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Stearoyl-CoA Desaturase Is Involved in the Control of Proliferation, Anchorage-independent Growth, and Survival in Human Transformed Cells*[S]

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Saturated and monounsaturated fatty acids are the most abundant fatty acid species in mammalian organisms, and their distribution is regulated by stearoyl-CoA desaturase, the enzyme that converts saturated into monounsaturated fatty acids. A positive correlation between high monounsaturated fatty acid levels and neoplastic transformation has been reported, but little is still known about the regulation of stearoyl-CoA desaturase in cell proliferation and apoptosis, as well as in cancer development. Here we report that simian virus 40-transformed human lung fibroblasts bearing a knockdown of human stearoyl-CoA desaturase by stable antisense cDNA transfection (hSCD*as* cells) showed a considerable reduction in monounsaturated fatty acids, cholesterol, and phospholipid synthesis, compared with empty vector transfected-simian virus 40 cell line (control cells). hSCD*as* cells also exhibited high cellular levels of saturated free fatty acids and triacylglycerol. Interestingly, stearoyl-CoA desaturase-depleted cells exhibited a dramatic decrease in proliferation rate and abolition of anchorage-independent growth. Prolonged exposure to exogenous oleic acid did not reverse either the slower proliferation or loss of anchorage-independent growth of hSCD*as* cells, suggesting that endogenous synthesis of monounsaturated fatty acids is essential for rapid cell replication and invasiveness, two hallmarks of neoplastic transformation. Moreover, apoptosis was increased in hSCD*as* cells in a ceramide-independent manner. Finally, stearoyl-CoA desaturase-deficient cells were more sensitive to palmitic acid-induced apoptosis compared with control cells. Our data suggest that, by globally regulating lipid metabolism, stearoyl-CoA desaturase activity modulates cell proliferation and survival and emphasize the important role of endogenously synthesized monounsaturated fatty acids in sustaining the neoplastic phenotype of transformed cells.

Saturated (SFA)[1] and monounsaturated (MUFA) fatty acids are the most abundant fatty acids present in mammalian organisms. As building blocks of phospholipids, triacylglycerols (TAGs), and cholesteryl esters (CEs), fatty acids are fundamental constituents of membranes, vital stores of metabolic energy, and relevant mediators/signals that regulate major cellular activities. Because changing levels of SFA and MUFA can influence a broad spectrum of cellular functions, the content and distribution of SFA and MUFA within the cell must be tightly controlled. One key regulator of fatty acid composition is stearoyl-CoA desaturase (SCD), the endoplasmic reticulum-resident enzyme that catalyzes the introduction of the first double bond in the *cis*-Δ9 position of several saturated fatty acyl-CoAs, principally palmitoyl-CoA and stearoyl-CoA, to render palmitoleyl- and oleyl-CoA (1).

The presence of SCD has been described in a wide variety of organisms (2). Among the best studied mammalian models, rodents have four SCD isoforms (SCD1–4) expressed in a tissue-specific manner (3). SCD expression and activity are regulated by a large number of factors, including dietary cholesterol and fatty acids, several hormones, and growth factors (4). Most of these studies have been done using rodent models, and little is known about human SCD. Two isoforms of SCD (SCD and ACOD4) have been described for humans, both exhibiting ~85% homology with murine SCD1 (5, 6). ACOD4 is almost exclusively expressed in fetal brain, with little or no detectable expression in adult tissues (6). The SCD gene is ubiquitously expressed, although the overall level of expression differs among tissues, with high levels in brain, liver, heart, and lung (5).

Because the lipid products of SCD are used for modulating membrane structure (7, 8) and/or energy metabolism (3), SCD has been suggested to play a key role in cell proliferation and in lipid-mediated cytotoxicity. In this regard, it has been observed that SCD is a target of growth factors and hormones that regulate key cell cycle events. Retinoic acid and transforming growth factor-β are reported to regulate SCD expression in a number of untransformed human cell types (9, 10). Very recently, it was demonstrated that platelet-derived growth factor and fibroblast growth factors FGF-2 and FGF-4 up-regulate SCD expression in human fibroblasts through the activation of sterol regulatory element-binding protein 1 (SREBP-1) by phosphatidylinositol 3-kinase (11). In this work, the activation of SCD by growth factors was paralleled by an increase in the

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[S] The on-line version of this article (available at http://www.jbc.org) contains Fig. S1.

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[1] The abbreviations used are: SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; SCD, stearoyl-CoA desaturase; TAG,: triacylglycerol; CE, cholesterol ester; FFA, unesterified fatty acid; hSCD, human stearoyl-CoA desaturase; FBS, fetal bovine serum; BSA, bovine serum albumin; MEM, minimal essential medium.

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synthesis of phospholipids and cholesterol, supporting the idea that MUFA synthesis is an integral part of the program of membrane synthesis. SCD has a role in lipid-mediated apoptosis, because it has been shown that overexpression of SCD1 in Chinese hamster ovary cells protects against the cytotoxic effects of excess SFA (12).

A linkage between SCD activity and tumor cell proliferation has also been proposed. Increased expression level of SCD was found in colonic and esophageal carcinoma and in hepatocellular adenoma (13), as well as in chemically induced tumors (14). In addition, we recently reported that SV40-transformed human lung fibroblasts (SV40-WI38 cells) show a ~5-fold higher fatty acid synthesis and a ~3-fold increased protein and activity levels of SCD with respect to their parental normal WI38 cell line, and this results in an increased MUFA/SFA ratio and a more fluid cell membrane environment (15). Because SV40-WI38 cells display enhanced phospholipid synthesis together with a fast replication rate (16), the increased synthesis of MUFA in these cells may satisfy the high demand for this fatty acid species for membrane lipid synthesis.

We hypothesize that SCD, by modulating the relative levels of SFA and MUFA, is a primary regulator of essential events of lipid homeostasis, such as membrane lipid synthesis and remodeling as well as buffering signaling and/or toxic fatty acids. As such, low SCD activity would lead to reduced proliferation and/or increased cell death. To test this hypothesis, we designed a model of reduced SCD gene expression in human cells. To this end, we employed SV40-transformed human lung fibroblasts, because this cell line bears several biological and biochemical features that make these cells an advantageous model to test the effects of SCD deficiency in lipid homeostasis as well as in cell proliferation, carcinogenesis, and programmed cell death as follows: (i) SCD protein and activity levels are significantly up-regulated by SV40-transformation, leading to a high MUFA synthesis and content (15); (ii) As a human cell strain, SV40-WI38 cells contains the main SCD isoenzyme present in human cells, if not the only active isoform (5, 6, 17), which may increase the understanding of SCD functions in cell physiology; (iii) Also, these human cells possess the advantage of a well known mechanism for transformation based on the inactivation of p53 and Rb activities, a condition that is present in the most frequent human cancers (18, 19).

In the present study, we show that the stable knockdown of SCD gene expression by a cDNA antisense strategy in human neoplastic SV40-WI38 cells decreases both MUFA and phospholipid synthesis and promotes the accumulation of SFA-enriched unesterified fatty acids (FFAs) and TAG. Concomitantly, SCD deficiency leads to a slower rate of cell proliferation, a loss of anchorage-independent growth, and higher rate of ceramide-independent apoptosis. Furthermore, cells that express only low levels of SCD are more sensitive to the cytotoxic effects of exogenous palmitic acid. Because the biological phenotype of SCD-deficient cells was not reversed by the addition of exogenous oleic acid, endogenously synthesized MUFA must be essential lipid molecules to maintain cellular lipid homeostasis. We take this to suggest that SCD action is important in the physiology of proliferating cells, even in conditions of normal supply of MUFA.

EXPERIMENTAL PROCEDURES

Materials—SV40-transformed human lung fibroblasts (SV40-WI38) were obtained from the American Type Culture Collection (Manassas, VA). The human SCD cDNA fragment, corresponding to bp 278–2005 of the sequence (GenBank™, accession no. AF097514) was a kind gift of Dr. Stephen M. Prouty, R. W. Johnson Research Institute, PA, and Dr. William Samuel, National Institutes of Health. Lipofectamine™, pcDNA3 plasmid, Geneticin™, cell culture media, and other culture reagents were from Invitrogen. Ultrafiltered fetal bovine serum (FBS) was from Gensa (Buenos Aires, Argentina). Cell culture supplies were from Greiner BioOne (Frickenhausen, Germany). Restriction enzymes and other molecular biology reagents were from Promega (Madison, WI). [1-^{14}C]Stearic acid and [2-^{3}H]glycerol were from American Radiolabeled Chemicals, Inc. (St. Louis, MO). [1-^{14}C]Acetic acid, [1-^{14}C]oleic acid, [*methyl*-^{14}C]choline chloride, [γ-^{32}P]ATP, and [6-^{3}H]thymidine were from Amersham Biosciences. Fatty acid-free bovine serum albumin (BSA), mouse anti-β-actin monoclonal antibody, anti-mouse IgG peroxidase conjugate, protease inhibitors mixture, and etoposide were purchased from Sigma. Pure lipid standards were from Doosan Serdary (Yongin, Korea). Silica gel 60 chromatography plates were from Merck (Darmstadt, Germany). Analytical-grade solvents were from Carlo Erba (Milano, Italy). Hoechst 33258 was purchased from Aldrich, and CaspACE™ Assay System was from Promega. *Escherichia coli* diacylglycerol kinase was purchased from Calbiochem-EMD Biosciences (San Diego, CA).

Cell Culture—SV40-WI38 cells as well as the derived transfected cell lines were routinely cultured in bicarbonate buffered minimum essential medium with Earle's salts (MEM) supplemented with 10% heat-inactivated FBS, penicillin (100 units/ml), streptomycin (10 μg/ml), 1% non-essential amino acids, and 1% MEM vitamin solution (growing medium), at 37 °C, 5% CO_2, and 100% humidity.

Stable SCD Knockdown in SV40-transformed Cells—The human SCD cDNA fragment was subcloned into a pcDNA3 vector in an antisense orientation into EcoRI and HindIII restriction sites. The construct pcDNA3-hSCD*as* (for "human Stearoyl-CoA Desaturase antisense" cDNA) was transfected into SV40-WI38 cells using Lipofectamine™ reagent according to the manufacturer's instructions. Positively transfected cells were selected with 600 μg/ml Geneticin™ in culture medium for 14 days. Several individual cell colonies (hSCD*as*) were then isolated using cloning cylinders. Empty pcDNA3 vector was stably transfected into SV40-WI38 cells, and these cells were considered the control cell line.

Immunoblotting—Western blot analysis was performed as previously described (15). Polyclonal rabbit anti-SCD (1:500 dilution) or monoclonal mouse anti-β-actin (1:10000 dilution) were used as primary antibodies.

Lipid Extraction—Cell monolayers were washed twice with ice-cold PBS and scraped from the Petri dishes with two additions of 1 ml of ice-cold methanol. 1 ml of chloroform and 0.5 ml of distilled water were added to complete the extraction as described by Bligh and Dyer (20).

Determination of SCD Activity—Preconfluent hSCD*as* and control cells were incubated with trace amounts (2.3 μM) of [^{14}C]stearic acid (0.25 μCi/dish) in culture medium containing 0.5% BSA for 6 h. At the end of the incubation, total cell lipids were extracted as described. Total cellular lipids were saponified, and released fatty acids were then esterified with boron trifluoride in methanol for 3 h at 64 °C. The derivated methyl esters were separated by argentation TLC following the procedure of Wilson and Sargent (21), using a solvent phase consisting of hexane:ethyl ether (90:10, by vol). Pure stearic acid and oleic acid standards were run in parallel to the samples. Fatty acid spots on TLC were detected by radiometric scanning as well as by lipid staining with iodine vapors. Spots were scraped from the TLC plate and quantified with a scintillation counter. The level of [^{14}C]oleic acid produced was calculated from specific activity of [^{14}C]stearic acid and normalized to cellular protein content.

Metabolic Labeling—Preconfluent cells were incubated for up to 24 h either with 0.25 μCi/dish [^{14}C]oleic acid (2.1 μM), 0.5 μCi/dish [^{14}C]acetate (4.2 μM), or 0.25 μCi/dish [^{14}C]stearic acid (2.3 μM), in 10% FBS MEM media supplemented with 0.5% BSA. At the end of each labeling period, the radioactive medium was recovered, and cell monolayers were washed twice with 0.1% BSA in ice-cold PBS, to eliminate residual label on the cell surface. Lipid extraction was carried out as described.

Lipid Quantification and Analysis—Analysis of mass and radioactivity of neutral and polar lipids species were carried out as described by Bagnato and Igal (16). Briefly, neutral and polar lipids species were separated on silica gel 60 TLC plates using one-dimensional single development procedures. Neutral lipid separation was carried out with hexane:ethylether:acetic acid, 80:20:2 (by vol) as solvent system. Polar lipid species were resolved in a solvent system consisting of chloroform: methanol:ammonium hydroxide:water, 50:37.5:3.5:2 (by vol). Pure lipid standards were seeded and run on the TLC plates in parallel to samples. For the ^{14}C labeling experiments, individual lipid spots detected by radiometric scanning were scraped into plastic vials, and radioactivity levels were determined in a liquid scintillation counter. The amount of ^{14}C tracer incorporated in each lipid class was calculated from the specific activity of each substrate and normalized to cellular protein content.

For the analysis of fatty acid composition of phospholipids and TAG,

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lipids species were resolved by TLC as stated above and scraped from chromatoplates, and fatty acid methyl esters from each lipid were obtained as described above. Methyl esters of fatty acids were analyzed by gas chromatography in a Hewlett Packard HP6890 GC apparatus, fitted with a flame ionization detector, and equipped with an Omega-Wax 250 capillary column. Chromatographic peaks were identified by comparison of their retention times with those of pure fatty acid standards, and percent distribution was calculated. Quantification of TAG and FFA mass was performed using 20 μg of heptadecanoic acid as an internal standard.

Cell Proliferation—To determine the growth rate of control and hSCD*as* cell lines, 1.6×10^5 cells were seeded in triplicate 60-mm Petri dishes. Twenty-four hours later, medium was replaced with fresh medium. Medium was then changed every 2 days. At different times up to 144 h, cells were trypsinized and counted in a hemocytometer. At all time points, cell viability was determined by trypan blue exclusion.

[^{3}H]Thymidine Incorporation into Cell DNA—The rate of DNA synthesis of both hSCD*as* and control cells was analyzed by determining the levels of [^{3}H]thymidine-labeled DNA as described by Bagnato and Igal (16). In some experiments, both groups of cells were incubated with 100 μM oleate for 72 h before the incubation with [^{3}H]thymidine.

Colony Formation Assay—Control and hSCD*as* cells were plated at 1×10^4 cells per 60-mm dishes in quadruplicate in MEM containing 10% FBS, in the presence or absence of 200 μM oleate and 0.3% (w/v) agar plated onto a bottom layer of 0.6% (w/v) agar in MEM. After 4 weeks, the presence or absence of multicellular colonies (more than eight cells) in both cell groups was observed under the microscope. For macroscopic visualization, cell colonies were stained with ethidium bromide in PBS for 2 h, and photographed under UV light.

Apoptosis Analysis I: Fragmentation of [^{3}H]Thymidine-labeled DNA—The determination of DNA fragmentation was performed essentially as described by Venable *et al.* (22). DNA samples from preconfluent hSCD*as* cells and control cells were labeled with 0.4 μCi of [^{3}H]thymidine in regular growing medium for 24 h. Medium was removed, the cell monolayers were washed twice with PBS at 37 °C, and cells were incubated in serum-free medium with or without different concentrations of palmitic acid and/or oleic acid complexed to BSA (fatty acid/BSA ratio 2:1 or 5:1) for 24 h. Next, the chase medium containing detached apoptotic cells was collected, and the [^{3}H]radioactivity was determined in a scintillation counter. Cell monolayers were lysed in PBS with 1% Triton X-100 and 0.2 μM EDTA, collected, and sedimented by centrifugation at 14,000 rpm for 15 min. Radioactivity was quantified in the supernatant, containing fragmented [^{3}H]DNA, and in the pellet containing the intact cellular DNA, which was washed and resuspended in 1% Triton X-100 and 0.2 μM EDTA. The percentage of fragmented DNA was estimated according to the following calculation: (chase medium disintegrations/min (dpm) plus supernatant dpm)/total dpm.

Apoptosis Analysis II: Detection of Nuclear Fragmentation/Condensation by Fluorescence Microscopy—The presence of nuclear condensation or fragmentation during apoptosis was examined by Hoescht 33258 staining and fluorescence microscopy following the procedure described by Kasai *et al.* (23).

Apoptosis Analysis III: Determination of Caspase-3 Activity—Control and hSCD*as* cells were grown in 60-mm dishes until 80–90% confluent. Cells were scraped from the dishes in ice-cold PBS and pelleted by centrifugation. Next, cells were resuspended in ice-cold 10 mM Tris-HCl buffer and disrupted by several cycles of freezing-thawing, followed by sonication in an ice-water bath. Caspase-3 activity was assayed in cell homogenates using a CaspACE™ Assay System (Promega) according the manufacturer's instructions. Cells treated with 40 μM etoposide were used as positive control for apoptosis (15).

Apoptosis Analysis IV: Quantitation of Ceramide Levels—To determine the content of cellular ceramide, we performed a diacylglycerol kinase assay as described by Preiss *et al.* (24) and modified by Luberto and Hannun (25). Ceramide content was normalized to cellular protein content.

Determination of Cellular Protein—Total cellular protein content was measured as described by Lowry *et al.* (26), using BSA as a standard.

Statistical Analysis—All experiments were performed in triplicate or quadruplicate unless otherwise stated. Results are presented as means ± S.D. and statistical significance of the data were determined by Student's *t* test or analysis of variance.

RESULTS

A Stable Reduction of SCD Levels Decreases the MUFA Content in Cell Lipids—We have previously reported that the oncogenic transformation of normal human fibroblast with SV40 enhances the expression of SCD, leading to a significantly high MUFA/SFA ratio in cell membrane and a more fluid membrane environment (15). Unlike normal fibroblasts, SV40-transformed cells exhibit a neoplastic behavior *in vitro*, showing a fast replication rate and loss of anchorage independent-growth (16). In the present work, we examined whether SCD is relevant for sustaining the biochemical and biological phenotype of transformed cells by generating SV40-transformed cells deficient in SCD expression. For this purpose, several clones of SCD-ablated cells were isolated from a pool of SV40-transformed fibroblasts stably transfected with a fragment of human SCD cDNA in antisense orientation. Three clonal populations of antisense SCD cDNA-transfected cells, hSCD*as*-A, hSCD*as*-C, and hSCD*as*-F, were chosen for experiments, because these clones exhibited the lowest SCD mRNA expression and enzyme activity. In these clones, SCD activity, measured by the conversion of exogenous [^{14}C]stearic acid to oleic acid, was significantly reduced compared with mock-transfected cells, although to different extents (*Fig. 1A*). The hSCD*as*-A clone showed the lowest SCD activity (~70% decrease with respect to control cells), followed by hSCD*as*-C and hSCD*as*-F cells, in which SCD activity levels were reduced by 50 and 40%, respectively, compared with control cells. As expected, the lower SCD activity was caused by a reduction of SCD protein level in the hSCD*as* cells; in our Western blot analysis of the SCD-ablated clone hSCD*as*-A, the content of SCD enzyme was decreased when compared with control levels (Fig. 1*B*). These observations indicate that the stable transfection of antisense SCD cDNA into SV40-transformed cells led to a depletion of SCD protein and a concomitant loss in Δ9-desaturating activity. Because the hSCD*as*-A clonal line exhibited the lowest SCD activity, this cell line was selected for performing most of the experiments carried out in the present work.

To determine whether the decrease in SCD activity was effectively translated into a significant change in cellular fatty acid composition, the fatty acid profile of phospholipids was analyzed (Table I*A*). As expected, MUFA species were significantly diminished in hSCD*as*-A cells as compared with the controls. Palmitoleic acid (16:1*n*-7) was reduced by 56%, whereas oleic acid (18: 1*n*-9) and *cis*-vaccenic acid (18:1*n*-7) decreased by 14 and 23%, respectively. Stearic acid (18:0) levels increased by 43% in the SCD-deficient cell line with respect to mock-transfected cells. However, total palmitic acid levels were not different between the knockdown and control cells. Overall, the MUFA/SFA ratio, which indirectly expresses the rate of Δ9-desaturation, was reduced by 32% in the hSCD*as*-A line when compared with controls. Similarly, the ratio of MUFA/SFA in total lipids extracted from the hSCD*as*-C clone was reduced by 19% with respect to controls (data not shown). This indicates that the decrease in SCD activity is paralleled by an equivalent reduction in the MUFA/SFA ratio of cellular lipids. When the fatty acid profile of the main polar lipid species, phosphatidylcholine and phosphatidylethanol amine, was analyzed the changes in SFA and MUFA were similar to those seen in total phospholipids of the SCD-deficient hSCD*as*-A cell line (Table I, *B* and *C*).

SCD-deficient Cells Segregate SFA away From Phospholipids and into Neutral Lipids—It has been shown that SCD activity is mainly linked to neutral lipid metabolism in rodents (27, 28). However, recently published data suggest that SCD activation in normal and neoplastic human fibroblasts is associated with membrane lipid synthesis (11, 15). To elucidate the relationship between SCD and lipid synthesis in human cells, we investigated whether the incorporation of exogenous [^{14}C]stearic acid into cellular lipids was altered in SCD-depleted cells. For this purpose, preconfluent SCD*as*-A cell clone

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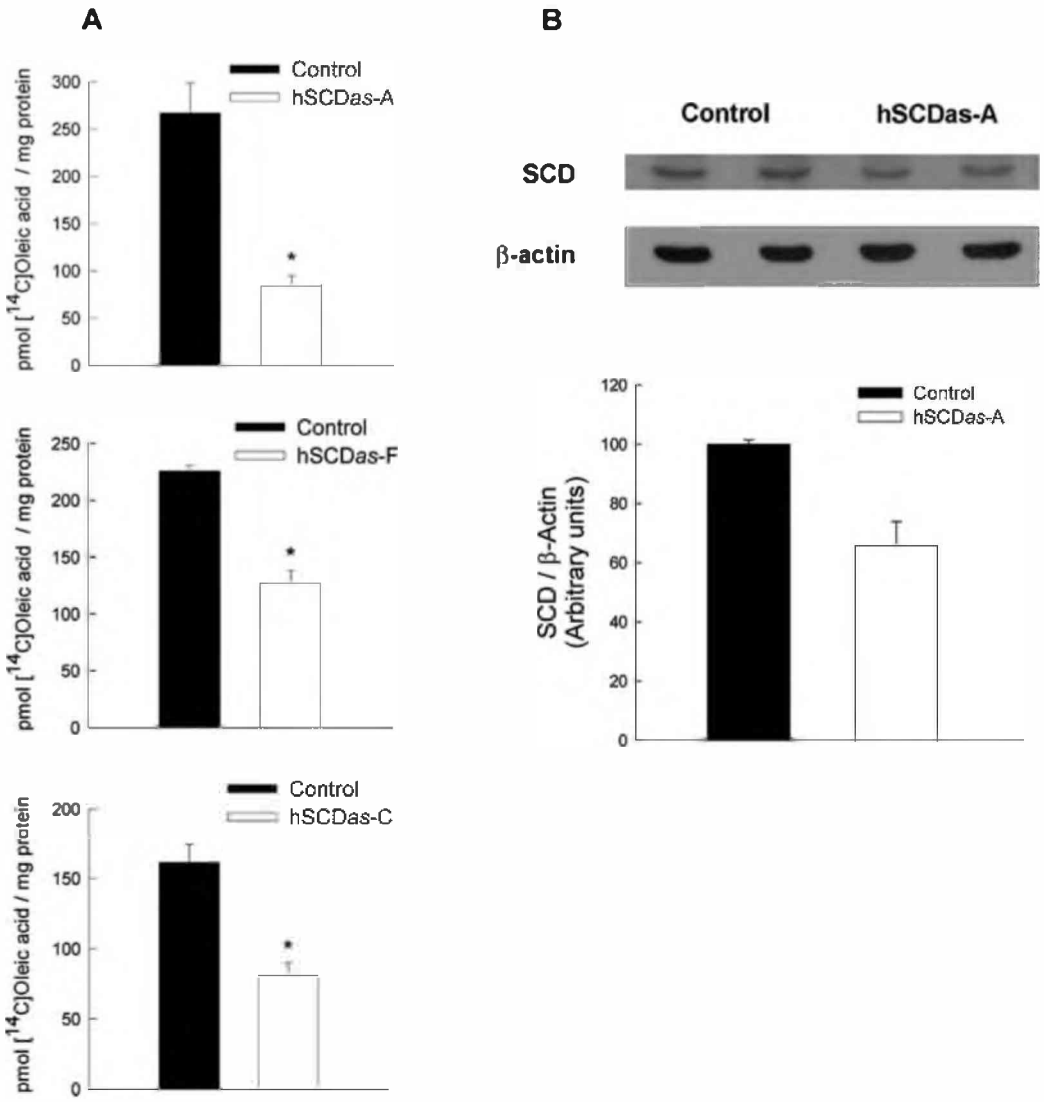

FIG. 1. **Determination of SCD activity and protein levels in hSCD*as* cells.** For the determination of desaturase activity (*A*), controls and hSCD*as* cells (clones A-, C-, and F-) were grown to 80–90% confluency and then incubated with [^{14}C]stearic acid (0.25 μCi/dish) for 6 h in 10% FBS, MEM. Conversion of [^{14}C]stearic acid into oleic acid was measured following the separation of fatty acids on TLC plates impregnated with silver nitrate and determination of radioactivity. Values represent the mean ± S.D. of three samples. *, $p < 0.01$, by Student's *t* test. For Western blot determinations (*B*), preconfluent cells from control and hSCD*as*-A groups were harvested and disrupted by sonication on ice. Proteins were resolved by SDS-PAGE, and the presence of SCD and β-actin was detected by Western blot and quantitated by densitometric analysis. SCD levels were normalized to β-actin signal.

and the mock-transfected controls were incubated with [^{14}C]stearic acid for 6 h, and levels of the major radiolabeled lipids were determined (Fig. 2*A*) As expected, most of the exogenous ^{14}C-fatty acid (~90%) was incorporated into the phospholipid fraction, with the remaining ^{14}C label incorporated into TAG and CE. In addition, the ^{14}C-fatty acid-labeled CE and PL fractions remained virtually unchanged between both SCD-deficient and control cell lines. On the other hand, we observed a 93% higher incorporation of the ^{14}C-fatty acid into TAG in SCDas-A cells compared with control cells (Fig. 2*A*). Increases of 55 and 53% in TAG labeling inhSCD*as*-C and hSCD*as*-F cells, respectively, with respect to controls were also detected (data not shown). We take these latter observations to indicate that the level of TAG formation from exogenous [^{14}C]stearic acid is inversely correlated to the degree of SCD deficiency.

The differences in the labeling of ^{14}C-lipid between hSCD*as* and control cells may be a consequence of the unequal levels of [^{14}C]stearic acid and its desaturated product oleic acid resulting from SCD-deficiency, hence we incubated the cells with exogenous [^{14}C]oleic acid for 6 h and analyzed the levels of ^{14}C-labeled lipids. As shown in Fig. 2*B*, the incorporation of oleic acid was 20% higher into total phospholipids of hSCD*as* cells compared with empty vector-transfected cells. Interestingly, [^{14}C]oleate-labeled TAG and CE fractions decreased by ~35% in hSCD*as* cells, indicating a differential acylation pattern for saturated and unsaturated fatty acids in SCD-deficient cells, with a preferential utilization of oleic acid for the formation of phospholipids.

Depletion of SCD Leads to a Decrease in de Novo Synthesis of Phospholipids and Neutral Lipids—Because the incorporation of exogenously added ^{14}C-fatty acid into both polar and neutral lipid fractions was differentially affected in hSCD*as* cells, we next examined the *de novo* synthesis of phospholipids and the major neutral lipids using [^{14}C]acetate as tracer. After incubating the cells with the tracer for 24 h, the levels of different ^{14}C-labeled lipids were analyzed. The synthesis of ^{14}C-phospholipids and [^{14}C]cholesterol was reduced by 40% and 33%, respectively, in SCD-deficient cells compared with control cells (Fig. 3, *A* and *B*). In addition, [^{14}C]acetate labeling of FFA,

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TABLE I
Distribution of fatty acids in phospholipids from control and hSCDas-A cells

Fatty acids from total cellular phospholipids (A), phosphatidylcholine (B), and phosphatidylethanolamine (C) were converted into methyl ester derivatives and analyzed by capillary GC. Part A shows percent distribution of total phospholipids; parts B and C show fatty acid desaturation indexes in phosphatidylcholine and phosphatidylethanolamine, respectively. Values express the mean ± S.D. of three samples.

Fatty acid	Control	hSCD*as* -A
%		
A)		
16:0	23.51 ± 0.59	24.45 ± 2.18
16:1*n*-7	4.77 ± 0.20	2.10 ± 0.17[a]
18:0	13.21 ± 1.18	19.00 ± 1.23[b]
18:1*n*-9	38.02 ± 0.63	32.88 ± 0.78[a]
18:1*n*-7	11.25 ± 0.05	8.69 ± 0.20[a]
18:2*n*-6	4.38 ± 0.05	6.62 ± 0.15[a]
X[c]	2.03 ± 0.08	2.46 ± 0.42
20:4*n*-6	2.84 ± 0.07	3.80 ± 0.22[b]
16:1/16:0	0.20 ± 0.01	0.09 ± 0.001[a]
16:1 + 18:1*n*-7/16:0	0.68 ± 0.01	0.44 ± 0.04[a]
18:1*n*-9/18:0	2.90 ± 0.28	1.74 ± 0.11[b]
MUFA/SFA	1.47 ± 0.06	1.01 ± 0.04[a]
B)		
16:1/16:0	0.17 ± 0.03	0.10 ± 0.01[a]
16:1 + 18:1*n*-7/16:0	0.41 ± 0.09	0.27 ± 0.01[b]
18:1*n*-9/18:0	3.62 ± 0.50	1.77 ± 0.19[a]
MUFA/SFA	0.98 ± 0.16	0.67 ± 0.03[a]
C)		
16:1/16:0	0.23 ± 0.07	0.09 ± 0.03[b]
16:1 + 18:1*n*-7/16:0	0.48 ± 0.03	0.24 ± 0.05[a]
18:1*n*-9/18:0	1.71 ± 0.09	0.82 ± 0.16[a]
MUFA/SFA	0.99 ± 0.03	0.53 ± 0.07[a]

[a] $p < 0.001$, by Student's *t* test.
[b] $p < 0.01$, by Student's *t* test.
[c] X, unidentified fatty acid.

TAG, and CE fractions was decreased by 50%, 40%, and 48%, respectively, in hSCD*as* cells relative to mock-transfected cells (Fig. 3*C*). These results were further confirmed by analyzing the formation of phospholipids and triacylglycerol using [^{3}H]glycerol as a tracer of the glycerolipid backbone. The incorporation of [^{3}H]glycerol into both phospholipids and triacylglycerol were significantly decreased in hSCD*as*-A cells (data not shown). Further studies using [^{14}C]choline as a marker indicated that the synthesis of the main membrane phospholipid in mammalian cells, phosphatidylcholine, was substantially decreased in the hSCD*as*-A cells (data not shown).

Accumulation of TAG and FFA in SCD-deficient Cells—Published reports have suggested that excess TAG content may be deleterious for survival of cardiac and pancreas β-cells (29, 30). On the other hand, it also has been proposed that TAG accumulation protects cells from toxic FFA by segregating these lipids away from apoptotic pathways (31). Having observed that the SCD-deficient hSCD*as* cells exhibit a TAG pool that is highly labeled with [^{14}C]stearic acid but also have a diminished TAG formation when traced with [^{14}C]acetate, we determined whether SCD-deficient cells had indeed altered their TAG mass in response to the observed changes in *de novo* lipid synthesis and fatty acid turnover. To assess this, we quantified the cellular TAG mass in both control and hSCD*as*-A cells. As shown in Fig. 4*A*, TAG content increased by 46% in the SCD-deficient cells with respect to controls. Interestingly, when the acyl composition of the TAG was analyzed, a ~40% increase in the content of both palmitic and stearic acids was observed (Fig. 4*A*, *right panels*). The amount of MUFA in TAG remained unchanged (data not shown). These results clearly indicate that, despite low *de novo* synthesis, the TAG pool expanded at the expense of using fatty acids, mainly SFA, likely generated by a greater phospholipid turnover.

Excess levels of FFA, mainly the saturated forms, within the cells have been proposed to induce cell death by both ceramide-dependent and -independent mechanisms (29, 32). However, the intracellular content and molecular species of FFA were not measured in these published studies. Hence, we asked whether the FFA mass and composition were altered in the hSCD*as*-A cell line relative to their controls. Stably transfected SCD-deficient cells showed a 2-fold higher FFA content compared with the corresponding mock-transfected cell line (Fig. 4*B*). Concomitantly, unesterified palmitic and stearic acid levels were 2- and 2.3-fold increased (Fig. 4*B*, *right panels*), indicating that the expansion of the FFA pool was due to the increase in the SFA fraction. Accumulation of FFA in SCD-deficient cells was further confirmed in cells incubated with [^{14}C]stearic acid for 6 h; ^{14}C-labeled-FFA increased by ~65% in hSCD*as*-A when compared with controls (Fig. 5*A*). This expansion of the FFA pool seems to be specific for SFA, because no change in [^{14}C]FFA labeling was observed following the incubation of hSCD*as*-A and control cells with [^{14}C]oleic acid for 6 h (Fig. 5*B*).

Reduced Expression of SCD Slows the Rate of Cell Proliferation and Abolishes the Anchorage-independent Growth in SV40-transformed Cells—Given our observations that diminished SCD expression lowers MUFA levels in phospholipids, as well as the rate of synthesis of membrane phospholipids and cholesterol, we addressed the question as to whether cell growth would be compromised by these alterations in lipid metabolism. First, we estimated the rate of DNA synthesis in three SCD-deficient clones and control cells by measuring [^{3}H]thymidine incorporation into total cellular DNA. As shown in Fig. 6*A*, all hSCD*as* cell clones had a significantly reduced synthesis of DNA when compared with the control cells. Remarkably, hSCD*as*-A cells, which had the lowest SCD activity, showed the most profound reduction in [^{3}H]DNA formation (62% lower than controls) and the greatest effect on reducing cell proliferation. For hSCD*as*-C and -F cell clones, which held greater levels of SCD expression, [^{3}H]DNA synthesis diminished by 49 and 47%, respectively, compared with control cells. Interestingly, the addition of 100 μM oleic acid to the growth medium for to 72 h did not change [^{3}H]thymidine incorporation into DNA of hSCD*as* cells (Fig. 6*B*), suggesting that an endogenous pool of oleic acid is required for sustaining a high DNA synthetic rate. Additionally, growth curves for hSCD*as*-A cells and the mock-transfected cells were determined (Fig. 6*C*). The empty vector-transfected SV40 cells displayed the most rapid growth rate; this rate of growth was comparable to the neoplastic growth pattern of non-transfected cells (16). In sharp contrast, the SCD-deficient cells grew at a significantly slower rate, showing increasing differences with the control cells at longer incubation times.

Because SCD deficiency dramatically decreased the proliferation rate of this neoplastic SV40-transformed cell line, we tested if other alterations in the transformed phenotype were taking place in hSCD*as* cells. Anchorage-independent growth is a typical feature of neoplastic cell transformation, hence we proceeded to analyze the ability of control and hSCD*as*-A cells to form colonies on a soft agar surface, in presence or absence of 200 μM oleic acid. Cells were seeded on agar and grown for up to 4 weeks to allow the formation of multicellular colonies. As shown in Fig. 6*D*, mock-transfected cells gave rise to numerous colonies, whereas hSCD*as*-A cells failed to produce multicellular colonies, indicating that SCD-deficiency completely suppressed anchorage-independent proliferation of the SV40-transformed cells. Interestingly, oleate treatment did not reverse the incapacity of hSCD*as*-A cells to form multicellular colonies, indicating that endogenously synthesized MUFA are required by SV40-transformed cells to sustain their capacity for anchorage-independent growth.

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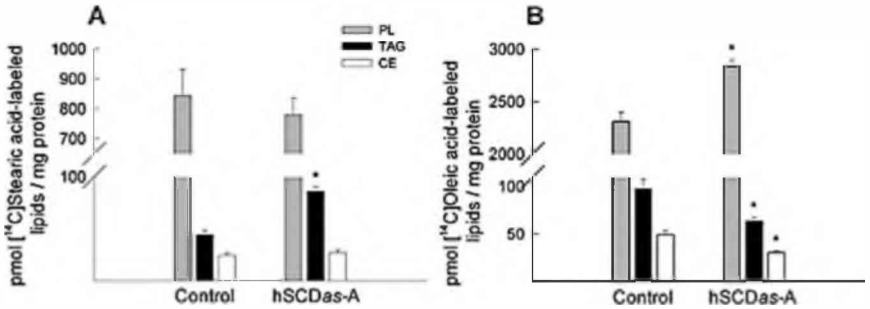

FIG. 2. **[^{14}C]Stearic acid incorporates preferentially into TAG, whereas exogenous [^{14}C]oleic acid is selectively incorporated into phospholipids of SCD-deficient cells.** hSCD*as*-A and empty-vector transfected control cells were grown until 80–90% confluent. Cells were incubated with either [^{14}C]stearic acid or [^{14}C]oleic acid for 6 h, and cell lipids were extracted and separated by TLC. Lipid spots were scraped, and their radioactivity was quantified by scintillation counting. *PL*, total phospholipids; *TAG*, triacylglycerols; *CE*, cholesterol esters. *A*, [^{14}C]stearic acid-labeled lipids; *B*, [^{14}C]oleic acid-labeled lipids. Values express the means ± S.D. of three determinations. *, $p < 0.01$, by Student's *t* test.

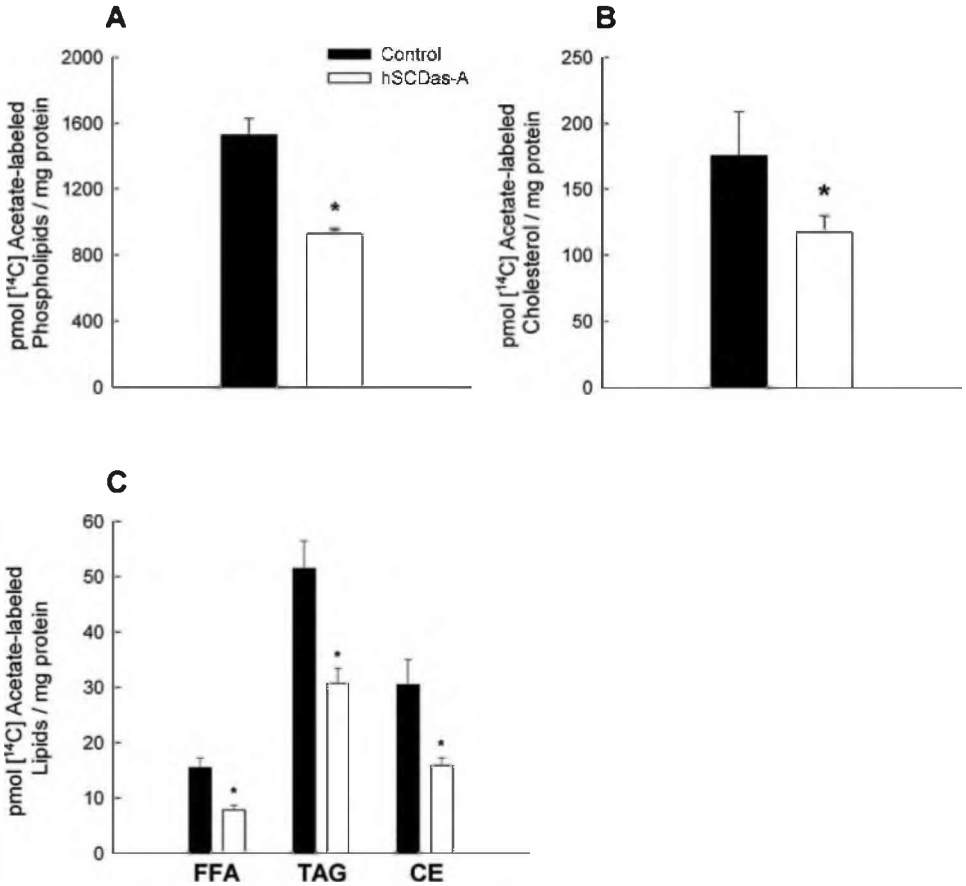

FIG. 3. **The *de novo* synthesis of neutral and polar lipids is decreased in SCD-depleted cells.** Preconfluent control and hSCD*as*-A cells were incubated with [^{14}C]acetate (0.50 μCi/dish) for 24 h. Phospholipids (*A*), cholesterol (*B*), unesterified fatty acids (*FFA*), triacylglycerols (*TAG*), and cholesterylesters (*CE*) (*C*) were resolved on TLC plates, and the radioactivity levels were measured by scintillation counting. Values represent the mean ± S.D. of quadruplicate samples. *, $p < 0.05$, by Student's *t* test.

SCD Deficiency Promotes Apoptosis in a Ceramide-independent Manner and Enhances Palmitic Acid-mediated Cytotoxicity—Excess SFAs in serum are suggested to promote apoptosis. Several mechanisms have been proposed to be responsible for this lipotoxic phenomenon, including excess ceramide synthesis (29), alterations in mitochondrial membranes (33), and exacerbated generation of reactive oxygen species (12). Most of the studies that investigate the lipotoxic effects of SFA on cell viability were performed with short term exposures to cytotoxic concentrations of these fatty acids. Hence, the question of whether SCD acts to reduce the cytotoxicity of increased levels of SFA either derived from exogenous or endogenous sources is still not fully understood. To this end, we tested the hypothesis that the reduction of SCD expression would induce apoptosis by promoting SFA accumulation. The degree of fragmentation of [^{3}H]thymidine-labeled DNA was analyzed as a marker for programmed cell death in hSCD*as*-A cells and control cells. As displayed in Fig. 7*A*, the percentage of apoptosis in SCD-deficient cells was ~70% higher than in control cells. The greater rate of apoptosis in hSCD*as* cells was confirmed by the presence of fragmented/condensed nuclei by fluorescence microscopy after staining with Hoescht fluorophore (Supplemental Fig. S1*A*). In addition, caspase-3 activity was measured in both cell groups using etoposide-treated cells as positive controls. As shown in Fig. 7*B*, hSCD*as*-A cells showed a 4-fold increase in caspase activity compared with mock-transfected cells, further corroborating the positive correlation between SCD depletion and programmed cell death.

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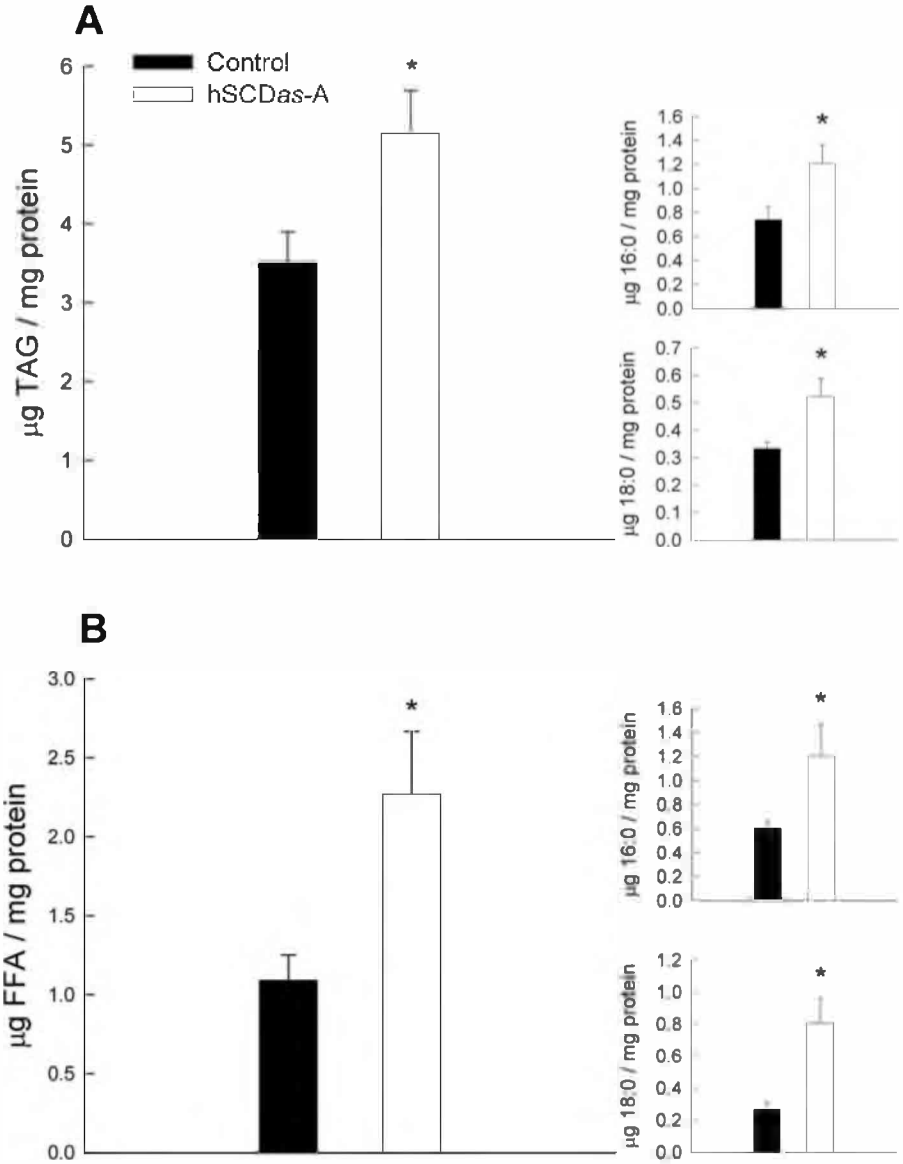

FIG. 4. **High levels of TAG and FFA in SCD-deficient SV40-cells.** Total lipids from preconfluent control and hSCD*as*-A cells were extracted and TAG and FFA were resolved by TLC. Mass and fatty acid composition of both lipid fractions were determined by gas chromatography. *A*, triacylglycerol mass (*TAG*), *insets:* 16:0 (*upper*); 18:0 (*lower*). *B*, unesterified fatty acids (*FFA*), *insets*: 16:0 (*upper*), 18:0 (*lower*). Values represent the mean ± S.D. of triplicate samples. *, $p < 0.01$, by Student's *t* test.

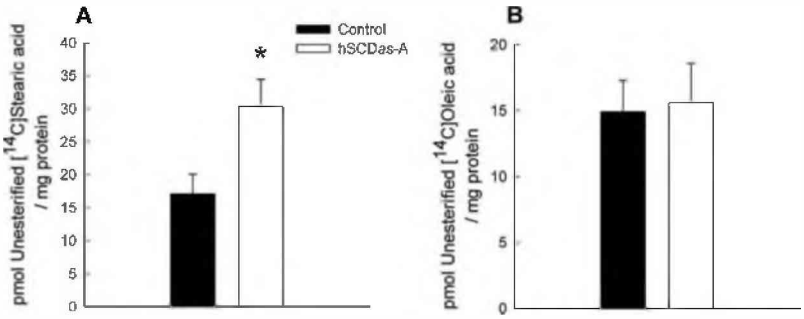

FIG. 5. **Stearic acid, but not oleic acid, increases in the FFA fraction of SCD-deficient cells.** Preconfluent control and hSCD*as*-A cells were labeled with either [^{14}C]stearic acid or [^{14}C]oleic acid for 6 h, total lipids were extracted, and FFA fractions were resolved by TLC. Spots corresponding to unesterified [^{14}C]stearic acid (*A*) and unesterified [^{14}C]oleic acid (*B*) were scraped into vials, and radioactivity was measured. Data represent the mean ± S.D. of three determinations. *, $p < 0.01$, by Student's *t* test.

The role of ceramide in the induction of apoptosis has been firmly established, but the relationship of ceramide levels and lipid accumulation is still unclear. It has been observed that accumulation of long chain SFA, which are substrates in *de novo* ceramide synthesis, leads to cell death in pancreatic β-cells (29). However, in other cell types, SFA-mediated apoptosis seems to occur in a ceramide-independent manner (32). Thus, we tested the hypothesis that the excess of largely saturated FFA and TAG caused by the decrease in SCD expression alter intracellular ceramide levels. Ceramide content was measured in hSCD*as*-A and control cells cultured under regular growth conditions. We observed that the levels of this lipid mediator were not significantly different between both types of cells (Fig. 7*C*). Taken as a whole, the results of these experiments support a role for SCD in cell survival. Moreover, the events of programmed cell death promoted by SCD deficiency are not mediated through a ceramide-dependent mechanism.

Previous studies have indicated that high levels of exogenous SFA promote apoptosis, whereas the addition of oleic acid or overexpression of rat SCD1 prevents the SFA-induced cell death (12). In an attempt to extend this observation, we incubated hSCD*as*-A and control cells with different concentrations

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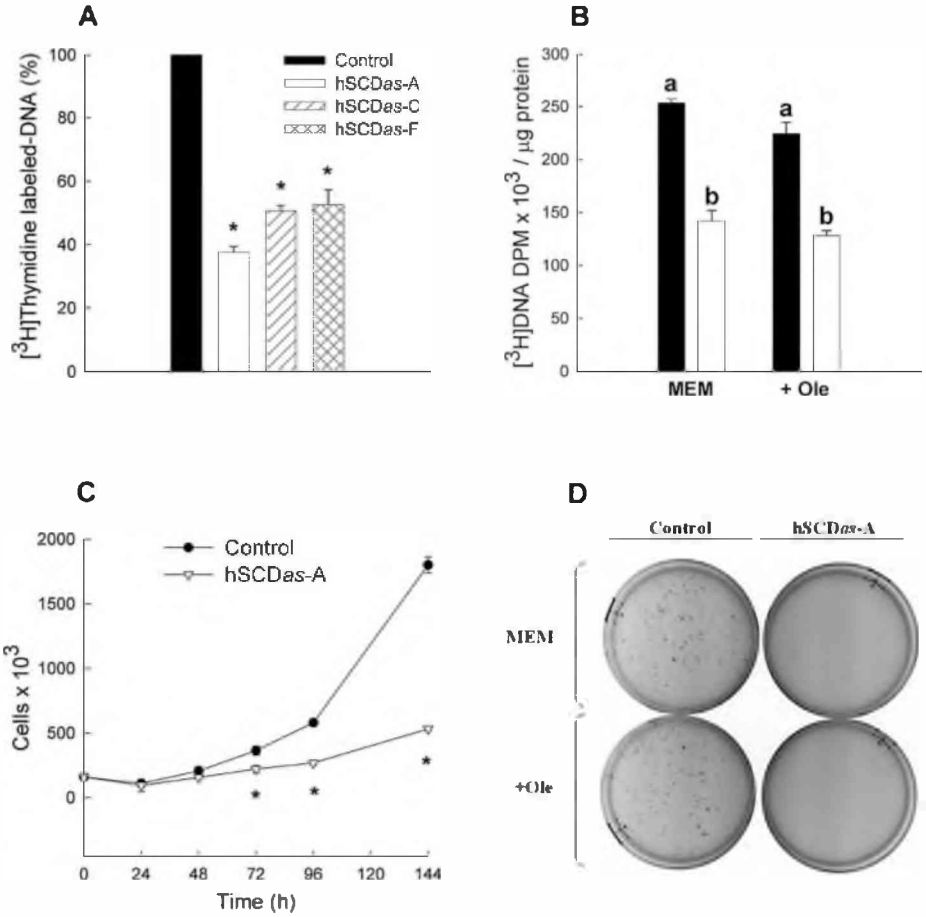

FIG. 6. **SCD gene knockdown slows proliferation rate and suppresses the anchorage-independent growth of SV40-transformed cells.** *A*, near confluent control cells and hSCD*as*-A, -C, and F-clones were pulsed with [^{3}H]thymidine (1 μCi/dish) in MEM with 10% FBS for 2 h at 37 °C, and total [^{3}H]DNA was quantified. Values represent the mean ± S.D. of triplicate samples. *B*, control and hSCD*as*-A cells were incubated with 100 μM oleate in 10% FBS MEM for 72 h. At the end of the treatment, cells were pulsed with 1 μCi/dish of [^{3}H]thymidine for 2 h, and formation of total cell [^{3}H]DNA was estimated. Values correspond to the mean ± S.D. of four determinations. Values bearing *different letters* are significantly different at $p < 0.01$ (analysis of variance). *C*, control and hSCD*as*-A cells were seeded in 60-mm Petri dishes and grown in MEM with 10% FBS. Cells were trypsinized and counted at time points up to 144 h. Data correspond to triplicate dishes. Some *error bars* are *hidden by symbols*. *, $p < 0.01$, by Student's *t* test. *D*, 1×10^5 hSCD*as*-A and controls cells were seeded in MEM containing 10% FBS, and 0.3% agar, with or without 200 μM oleate, over a layer of 0.6% agar. After 4 weeks, cells colonies were stained with ethidium bromide and photographed under UV light. A single cell culture dish, representative of four independent experiments, is shown for each cell strain and treatment.

of palmitic acid for 24 h and determined the rate of apoptosis by analyzing the degree of [^{3}H]thymidine-labeled DNA fragmentation. To avoid the influence of serum fatty acids, these studies were carried out in FBS-depleted medium. To ensure a high unbound FFA concentration in the incubation medium, we used a 5:1 fatty acid:BSA ratio. As shown in Fig. 7*D*, untreated hSCD*as* cells exhibited a 72% higher degree of DNA fragmentation than control cells. Palmitic acid supplementation significantly increased apoptosis in a dose-dependent manner in both cell groups. In mock-transfected cells, treatments with 250 and 500 μM palmitic acid increased the cell death rate by 1.8- and 2.7-fold, respectively, compared with untreated control cells. Interestingly, SCD deficiency made the cells more susceptible to the cytotoxic action of palmitic acid. At 250 and 500 μM palmitic acid, the percentage of apoptotic hSCD*as*-A cells increased by ~2.5- and ~4.5-fold, respectively, with respect to untreated SCD-deficient cells. To test the protective effect of MUFA on SFA-induced apoptosis, control and hSCD*as*-A cells were co-treated with 500 μM palmitic acid and 250 μM oleic acid, and the level of apoptosis was determined. Interestingly, co-supplementation with 250 μM oleic acid reduced apoptosis in both 500 μM palmitate-treated hSCD*as* and control cells to levels observed in conditions of no treatment for each cell group. Additionally, groups of both cell lines received 250 and 750 μM oleate alone, showing no changes with respect to their non-treated cell counterparts. However, oleic acid treatment did not reverse the higher basal apoptosis rate seen in hSCD*as*-A cells.

In experiments resembling more physiological conditions (low unbound FFA, achieved by a ratio fatty acid:BSA of ≤2), neither 250 nor 500 μM palmitate supplement increased apoptosis over the rate seen in cells without fatty acid treatment (Supplemental Fig. S1*B*). Moreover, at high and low fatty acid:BSA ratios, 100 μM palmitic acid, a concentration almost similar to that found in human serum (34), did not trigger cell death beyond basal levels in either control or SCD-deficient cell strains (data not shown).

DISCUSSION

In our studies, the stable transfection of SV40-transformed cells with antisense human SCD cDNA led to a significant decrease in the expression and, consequently, the activity of SCD. This was accompanied by a substantial reduction in the MUFA/SFA ratio of the major membrane phospholipids, PC and PE, and in TAG and FFA fractions. The different grade of SCD deficiency in independent hSCD*as* clones was mirrored by a proportionate decay in MUFA levels, indicating that SCD is the main regulator of MUFA content in SV40-transformed cells. Moreover, the relevance of SCD in controlling the relative abundance of SFA and MUFA is further supported by the observation that the alterations in MUFA levels in SCD-deficient cells persisted even in the presence of significant amounts of MUFA (~200 μM) present in the FBS-supplemented growth media.

In addition to the profound alterations in fatty acid distribution in the main phospholipid fractions promoted by SCD knockdown, the overall synthesis of phospholipids was significantly reduced in the SCD-deficient cell lines. This provides evidence that SCD may play a role not only in the regulation of fatty acid remodeling of pre-existing membrane lipids but also in the *de novo* synthesis of phospholipids in the fast replicating SV40-transformed cells. Because a drastic reduction in endogenous fatty acid synthesis occurred in SCD-ablated cells, the diminished phospholipid synthesis may be produced by a decrease in *de novo* synthesized glycerolipid substrates. In addition, SCD seems to play a central role in the control of the

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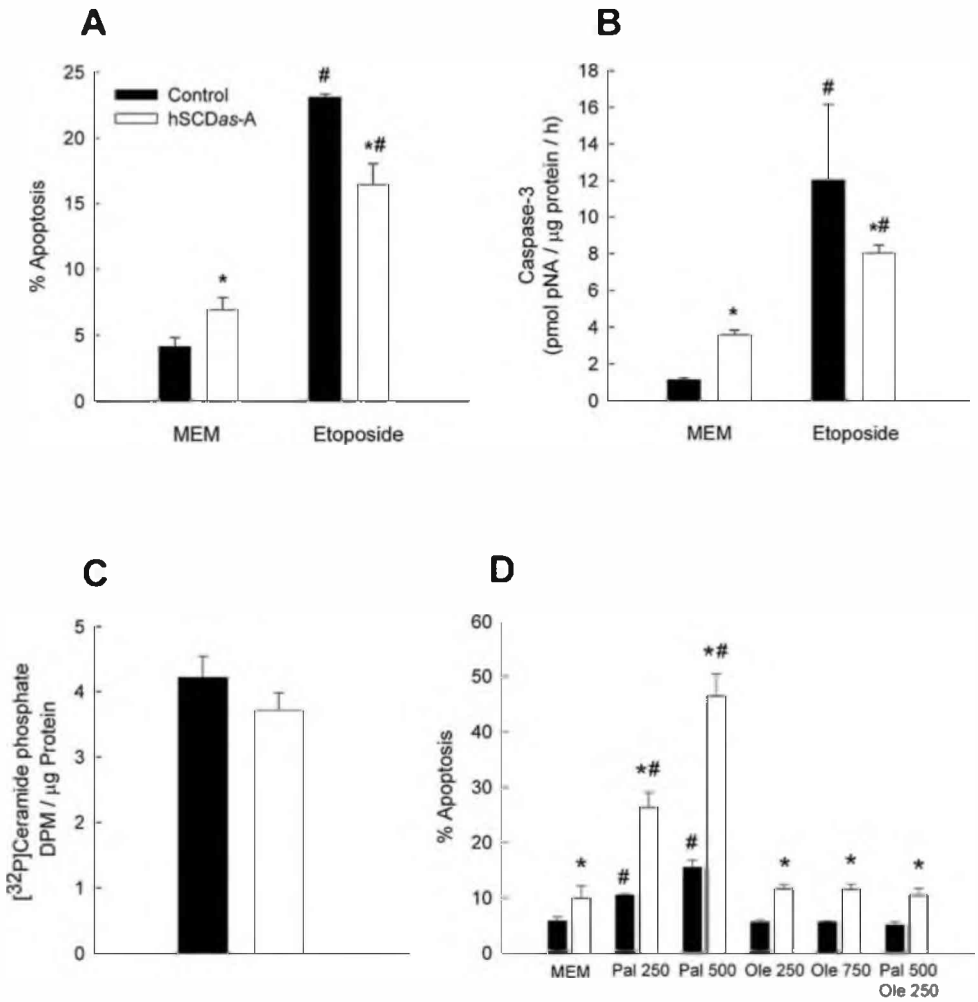

FIG. 7. **hSCD*as* cells exhibit a greater rate of apoptosis and are more sensitive to palmitic acid-mediated cytotoxicity.** The rate of programmed cell death in control and hSCD*as*-A cells, incubated in MEM with 10% FBS in presence of 40 μM etoposide or vehicle, was determined by estimating the fragmentation index of [^{3}H]thymidine-labeled DNA (*A*). Caspase-3 activity was quantified in homogenates from preconfluent hSCD*as*-A and control cells treated with 40 μM etoposide or vehicle (*B*). Ceramide content was measured in total lipids extracted from controls and hSCD*as*-A cells grown to near confluence (*C*). The rate of apoptosis was determined by the analysis of DNA fragmentation in control and hSCD*as*-A cells incubated in MEM without FBS in the presence of palmitic acid (*Pal*, 250 μM or 500 μM), oleic acid (*Ole*, 250 μM or 750 μM), or both for 24 h (*D*). Data represent the mean ± S.D. of three or four determinations. *, $p < 0.05$, compared with control cells; #, $p < 0.05$ compared with same cell group with different treatment (analysis of variance).

synthesis of membrane lipids, because SCD knockdown led to a down-regulation in the synthesis of cholesterol, the other major structural lipid in cell membranes. In this context, it has been reported that the induction of cell replication with growth factors in human cells triggers a severalfold increase in expression of SCD, together with the activation of enzymes needed for cholesterol and phospholipid synthesis (11).

Under normal conditions, cellular phospholipid concentrations remain essentially constant, and therefore this lipid pool can accommodate a limited amount of fatty acids. Thus, excess content of a particular species of fatty acid in this pool may alter the composition and membrane properties of cellular membranes. Consequently, excess fatty acids must be channeled away into an expandable lipid pool like TAG. Indeed, we found that hSCD*as* cells showed a significant increase in the incorporation of [^{14}C]stearic acid into FFA and TAG together with the expansion of the TAG and FFA pools. Both FFA and TAG were greatly enriched with SFA species, with no significant changes in MUFA levels. We take this to suggest that in a state of SCD deficiency, to preserve the membrane physical-chemical homeostasis, cells segregate excess SFA away from the phospholipid remodeling pool and store these fatty acid species under the form of TAG. This hypothesis is also supported by the observation that modifications in the content and composition of the FFA fraction, a pool of intermediates for the formation of both phospholipids and TAG, occurs in a manner and degree similar to TAG. Exogenous [^{14}C]oleate was excluded from TAG and FFA pools and segregated preferentially into phospholipid fractions of SCD-deficient cells, further indicating a differential channeling of SFA and MUFA into the different lipid species.

Our knockdown of SCD promoted dramatic effects on cell proliferation. The growth rate for hSCD*as* cells was significantly decreased leading to a doubling time that was ~70% slower than that of mock-transfected cells and approximately similar to normal parental WI38 fibroblasts (16). It is noteworthy that the magnitude of growth inhibition was strongly correlated to the degree of SCD depletion in the three clones of SCD-deficient cells. This firmly establishes a direct relationship between Δ9-desaturation and cell replication. Because SV40-WI38 cells exhibit neoplastic behavior, the effect of SCD knockdown on their rapid proliferation rate may be an indication of a reversion of the neoplastic cell phenotype. This is supported by the observation that SCD depletion in SV40-WI38 cells also suppresses anchorage-independent growth, another hallmark of malignant transformation. These data indicate that the high cellular SCD activity is responsible, at least

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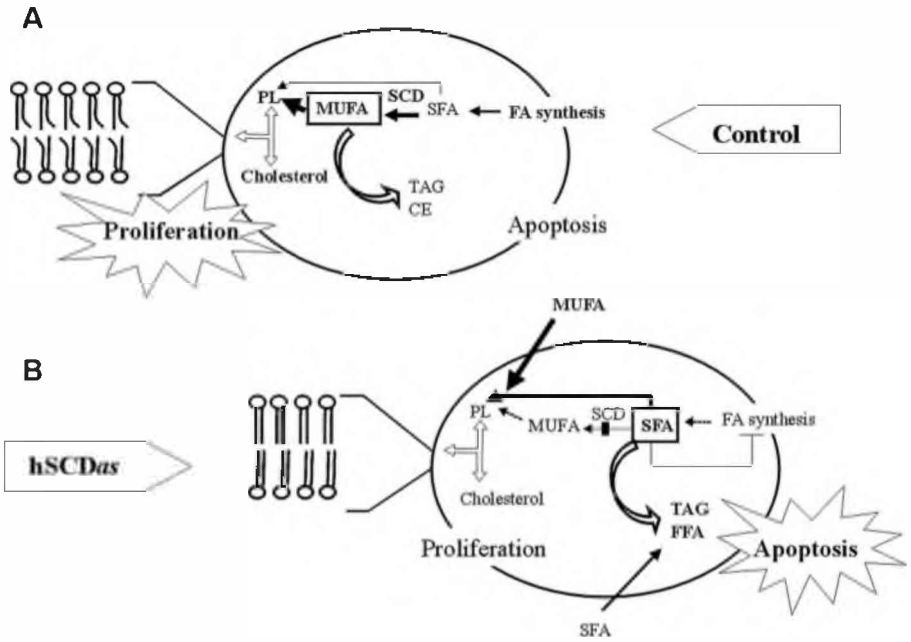

FIG. 8. **Hypothetical model for the regulation of cell proliferation and apoptosis by SCD in SV40-transformed cells.** In empty-vector transfected SV40-cells (*A*, control cells), oncogenic transformation activates a global program for the formation of new membrane lipids that includes a high rate *de novo* synthesis and Δ9-desaturation of fatty acids and greater production of phospholipids and cholesterol. MUFA are preferentially channeled toward the phospholipid fraction to create a more fluid physical-chemical membrane environment that favors an active cell replication. Ablation of SCD expression and activity in the transformed cells (*B*, hSCD*as* cells) leads to lower levels of MUFA and accumulation of SFA in membrane phospholipids. The synthesis of phospholipids and cholesterol is impaired by SCD deficiency, causing a reduction in the cell proliferation rate. In addition, excess content of SFA inhibits fatty acid synthesis and accumulates as FFA and TAG, two events that are known to trigger the cell death program.

partially, for the abnormal cell proliferation and invasiveness capacity of SV40-transformed cells.

At this point, we cannot explain how a reduction in SCD expression affects the molecular mechanisms of cell proliferation and invasiveness in SV40-transformed cells. Some of the alterations in the growth properties of SCD-deficient cells are likely to be caused by the down-regulation of either phospholipid and/or cholesterol synthesis needed to maintain a rapid rate of membrane formation. Alternatively, changes in the MUFA/SFA ratio of phospholipids may alter the physical-chemical properties of cell membranes, and this may account for the observed effects. Phospholipid synthesis is required for normal cell cycle progression (35), whereas deficient formation of phosphatidylcholine stops cell growth and promotes apoptosis (36). Impaired phospholipid synthesis leads to a loss in cell growth and invasiveness in human neoplastic cells (16, 37) and mouse lymphoma cells (38). In addition, the proportional enrichment of phospholipids with SFA promotes cytostatic effects in cancer cells (39). Furthermore, sterculic acid, an inhibitor of SCD activity, exhibits an anti-proliferative action in neoplastic cells likely by increasing the relative abundance of SFAs (40). Finally, the inhibition of fatty acid synthesis observed in SCD-depleted cells may be also responsible for the decrease in cell proliferation. In many cancer cells the active *de novo* fatty acid synthesis appears essential for sustaining a rapid cell growth, whereas inactivation of fatty acid synthase by means of pharmacological inhibitors reduces cell proliferation and triggers programmed cell death (41).

It is also tempting to speculate that, by regulating SCD activity and, consequently, the MUFA/SFA ratio of phospholipids, cells may be able to modulate the structural properties of cellular membranes by altering the organization of lipid domains which may, in turn, influence signaling receptor functions that favor neoplastic cell features like fast proliferation, adhesion and invasion. In this regard, SCD activity is known to regulate the activity of proteins that are resident in the plasma membrane and inner cellular membranes (7, 8). Furthermore, changes in oleic acid abundance are known to produce important effects on signal transduction networks related to cell proliferation and death, like phosphatidylinositol 3-kinase (42) and protein kinase C (43).

Our experiments provide the first clear evidence that SCD is regulating programmed cell death and add valuable information to previous studies that suggest a role for SCD in protecting mammalian cells against SFA-mediated lipotoxicity. Interestingly, SCD-deficient cells undergo apoptosis even in regular growing conditions, strongly indicating that SCD activity is crucial for cell survival. The relevance of the anti-apoptotic role of SCD is twofold. First, SCD activity is required to avoid the cytotoxic effect of SFA overload. SCD-depleted cells were more sensitive to SFA-induced lipotoxicity, supporting previous studies that indicated that SCD1-overexpressing Chinese hamster ovary cells are highly resistant to palmitate-induced apoptosis (12). The observation that, unlike Chinese hamster ovary cells, SFA-mediated apoptosis was triggered in SV40-cells only at a high SFA:BSA ratio, suggests that sensitivity to SFA-induced cytotoxicity likely depends on cell type and specie. Second, the alterations in FFA and TAG composition and mass observed in the SCD-deficient cells are likely to contribute to the process of programmed cell death. Accumulation of TAG in non-adipose cells triggers the apoptosis program by providing high levels of SFA substrates for the *de novo* synthesis of ceramide (30). Although excess contents of FFA and TAG were found in this study, the observations that ceramide levels were unaltered in hSCD*as*-A cells rules out the involvement of a ceramide-dependent mechanism of programmed cell death caused by SCD ablation.

To summarize the current results and our previous data (15), we have shown that neoplastic SV40 transformation imposes a constitutive activation of MUFA synthetic machinery by triggering a series of events that increases the fatty acid synthesis and SCD activity with a consequent enrichment of membrane phospholipids with MUFA (Fig. 8*A*). MUFA production is an essential component for the adequate synthesis of membrane lipids, because transformed cells show a parallel activation of the formation of the main structural membrane lipids, phospholipids, and cholesterol (this work and Ref. 15). Moreover, highly fluid cell membranes are required for neoplastic cells to establish appropriate structural and metabolic conditions to sustain high rates of proliferation and invasion and to avoid apoptosis.

In addition, we provide evidence indicating that SCD is, indeed, essential for maintaining the biochemical and biological phenotype of neoplastic transformation. SCD deficiency generated by ablating SCD expression in SV40 cells leads to a decrease in the MUFA:SFA ratio in phospholipids, likely pro-

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ducing a more rigid cellular membrane (Fig. 8*B*). As a result of the diminished provision of endogenously synthesized MUFA, the incorporation of exogenous oleic acid into phospholipids of hSCD*as* cells is enhanced, but this cannot compensate for the more saturated fatty acid profile of membrane lipids and its consequences. The block in SCD expression also reduces the synthesis of phospholipids, especially phosphatidylcholine, and cholesterol. These changes in the content and quality of membrane lipids may lead to alterations in cell proliferation and invasiveness, as observed in the hSCD*as* clones. Additionally, SFA accumulates and consequently inhibits *de novo* fatty acid synthesis, but FFA and TAG pools are expanded because of the preferential channeling of SFA into these lipid fractions. Due to the highly saturated nature of both FFA and TAG, these lipids may display increased cytotoxic effects in the transformed cells, thereby triggering apoptosis.

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REFERENCES

1. Enoch, H. G., Catalá, A., and Strittmatter, P. (1976) *J. Biol. Chem.* **251,** 5095–5103
2. Sperling, P., Ternes, P., Zank, T. K., and Heinz, E. (2003) *Prost. Leukotr. Essential Fatty Acids* **68,** 73–96
3. Ntambi, J. M., and Miyazaki, M. (2004) *Prog. Lipid Res.* **43,** 91–104
4. Ntambi, J. M. (1999) *J. Lipid Res.* **40,** 1549–1558
5. Zhang, L., Ge, L., Parimoo, S., Stenn, K., and Prouty, S. M. (1999) *Biochem. J.* **340,** 255–264
6. Beiraghi, S., Zhou, M., Talmadge, C. B., Went-Sumegi, N., Davis, J. R., Huang, D., Saal, H., Seemayer, T. A., and Sumegi, J. (2003) *Gene (Amst.)* **309,** 11–21
7. Sun, Y., Hao, M., Luo, Y., Liang, C. P., Silver, D. L., Cheng, C., Maxfield, F. R., and Tall, A. R. (2003) *J. Biol. Chem.* **278,** 5813–5820
8. Hao, M., Mukherjee, S., Sun, Y., and Maxfield, F. R. (2004) *J. Biol. Chem.* **279,** 14171–14178
9. Samuel, W., Kutty, R. K., Nagineni, S., Gordon, J. S., Prouty, S. M., Chandraratna, R. A. S., and Wiggert, B. (2001) *J. Biol. Chem.* **276,** 28744–28750
10. Samuel, W., Nagineni, C. N., Kutty, R. K., Parks, W. T., Gordon, J. S., Prouty, S. M., Hooks, J. J., and Wiggert, B. (2002) *J. Biol. Chem.* **277,** 59–66
11. Demoulin, J. B., Ericsson, J., Kallin, A., Rorsman, C., Ronnstrand, L., and Heldin, C. H. (2004) *J. Biol. Chem.* **279,** 35392–35402
12. Listenberger, L. L., Han, X., Lewis, S. E., Cases, S., Farese, R.V., Jr., Ory, D. S., and Schaffer, J. E. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100,** 3077–3082
13. Li J., Ding, S. F., Habib, N. A., Fermor, B. F., Wood, C. B., and Gilmour, R. S. (1994) *Int. J. Cancer* **57,** 348–352
14. Thai, S. F., Allen, J. W., DeAngelo, A. B., George, M. H., and Fuscoe, J. C. (2001) *Carcinogenesis* **22,** 1317–1322
15. Scaglia, N., Caviglia, J. M., and Igal, R. A. (2005) *Biochim. Biophys. Acta* **1687,** 141–151
16. Bagnato, C., and Igal, R. A. (2003) *J. Biol. Chem.* **278,** 52203–52211
17. Zhang, L., Ge, L., Tran, T., Stenn, K., and Prouty, S. M. (2001) *Biochem. J.* **357,** 183–193
18. Hollstein, M., Hergenhahn, M., Yang, Q., Bartsch, H., Wang, Z. Q., and Hainaut, P. (1999) *Mutat. Res.* **431,** 199–209
19. Ortega, S., Malumbres, M., and Barbacid, M. (2002) *Biochim. Biophys. Acta* **1602,** 73–87
20. Bligh, E. G., and Dyer, W. J. (1959) *Can. J. Med. Sci.* **37,** 911–917
21. Wilson, R., and Sargent, J. R. (2001) *J. Chromatogr. A* **905,** 251–257
22. Venable, M. E., Lee, J. Y., Smyth, M. J., Bielawska, A., and Obeid, L. M. (1995) *J. Biol. Chem.* **270,** 30701–30708
23. Kasai, T., Ohguchi, K., Nakashima, S., Ito, Y., Naganawa, T., Kondo, N., and Nozawa, Y. (1998) *J. Immunol.* **161,** 6469–6474
24. Preiss, J., Loomis, C. R., Bishop, W. R., Stein, R., Niedel, J. E., and Bell, R. M. (1986) *J. Biol. Chem.* **261,** 8597–8600
25. Luberto, C., and Hannun, Y. A. (1998) *J. Biol. Chem.* **273,** 14550–14559
26. Lowry, O. R., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275
27. Miyazaki, M., Kim, Y.-C., Gray-Keller, M. P., Attie, A. D., and Ntambi, J. M. (2000) *J. Biol. Chem.* **275,** 30132–30138
28. Miyazaki, M., Kim, Y.-C., and Ntambi, J. M. (2001) *J. Lipid Res.* **42,** 1018–1024
29. Shimabukuro, M., Zhou, Y. T., Levi, M., and Unger, R. H. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 2498–2502
30. Zhou, Y. T., Grayburn, P., Karim, A., Shimabukuro, M., Higa, M., Baetens, D., Orci, L., and Unger, R. H. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 1784–1789
31. Cnop, M., Hannaert, J. C., Hoorens, A., Eizirik, D. L., and Pipeleers, D. G. (2001) *Diabetes* **50,** 1771–1777
32. Listenberger, L. L., Ory, D. S., and Schaffer, J. E. (2001) *J. Biol. Chem.* **276,** 14890–14895
33. Hardy, S., El-Assaad, W., Przybytkowski, E., Joly, E., Prentki, M., and Langelier, Y. (2003) *J. Biol. Chem.* **278,** 31861–31870
34. Richieri, G. V., and Kleinfeld, A. M. (1995) *J. Lipid Res.* **36,** 229–240
35. Jackowski, S. (1994) *J. Biol. Chem.* **269,** 3858–3867
36. Cui, Z., Houweling, M., Chen, M. H., Record, M., Chap, H., Vance, D. E., and Terce, F. (1996) *J. Biol. Chem.* **271,** 14668–14671
37. Miquel, K., Pradines, A., Terce, F., Selmi, S., and Favre, G. (1998) *J. Biol. Chem.* **273,** 26179–26186
38. Schaider, H., Seidl, H., Berger, M. R., Hofmann-Wellenhof, R., Tritthart, H. A., and Smolle, J. (1998) *Anticancer Res.* **18,** 995–998
39. Habib, N. A., Wood, C. B., Apostolov, K., Barker, W., Barker, M. J., Hershman, M. J., Aslam, M., Heinemann, D., Fermor, B., Williamson, R. C. N., Jenkins, W. E., Masters, J. R. W., and Embleton, M. J. (1987) *Br. J. Cancer* **56,** 455–458
40. Khoo, D. E., Fermor, B., Miller, J., Wood, C. B., Apostolov, K., Barker, W., Williamson, R. C. N., and Habib, N. A. (1991) *Br. J. Cancer* **63,** 97–101
41. Baron, A., Migita, T., Tang, D, and Loda, M. (2004) *J. Cell. Biochem.* **91,** 47–53
42. Hardy S., Langelier, Y., and Prentki, M. (2000) *Cancer Res.* **60,** 6353–6358
43. Park, J. Y., Kim, Y. M., Song, H. S., Park, K. Y., Kim, Y. M., Kim, M. S., Pak, Y. K., Lee, I. K., Lee, J. D., Park, S. J., and Lee, K. U. (2003) *Biophys. Res. Commun.* **303,** 891–895

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