RNA Interference–Mediated Silencing of the Acetyl-CoA-Carboxylase-α Gene Induces Growth Inhibition and Apoptosis of Prostate Cancer Cells

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Abstract

Overexpression of lipogenic enzymes is a common characteristic of many cancers. Thus far, studies aimed at the exploration of lipogenic enzymes as targets for cancer intervention have focused on fatty acid synthase (FAS), the enzyme catalyzing the terminal steps in fatty acid synthesis. Chemical inhibition or RNA interference (RNAi)–mediated knockdown of FAS consistently inhibits the growth and induces death of cancer cells. Accumulation of the FAS substrate malonyl-CoA has been implicated in the mechanism of cytotoxicity of FAS inhibition. Here, using RNAi technology, we have knocked down the expression of acetyl-CoA carboxylase-α (ACC-α), the enzyme providing the malonyl-CoA substrate. Silencing of the ACC-α gene resulted in a similar inhibition of cell proliferation and induction of caspase-mediated apoptosis of highly lipogenic LNCaP prostate cancer cells as observed after FAS RNAi. In nonmalignant cells with low lipogenic activity, no cytotoxic effects of knockdown of ACC-α or FAS were observed. These findings indicate that accumulation of malonyl-CoA is not a prerequisite for cytotoxicity induced by inhibition of tumor-associated lipogenesis and suggest that in addition to FAS, ACC-α is a potential target for cancer intervention. (Cancer Res 2005; 65(15): 6719-25)

Introduction

Enhanced expression of lipogenic enzymes is increasingly recognized as a common characteristic of a wide variety of tumors (1–3). Overexpression of fatty acid synthase (FAS), a key lipogenic enzyme that catalyzes the terminal steps in the de novo biosynthesis of long-chain fatty acids (4), is found already in the earliest stages of tumor development (5, 6). In many tumor types, FAS overexpression is further pronounced as the tumor progresses towards a more advanced stage (2, 5, 7). In breast and prostate cancers, similar changes have been found in the expression of acetyl-CoA-carboxylase-α (ACC-α), the rate-limiting enzyme of fatty acid synthesis that catalyzes the condensation of malonyl-CoA using acetyl-CoA and CO₂ as precursors (1, 8).

The finding that lipogenesis is low in nearly all nonmalignant adult tissues (9, 10), whereas it is up-regulated in many tumors, has led to the exploration of endogenous lipogenesis as a novel target for prevention and/or treatment of cancer. Up to now, nearly all attempts to examine this possibility have focused on FAS. Both chemical inhibitors of FAS (cerulenin, cet, Orlistat, EGCG) and the use of more selective approaches such as gene silencing with small interfering RNA (siRNA) targeting FAS have resulted in growth arrest and cell death in tumor cells (2, 11–21). Although it has been suggested that cytotoxicity induced by FAS inhibition is the result of accumulation of the toxic intermediate malonyl-CoA (22, 23), the exact mechanism by which inhibition of FAS induces tumor cell death remains a matter of debate.

In the present work, we examined the effect of siRNA-mediated knockdown of ACC-α on prostate cancer cells and compared the effects with those of knockdown of FAS. It is shown that inhibition of ACC-α induces a similar growth arrest and tumor cell death as observed after blockage of FAS, despite the fact that there is no accumulation of malonyl-CoA.

Materials and Methods

Cell culture. The human LNCaP prostate cancer cell line was obtained from the American Type Culture Collection (Manassas, VA). Nonmalignant skin fibroblasts were provided by Prof. Dr. J.J. Cassiman (K.U. Leuven, Belgium). Cells were cultured at 37°C in a humidified incubator with a 5% CO₂/95% air atmosphere in RPMI 1640 supplemented with 3 mmol/L L-glutamine and 10% FCS (Invitrogen, Carlsbad, CA). For experiments examining biological effects in the absence of androgens, charcoal-treated fetal calf serum (FCS) was used to reduce background steroid levels. For analyzing cellular responses to androgens, R1881 (methyltrienolone; DuPont/NEN, Boston, MA) was dissolved in ethanol and added to the cultures. For culturing cells in the presence of palmitate-bovine serum albumin (BSA) complex, palmitate (Sigma, St. Louis, MO) was first complexed to fatty acid–free BSA (Invitrogen).

RNA interference. Transfection procedures with siRNA using Oligofectamine (Invitrogen) have been described previously (17; siRNA oligonucleotides were purchased from Dharmacon (Lafayette, CO). Sequences of siRNA oligonucleotides targeting FAS and luciferase (Luc) have been described (17). The siRNA oligonucleotides targeting ACC-α were sense, CAAUUGCAUUGCAUGdTdT, and antisense, CACUCGUCGCAUCUGdTdT.

RNA analysis. An 800-bp cDNA probe for ACC was synthesized by PCR on human cDNA (generated by reverse transcription as described; ref. 26) using 5′-TTCTCAGAGCTTCCAATGTCT and 5′-CTAATCCGTGCTACATACCAAAGGCAAC as forward and reverse primer, respectively. PCR products were cloned into the pGEM-T vector (Promega, Madison, WI). Probes for FAS and 18S rRNA, RNA preparation, and Northern blot procedures have been described previously (26).

2-14C-acetate incorporation assay and TLC analysis. At 24, 48, 72, 96, or 120 hours after transfection with siRNA, 2-14C-labeled acetate (57 mCi/mL; 2 μCi/dish; Amersham International, Aylesbury, United Kingdom) was added to the cell culture medium. After 4 hours incubation, cells were collected by centrifugation and resuspended in 0.8 mL PBS. Lipids were extracted using the Bligh Dyer method as previously described (17);

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2-14C-acetate incorporation into cellular lipids was quantitated by scintillation counting. Obtained results were normalized for sample protein content. To analyze 2-14C-acetate incorporation into different lipid classes, TLC analysis was done and lipids were quantitated using a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA) as previously described (17).

**Proliferation/cytotoxicity assays and fluorescence-activated cell sorting analysis.** At the indicated time after transfection with siRNA, cells were collected and cell number and viability were determined using a trypan blue dye exclusion assay as described previously (17). Cell proliferation was quantitated using a bromodeoxyuridine (BrdUrd) labeling and detection kit (Roche, Basel, Switzerland). For fluorescence-activated cell sorting (FACS) analysis, cells were trypsinized; fixed in ice-cold 70% ethanol for 1 hour; washed twice with PBS containing 0.05% Tween 20; and resuspended in PBS containing 0.05% Tween 20, 0.5 mg/mL propidium iodide, and 1 mg/mL RNase A (Sigma). Samples were analyzed on a FACSort cytometer using the CellQuest and Modfit programs (Becton Dickinson, Franklin Lakes, NJ).

**Hoechst staining.** LNCaP cells were plated in Lab-Tek II chamber slides (Nalge Nunc International, Naperville, IL) at a density of 1.2 × 10^5 cells per chamber, incubated overnight, and transfected with siRNA as described (17). At 96 hours after transfection, Hoechst 33342 (Sigma) was used as internal control for Northern blot and Western blot analysis, respectively. C, measurement of 2-14C-labeled acetate incorporation into cellular lipids of LNCaP cells at 24, 48, 72, 96, and 120 hours after transfection with siRNA targeting ACC-α, FAS, or Luc, as revealed by scintillation counting. D, measurement of 2-14C-labeled acetate incorporation into different lipid classes of LNCaP cells at 72 hours after transfection with siRNA targeting ACC-α, FAS, or Luc, as quantitated by TLC analysis and Phosphor Imaging. PL, phospholipids; TG, triglycerides; Chol, cholesterol. Columns, means (n = 6-18); bars, ±SD. *, significantly different from control (Luc siRNA-transfected cells) by Tukey test.
added to the culture medium of living cells; changes in nuclear morphology were detected by fluorescence microscopy using a filter for Hoechst 33342 (365 nm). For quantification of Hoechst 33342 stainings, the percentages of Hoechst-positive nuclei per optical field were counted. To investigate the involvement of caspases, the irreversible caspase inhibitor z-VAD-fmk (N-benzyl-oxycarbonyl-Val-Ala-Asp-fluoromethylketone) was added 24 hours after siRNA transfection at a final concentration of 50 μmol/L.

**Immunoblot analysis.** At the indicated time after transfection with siRNA, cells were washed with PBS and lysed in a reducing buffer containing 62.5 mmol/L Tris (pH 6.8), 2% SDS, 0.715 mol/L 2-mercaptoethanol, and 8.7% glycerol. Protein concentrations were measured using the bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL). Generation of antibodies against FAS and Western blot procedures using antibodies against FAS, cytokeratin-18 (Santa Cruz Biotechnology, Santa Cruz, CA), ACC-α, or cleaved poly(ADP-ribose) polymerase (PARP; both from Cell Signaling Technology, Inc., Beverly, MA) have been previously described (17, 25).

**Malonyl-CoA measurement.** Sample preparation and malonyl-CoA quantification in LNCaP cells was done as described (27, 28). Cells were collected in PBS and after centrifugation resuspended in 0.6 mL ice-cold 0.6 N trichloroacetic acid. Cell suspensions were sonicated and 100 pmol of isobutyryl-CoA was added to each sample as internal standard. After centrifugation at 12,000 x g, supernatants were washed six times with 0.7 mL diethyl ether and were evaporated to dryness by vacuum centrifugation. Acyl-CoA esters were separated and quantitated using a reverse-phase high-performance liquid chromatography (HPLC) with a 3.5-μm Zorbax SB-C8 column (Rockland Technologies, Newport, DE), an LKB 2150 HPLC pump, an LKB 2152 LC controller, a UV detector (at 254 nm) with UV-M monitor, and an LKB 2210 recorder (all from Amersham Pharmacia Biotech, LKB, Uppsala, Sweden). The buffers used for the mobile phase were 0.1 mol/L potassium phosphate (pH 5.0; buffer A) and 0.1 mol/L potassium phosphate (pH 5.0) with 40% acetonitrile (buffer B). Using a constant flow rate of 1 mL/min, runs started with a 12-minute period with 100% buffer A; at 12 minutes, the percentage of buffer B gradually increased from 0% to 10% (over 28 minutes); at 40 minutes, buffer B was kept at 10% for 4 minutes; at 44 minutes, the percentage of buffer B was gradually increased to 80% (over 36 minutes). For identification, sample peaks and retention times were compared with those of pure malonyl-CoA and isobutyryl-CoA standards.

**Statistical analysis.** Obtained results were analyzed by one-way ANOVA using a Tukey test as post-test. *Ps < 0.05 were considered statistically significant. All data are means ± SD.

**Results**

RNA interference–mediated silencing of ACC-α and fatty acid synthase decreases the synthesis of fatty acids in prostate cancer cells. To specifically silence ACC-α and FAS gene expression in LNCaP prostate cancer cells, cells were transiently transfected with appropriate siRNA oligos or with siRNAs targeting Luc, which is not endogenously expressed in LNCaP cells, as a control. Northern blot and Western blot analysis showed a marked suppression of ACC-α and FAS expression in LNCaP cells after transfection with ACC-α siRNA and FAS siRNA, respectively, when compared with cells transfected with Luc siRNA (Fig. 1A–B).

**Figure 2.** Impact of ACC-α RNAi and FAS RNAi on LNCaP cell proliferation and viability. LNCaP cells were transfected with siRNA targeting ACC-α, FAS, or Luc. A, at the indicated time points, cells were collected and stained with trypan blue; viable cells were counted. B, at the indicated time points, cells were exposed to BrdUrd for 2 hours. Thereafter, BrdUrd incorporation in LNCaP cells was measured colorimetrically and normalized for the number of viable cells at the start of the BrdUrd exposure [expressed as percentage BrdUrd incorporation of the control (Luc siRNA-transfected cells)]. C, the percentage of dead cells was counted after staining the cells with trypan blue at the indicated time points. Columns, means (n = 6-7); bars, ± SD. *, significantly different from control (Luc siRNA-transfected cells) by Tukey test. D, cell cycle analysis on LNCaP cells at 72 hours after transfection with FAS siRNA, ACC-α siRNA, or Luc siRNA. Data are expressed as percentage of the total population of cells. The data from the FACScan-based cell cycle analysis are the means ± SD of three independent experiments.
To evaluate the effect of reduced ACC-α and FAS expression on endogenous lipid synthesis, LNCaP cells were exposed to 2-14C-labeled acetate for 4 hours at different time points after transfection with siRNA targeting ACC-α, FAS or Luc. Thereafter, cellular lipids were extracted and the incorporation of 2-14C-labeled acetate into cellular lipids was quantitated by scintillation counting. Starting from 72 hours after transfection, ACC-α RNA interference (RNAi) markedly reduced the synthesis of new lipids compared with the control levels, and this effect lasted for at least till 120 hours after transfection (Fig. 1C). A similar inhibition was observed for FAS siRNA (Fig. 1C).

To investigate the effect of ACC-α RNAi and FAS RNAi on different lipid species, lipid extracts of LNCaP cells were analyzed by TLC. The majority of 14C-label in control cells was incorporated into phospholipids and smaller but substantial amounts of label were found in triglycerides and in free cholesterol. Both ACC-α and FAS RNAi caused a significant and comparable decrease in the synthesis of phospholipids and triglycerides (Fig. 1D). The synthesis of cholesterol was not affected by FAS RNAi but was reduced by ACC-α RNAi.

Both ACC-α and fatty acid synthase RNA interference inhibit cell proliferation and induce apoptosis of prostate cancer cells. To investigate the effect of ACC-α RNAi and FAS RNAi on cell proliferation and cell survival, LNCaP cells were stained with trypan blue at different time points after transfection with siRNA targeting ACC-α, FAS or Luc. Cell counting showed that silencing of the ACC-α gene caused a stagnation of the number of viable cells, whereas control cells continued proliferating normally (Fig. 2A). BrdUrd incorporation experiments revealed that ACC-α RNAi significantly decreased proliferation of LNCaP cells (Fig. 2B). Counting the percentage of dead cells after trypan blue staining showed a significant increase in cell death starting at 72 hours after transfection (Fig. 2C). Similar effects on cell proliferation and survival were observed in LNCaP cells transfected with siRNA targeting FAS. In contrast, no effects on cell proliferation or viability were observed after transfection with siRNA targeting Luc.

Figure 3. ACC-α RNAi and FAS RNAi induce apoptosis of LNCaP cells. A, Western blot analysis showing the cleavage of PARP in LNCaP cells, transfected with siRNA targeting ACC-α, FAS, or Luc, at 24, 48, 72, 96, and 120 hours after transfection. Cytokeratin-18 (CK18) expression was used as an internal control. At 96 hours after transfection with Luc siRNA (B), ACC-α siRNA (C), or FAS siRNA (D), LNCaP cells were stained with Hoechst 33342 and apoptosis was analyzed by fluorescence microscopy. Addition of z-VAD-fmk, 24 hours after transfection, suppressed LNCaP cell apoptosis induced by inhibition of ACC-α and FAS as revealed at 96 hours after transfection with ACC-α siRNA (E) or FAS siRNA (F). Bar, 100 μm.

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To investigate the effects on cell proliferation more in detail, we analyzed the cell cycle distribution of LNCaP cells using a FACS. In agreement with the cell death observed after trypan blue staining, ACC-α and FAS RNAi increased the percentage of sub-G1 cells (which are assumed nonviable) at 72 hours after transfection (Fig. 2D). Both inhibition of ACC-α and of FAS also induced a 2-fold decrease of the fraction of cells in the S phase (Fig. 2D).

To study the mechanism of cell death induced by inhibition of fatty acid synthesis, we analyzed cleavage of PARP, a nuclear proteinase which is one of the main targets for cleavage by caspases during the apoptotic process. As revealed by Western blot analysis, LNCaP cells transfected with ACC-α or FAS siRNA showed positive staining for cleaved PARP, confirming apoptosis (Fig. 3a). No cleaved PARP was observed in control cells. In addition, Hoechst 33342 staining of LNCaP cells at 96 hours after transfection with siRNA targeting ACC-α or FAS (Fig. 3C-D) showed the presence of nuclear fragmentation and chromatin condensation, typical hallmarks of apoptosis, which were not observed in Luc siRNA–transfected LNCaP cells (Fig. 3B). To confirm the involvement of caspases, we examined the effects of z-VAD-fmk, a general caspase inhibitor, on the internucleosomal degradation of DNA. To this end, LNCaP cells were treated with z-VAD-fmk 24 hours after transfection with siRNA targeting ACC-α or FAS. Hoechst 33342 staining at 96 hours after transfection revealed that apoptosis induced by inhibition of ACC-α as well as by inhibition of FAS were both significantly suppressed by z-VAD-fmk (Fig. 3E-F). Using fluorescence microscopy, quantitative analysis of Hoechst 33342 stainings at 96 hours after transfection was done by counting the percentages of Hoechst-positive cells per optical field: 3.4 ± 1.9% for Luc siRNA, 21.2 ± 7.1% for ACC-α siRNA, 21.3 ± 4.9% for FAS siRNA, 6.3 ± 3.1% for ACC-α siRNA supplemented with z-VAD-fmk, and 5.8 ± 2.2% for FAS siRNA supplemented with z-VAD-fmk [ACC-α siRNA and FAS siRNA conditions in the absence of z-VAD-fmk were significantly different (P < 0.05) from the control (Luc siRNA), n = 7-12].

In an attempt to find out whether tumor cell death, observed after inhibition of fatty acid synthesis, is due to an accumulation of the toxic intermediate malonyl-CoA or to starvation of fatty acids, we quantitated the levels of malonyl-CoA in acid-soluble extracts from LNCaP cells transfected with siRNA targeting ACC-α, FAS, or Luc using reversed-phase HPLC. FAS inhibition markedly increased the levels of malonyl-CoA. Although inhibition of ACC-α induced tumor cell death, ACC-α RNAi had no effect on malonyl-CoA levels (Fig. 4).

In a second step, we investigated the effects of exogenous palmitate, the predominant product of the fatty acid synthesis pathway, on the viability of LNCaP cells transfected with siRNA targeting FAS, ACC-α, or Luc. The cytotoxicity induced by FAS inhibition was decreased ~2-fold after addition of palmitate (Fig. 5). To a lesser extent, palmitate also rescued the cytotoxic effects induced by ACC-α RNAi.

Effect of RNA interference–mediated inhibition of ACC-α and fatty acid synthase on nonmalignant human fibroblasts. To investigate whether the cytotoxic effects induced by silencing of ACC-α are specific for cancer cells, we analyzed the effect of ACC-α RNAi on nonmalignant human fibroblasts, which show a low lipogenic activity (17, 25). 2-14C-labeled-acetate incorporation assays revealed that ACC-α and FAS RNAi significantly inhibited the already low de novo synthesis of lipids in these fibroblasts (Fig. 6A). However, in contrast with LNCaP cells, silencing of the ACC-α gene or of the FAS gene did not affect proliferation nor viability of fibroblasts (Fig. 6B-C).

Discussion

To gain more insight into the mechanisms underlying cytotoxicity induced by FAS inhibition and to explore whether also other lipogenic enzymes are potential targets for cancer intervention, we have selectively knocked down ACC-α expression in prostate cancer cells using RNAi and have compared the effects with those of FAS RNAi. Our data show that down-regulation of ACC-α and FAS evoked a comparable inhibition of LNCaP cell proliferation and resulted in both cases in caspase-mediated apoptosis. These similar effects were observed despite the fact that FAS siRNA increased intracellular malonyl-CoA levels 9-fold, whereas no accumulation of this metabolite was observed after transfection with ACC-α siRNA. Moreover, administration of exogenous palmitate, the end product of FAS, rescued the cytotoxic effects of FAS RNAi and to a lesser extent also those of ACC-α RNAi, suggesting that depletion of end products (fatty acids) may be the major component in the observed cytotoxic effects. The observation that the rescue effect of palmitate was more outspoken in LNCaP cells transfected with FAS siRNA than in those transfected with ACC-α siRNA, may be explained by at least two facts: (a) In contrast to FAS, ACC-α is also involved in fatty acid elongation. (b) Whereas FAS RNAi did not affect the synthesis of cholesterol, ACC-α RNAi slightly reduced the synthesis of cholesterol, which fits in with previous studies in human liver reporting that malonyl-CoA rather than acetyl-CoA is incorporated in mevalonate, a precursor of cholesterol (29). Both fatty acid elongation and synthesis of cholesterol cannot be rescued by administration of palmitate. Additional involvement of changes in the NADPH levels in the induction of cell death can not be excluded (30). It is worth mentioning that ACC-α and FAS RNAi also cause inhibition of lipogenesis, growth arrest, and cell death in LNCaP cells cultured in the absence of androgens (charcoal-treated FCS) or in the presence of 0.1 nmol/L R1881 (data not shown), thereby indicating that ACC-α or FAS RNAi-mediated growth inhibition/cytotoxicity of prostate cancer cells does not depend on the presence or absence of androgens. Interestingly, nonmalignant fibroblasts with a low basal rate of lipogenesis show no growth retardation or cell death after

**Figure 4.** Effect of ACC-α RNAi and FAS RNAi on malonyl-CoA accumulation. LNCaP cells were transfected with siRNA targeting ACC-α, FAS, or Luc and after 72 hours intracellular malonyl-CoA levels were quantitated using reversed-phase HPLC. Columns, means (n = 8); bars, ±SD. *, significantly different from control (Luc siRNA–transfected cells) by Tukey test.
treatment with RNAi targeting ACC-α and this despite a marked further decrease in lipogenesis, suggesting that cytotoxic effects may have some selectivity for tumor cells overexpressing lipogenic enzymes. Why similar data where not obtained with TOFA [5-(tetradecyloxy)-2-furoic acid], an allosteric inhibitor of ACC, remains to be explored (23).

The significance of increased lipogenesis in tumor cells and the mechanisms by which interference with this increased lipogenesis may result in tumor cell death certainly merit further investigation. It is obvious that one of the basic characteristics of malignant cells is uncontrolled proliferation and that proliferation requires membrane synthesis. Both RNAi-mediated silencing of ACC-α and of FAS induced growth arrest of LNCaP cells in the G1 phase and membrane synthesis has been linked to the late G1 phase of the cell cycle (31, 32). It is less obvious why tumor cells would derive the required lipids for membrane synthesis preferentially from endogenous lipid synthesis. Moreover, lipogenesis is also increased in nonproliferating tumor cells (1). Enhanced endogenous lipogenesis may not only have quantitative effects on lipid and membrane synthesis but also qualitative effects. In fact, in view of the inability of human cells to synthesize polyunsaturated fatty acids from fully saturated precursors produced by FAS, the newly synthesized phospholipids in tumor cells are mainly saturated and monounsaturated. Recent reports indicate that saturated and monounsaturated phospholipids together with cholesterol and sphingolipids tend to partition into detergent-resistant membrane microdomains (33). Membrane microdomains or “lipid rafts” have been shown to be involved in several key cellular processes including signal transduction, intracellular trafficking, cell polarization, and migration (33–36). We observed that both inhibition of ACC-α and of FAS mainly affected the synthesis of raft-associated lipids in prostate cancer cells, whereas non–raft-associated lipids were less dependent on ACC-α and FAS activity (37).1 The finding that fatty acid synthesis in cancer cells preferentially affects the composition of membrane microdomains opens the possibility that the increased lipogenesis in cancer cells may also affect key processes such as signal transduction, intracellular trafficking, and tumor cell migration. Interestingly, in yeast, inactivation of ACC-α completely inhibits vegetative growth and causes cell death even after supplementation of fatty acids (38). To investigate the specific function of ACC-α in Saccharomyces cerevisiae, a temperature-sensitive mutant of ACC-α has recently been constructed. When

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Figure 5. Exogenous palmitate partially rescues the cytotoxicity induced by inhibition of fatty acid synthesis. LNCaP cells were transfected with siRNA targeting ACC-α, FAS, or Luc; 4 hours after transfection palmitate-BSA was added to the culture medium to a final concentration of 80 μmol/L. After 72 hours of incubation, cells were collected and stained with trypan blue and the percentage of dead cells was counted. Columns, means (n = 3); bars, ± SD. Representative of two independent experiments.

Figure 6. Effect of ACC-α RNAi and FAS RNAi on nonmalignant human fibroblasts. A, fibroblasts were transfected with siRNA targeting ACC-α, FAS, or Luc. After 72 hours, cells were treated with 2-14C-labeled acetate for 4 hours. Lipid extracts were prepared and 14C-incorporation was quantitated. Proliferation (B) and viability (C) of fibroblasts transfected with ACC-α, FAS, or Luc siRNA, as revealed by counting the number of viable cells and the percentage of dead cells respectively, using the trypan blue exclusion assay. Columns, means (n = 3–8); bars, ± SD. *, significantly different from control (Luc siRNA-transfected cells) by Tukey test.
grown at the restrictive temperature, these yeast cells developed an altered nuclear envelope, showed severe abnormalities in spindle formation, became arrested in the G2-M phase of the cell cycle, and finally lost viability (39). In this system, inactivation of ACC-α caused a sharp decline in the production of inositol-ceramides and very long chain fatty acids. It would certainly be worthwhile to study whether similar alterations in lipid synthesis are also observed in human cancer cells treated with RNAi-targeting ACC-α.

In conclusion, the present data suggest that not only FAS but also ACC-α may be an interesting target for the development of novel forms of cancer prevention and therapy. Selective inhibition of ACC-α indicates that at least in the prostate tumor cell line LNCaP cell death is not related to accumulation of malonyl-CoA. The ability to selectively inhibit different steps in the lipogenic pathway may contribute to a better understanding of the mechanisms by which increased lipogenesis and inhibition of this increased lipogenesis alters tumor cell behavior.

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References

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