Smad proteins to the Smad-binding elements (SBE) present in the promoter regions of target genes (48–52). The consensus sequence for SBE is the 8-base pair palindromic, GTCTAGAC (58, 59). We have identified the promoter sequence of human SCD reported by Zhang et al. (14) and Bene et al. (15) for the presence of putative SBE elements. Although the canonical SBE sequence was not detected in the SCD 5′-flanking region, a 5-base pair CAGAC sequence was found in four different positions in the −1510 to −3056 base pair region. This 5-base pair sequence, also called Smad-binding element, is often present in the proximal region of genes regulated through Smad pathway (48, 49, 58). The 5′-flanking region of SCD also contains binding regions for SP1, AP-1 family members, and TFE3 at −410, −1665, and −3391 base pairs, respectively. These transcription factors are reported to cooperate with Smad proteins during the TGF-β-induced transcriptional activation of target genes (60–62). Further investigation using promoter-reporter constructs is necessary to identify which of these putative SBE is involved in the transcriptional regulation of SCD by TGF-β.

The regulation of SCD expression by TGF-β may play an important role in the pathophysiology of the retinal pigment epithelium. TGF-β production in the eye is greatly increased during the retinal disorder known as proliferative vitreoretinopathy (20). Such an increase is also indicated in age-related macular degeneration (21). We have shown previously that retinoic acid increased the expression of SCD in human RPE cells (16). The relationship between the regulation of SCD by TGF-β and that by retinoic acid remains to be elucidated. Both TGF-β and retinoid signal transduction pathways are interrelated (63). Retinoic acid is reported to increase the expression of TGF-β and its receptors (64). The induction of a MUC4 gene expression by retinoic acid has been shown to be mediated through TGF-β (65). However, the effect of retinoic acid and TGF-β on the expression of CRBP-1 and α-smooth muscle actin in fibroblasts is independent or reciprocal, respectively (66). Both retinoic acid and TGF-β are known to regulate cell growth, differentiation, and apoptosis (23, 67). Interestingly, SCD by its ability to control the cell membrane fluidity can also regulate these processes. Thus, our results imply that SCD could act as a common factor in mediating the actions of both retinoid and TGF-β.

In summary, we have identified SCD as a gene regulated by TGF-β in human RPE cells and in several other cell lines. A signal transduction pathway involving Smad proteins appears to mediate the induction of SCD mRNA expression by TGF-β. The regulation of SCD, a key regulatory enzyme in the lipid metabolic pathway, by TGF-β, a pleiotropic cytokine, may play an important role in cellular pathophysiology and metabolism.

Acknowledgments—We thank Dr. Anita Roberts (NCI, National Institutes of Health, Bethesda, MD) for support and Dr. Joan Massague (Memorial Sloan-Kettering Cancer Center, New York, NY) for providing Smad2 and Smad4 plasmid constructs. We also thank Dr. Anupam Agarwal (University of Florida, Gainesville, FL), Michelle Mosher (NCI, National Institutes of Health, Bethesda, MD), Todd Duncan (NEI, National Institutes of Health, Bethesda, MD), and Dr. Shanyi Chen (NEI, National Institutes of Health, Bethesda, MD) for their help.

REFERENCES

1. Enoch, H. G., Catala, A., and Strittmatter, P. (1976) J. Biol. Chem. 251, 5095–5103
2. Kasturi, R., and Joshi, V. C. (1982) J. Biol. Chem. 257, 12224–12230
3. Ntambi, J. M. (1995) Prog. Lipid Res. 34, 139–150
4. Kates, M., Pugh, E. L., and Ferrante, G. (1984) in Membrane Fluidity (Kates, M., and Manson, L. A., eds) pp. 379–395, Plenum Press, New York
5. Ntambi, J. M., Sealsler, A. M., and Takova, T. (1996) Biochem. Biophys. Res. Commun. 220, 990–995
6. Waters, K. M., Miller, C. W., and Ntambi, J. M. (1996) Biochem. Biophys. Res. Commun. 233, 839–845
7. Hwang, J., and Ntambi, J. M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9443–9448
8. Kurebayashi, S., Hirose, T., Miyashita, Y., Kasayama, S., and Kishimoto, T. (1997) Diabetes 46, 2115–2118
9. Kaestner, K. H., Ntambi, J. M., Kelly, T. J., Jr., and Lane, M. D. (1989) J. Biol. Chem. 264, 14755–14761
10. Mihara, K. (1999) J. Biochem. (Tokyo) 126, 1022–1029
11. Zheng, Y., Prouty, S. M., Harman, A., Sundberg, J. P., Stenn, K. S., and Parimoo, S. (2001) Genomics 78, 182–191
12. Zhang, L., Ge, L., Parimoo, S., Stenn, K. K., and Prouty, S. M. (1999) Biochem. J. 340, 255–261
13. Zhang, L., Ge, L., Tran, T., Stenn, K., and Prouty, S. M. (2001) Biochem. J. 357, 183–193
14. Bene, H., Lasky, D., and Ntambi, J. M. (2001) Biochem. Biophys. Res. Commun. 284, 1194–1198
15. Samuel, W., Kutty, R. K., Naginensi, S., Gordon, J. S., Prouty, S. M., Chandraratna, R. A., and Wiggert, B. (2001) J. Biol. Chem. 276, 29744–29750
16. Bok, D. (1995) J. Cell Sci. 115, 189–195
17. Hicks, D., Bugra, K., Fauchex, B., Jeanry, J. C., Laurent, M., Malecze, F., Mascarelli, F., Raultis, D., Cohen, Y., and Courtois, T. (1991) in Progress in Retinal Research (Osbourn, N. N., and Chader, G. J., eds) pp. 333–374, Pergamon Press, Oxford
18. Hayashi, A., Nakae, K., Nakae, H., Ohji, M., and Tano, Y. (1996) Curr. Eye Res. 15, 497–499
19. Connors, T. B., Jr., Roberts, A. B., Sporn, M. B., Danielpour, D., Dart, L. L., Michels, R. G., de Bustros, S., Enger, C., Kato, H., Lansing, M., Hayashi, H., and Glaser, B. M. (1989) Br. J. Ophthalmol. 81, 154–162
20. Roberts, A. B., and Sporn, M. B. (1990) in Handbook of Experimental Pharmacology (Sporn, M. B., and Roberts, A. B., eds) pp. 419–472, Springer
