Acetyl-CoA Carboxylase α Is Essential to Breast Cancer Cell Survival

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Abstract

Activation of de novo fatty acid synthesis is a characteristic feature of cancer cells. We have recently described an interaction between acetyl-CoA carboxylase α (ACCα), a key enzyme in fatty acid synthesis, and BRCA1, which indicates a possible connection between lipid synthesis and genetic factors involved in susceptibility to breast and ovarian cancers. For this reason, we explored the role of ACCα in breast cancer cell survival using an RNA interference (RNAi) approach. We show that specific silencing of either the ACCα or the fatty acid synthase (FAS) genes in cancer cells results in a major decrease in palmitic acid synthesis. Depletion of the cellular pool of palmitic acid is associated with induction of apoptosis concomitant with the formation of reactive oxygen species (ROS) and mitochondrial impairment. Expression of a small interfering RNA (siRNA)–resistant form of ACCα mRNA prevented the effect of ACCα-RNAi but failed to prevent the effect of FAS gene silencing. Furthermore, supplementation of the culture medium with palmitate or with the antioxidant vitamin E resulted in the complete rescue of cells from both ACCα and FAS siRNA–induced apoptosis. Finally, human mammary epithelial cells are resistant to RNAi against either ACCα or FAS. These data confirm the importance of lipogenesis in cancer cell survival and indicate that this pathway represents a key target for antineoplastic therapy that, however, might require specific dietary recommendation for full efficacy. (Cancer Res 2006; 66(10): 5287-94)

Introduction

Numerous lines of evidence suggest that activation of fatty acid synthesis is required for carcinogenesis. Tumor cells synthesize 95% of saturated and monosaturated fatty acids de novo despite adequate nutritional lipid supply (for review, see ref. 1). Hence, fatty acid accumulation due to induction of de novo lipogenesis might lead to specific alterations that we previously reported in mammary epithelial cells, selective FAS or ACCα gene silencing, as well as selective FAS gene silencing, is correlated to a decline in fatty acid synthesis, growth arrest, and induction of apoptosis associated with alteration of mitochondrial function. On the contrary, in human mammary epithelial cells, selective FAS or ACCα inhibition leads to a decline in fatty acid synthesis without affecting both cell growth and cell survival. Our data provide evidence that fatty acids newly synthesized through de novo lipogenesis are essential to breast cancer cell survival and, thus, that ACCα might be considered as a key target for antineoplastic therapy. Our data also increase the potent interference between therapy based on lipogenesis inhibition and dietary lipids intake.

Materials and Methods

Cell culture. Human breast carcinoma cell lines MCF-7, MDA-MB-231, breast transformed cell line HBL100, and prostate cancer cell line LNCaP were used and tested for mycoplasm-free cell lines. Stock cultures were grown in RPMI 1640 containing 10% FCS and 1% antibiotics. The cells were grown in growth medium at 37°C in a 95% air, 5% CO2-humidified incubator. Human mammary epithelial cells (Cambrex) were grown in...
MEGM Bulletkit (Cambrex, Emerainville, France) containing growth factors, cytokines, and supplements according to the recommendation of the manufacturer.

**RNAi.** To design siRNA oligonucleotides targeting ACCα, DNA sequences of the type AA (N19): 5′-GATGAGAATGCGACAACTGG-3′ were selected according to the protocol of the manufactuer (Prologos Primers & Probes) and submitted to a BLAST search against human genome sequence to ensure reliable specificity. This sequence corresponded to exon 15, nucleotides 1,043 to 1,062 located 3′ to the first nucleotide of the start codon of the human ACCα cDNA (NM198836). The siRNA oligonucleotides targeting FAS gene were derived from previous report (18). As a non-specific siRNA control, the luciferase siRNA duplex was used as control. Transfections of human breast cancer cells with siRNA-targeting ACCα, FAS, or luciferase were done in six-well culture plates at a density of 1 × 10^6 per well using Lipofectamine 2000 (Invitrogen, Cergy-Pontoise, France) and 0.2 nmol of siRNA duplex, resulting in a final siRNA concentration of 100 nmol/ L. siRNA-transfected cells were collected at 0, 24, 48, 72, and 96 hours after transfection for real-time PCR analysis. Cells were used 72 hours after transfection for fatty acid analysis and 96 hours after transfection for Western blot analysis. Cells were harvested at different time points after transfection according to the cell type for apoptosis analysis (96 hours for HBL-100 and MDA-MB-231, 144 hours for MCF-7 and LNCaP).

**Construction of wild-type and siRNA-resistant ACCα minigenes.** The ACCα sequences corresponding to NH2-terminal (4019 bp) and COOH-terminal (3,090 bp) fragments were PCR amplified from human cDNA library using respectively the primer set ACCα-Nter(+)-5′-GCTTCGTGGGACTGAGCCAGGAGGAAATAGA-3′ and ACCα-Nter(-) 5′-AAATCTCAAGGGCAGAACTTTACCTG-3′ and the reverse set ACCα-Cter(+)-5′-GGCTCCGACGATCTGGGAGCTCTACGAGCAGAGATAAGGCTTCT-3′ and ACCα-Cter(-)-5′-GAGCGCGGCTGTGGAGGAAATGCTAACGATCTTCT-3′ oligonucleotides, subcloned into appropriate vector, sequenced, and used to substitute exon 15-exon 16 sequence of human ACCα gene and the first 1000 nucleotides of COOH-terminal mRNA sequence. To construct the siRNA-resistant ACCα mutant, four mutations were introduced in the siRNA-targeted sequence: 5′-GATGAGGCGGCTAGCCAAGCAGC-3′ (substituted nucleotides are underlined). The two ACCα constructs, ACCα-WT and ACCα-m, were built by cloning the restricted fragments XhoI/Kast and KasI/NotI containing respectively the NH2-terminal and the COOH-terminal coding sequences of ACCα into the pEGM1-112Zf- vector (Promega, Charbonnieres, France) and sequenced. For the construction of ACCα minigene, the ACCα sequence corresponding to exon 15-IVS15-exon 16 fragment was amplified from human DNA using the forward 5′-ATGGCCATGGGGATCTCTTCT-3′ and the reverse 5′-AATTGTTGCTCTTCCTGTTTCTTCT-3′ oligonucleotides, subcloned into appropriate vector, sequenced, and used to substitute exon 15-exon 16 sequence of ACCα-Nter-pEGM1-112Zf- intermediate vector. For the construction of the siRNA-resistant ACCα mutant, four mutations were introduced in the ACCα-targeted sequence: 5′-GATGAGGCCTAGCCAAGCAGC-3′ (substituted nucleotides are underlined). The two ACCα constructs, ACCα-WT and ACCα-m, were built by cloning the restricted fragments XhoI/Kast and Kast/NotI containing respectively the NH2-terminal and the COOH-terminal coding sequences of ACCα into the pEGM1-112Zf- vector (Promega, Charbonnieres, France) and sequenced.

**Real-time PCR analysis.** Quantitative reverse transcription-PCR (RT-PCR) analysis for ACCα gene was done as previously described (21) using a TaqMan PCR on a sequence system (ABI prism 7700; Applied Biosystems, Courtaboeuf, France). Primer sets for human FAS mRNA were forward primer 5′-AAACCAGGCTCTCTCTCTTCT-3′, reverse primer 5′-TTGGGCCTCCAGCATTGCTAT-3′, and FAS probe 5′-FAM-CTCAGGCGGCGAGTTCGGCCTG-3′ (substituted nucleotides are underlined). A standard curve for ACCα and FAS mRNA quantification was generated from a plasmid containing target sequences of ACCα and FAS in mammalian expression vector (Invitrogen).

**Western blot analysis.** Total protein extracts were obtained 96 hours after transfection using a reducing SDS buffer containing 50 mmol/L Tris-base, 6% formaldehyde, 20% glycerol, 1% SDS, and 0.1% bromophenol blue, followed by 25 μL of protein solution being loaded on a 6% SDS-PAGE gel. Proteins were transferred on a nitrocellulose membrane (Trans-Blot, Bio-Rad, Hercules, CA). Membranes were blocked with a 5% nonfat dry milk and incubated with antibodies against FAS (1:10000 dilution) kindly provided by Prof. J. Swinnen, Catholic University of Leuven, Leuven, Belgium), ACCα (1:1000 dilution; kindly provided by Nicole Dalla Venezia, CNRS-UMR 5290, Lyon, France; ref. 20), or myc (1:100 dilution; 9E10, Santa Cruz Biotechnology, Santa Cruz, CA). Peroxidase-conjugated secondary antibodies (Amersham, Orsay, France) were used for detection of immunoreactive proteins by enhanced chemiluminescence plus system (Amersham).

**Results**

**Effect of siRNA-targeting ACCα on mRNA and protein levels in human breast cancer cells.** To specifically silence the ACCα gene, breast tumor cell lines (MDA-MB-231 and MCF-7) and mammary epithelial transformed cell line (HBL100) were transfected with siRNA-targeting ACCα mRNA. To further compare ACCα and FAS inhibition effect, silencing of FAS gene by RNAi was also done according to previous report (18). As shown in Fig. 1, siRNA-targeting ACCα severely suppressed expression of ACCα when compared with control cells. For the HBL100 cell line, basal ACCα expression level was too low to determine ACCα protein level after siRNA treatment (see basal ACCα expression in Fig. 1A, top). In the same way, siRNA-targeting FAS suppressed expression of FAS in tumor cell lines when compared with control. Interestingly, although treatment of the three cell lines with siRNA-targeting ACCα had no effect on the levels of FAS, depletion of FAS by siRNA in both MCF-7 and MDA-MB-231 cells also slightly reduced the levels of ACCα (Fig. 1B).
Effect of siRNA-targeting ACCα on cell growth and cell apoptosis. To examine the effect of ACCα siRNA on cell phenotype, we examined several hallmarks of cell apoptosis and cell proliferation. ACCα siRNA–transfected cells were analyzed by three-color flow cytometry after staining with hydroethidium that stains cells producing reactive oxygen species (ROS) and with DiOC6 that stains cells with normal mitochondrial function. Indeed, some apoptotic pathways are linked to mitochondrial perturbations, such as the loss of the mitochondrial membrane integrity, resulting in the collapse of the mitochondrial membrane potential reflected by decreased binding of DiOC6 (for review, see ref. 22). Cells were also stained with Hoechst 33342, allowing quantification of DNA content. In parallel, the cell numbers were determined with a cell counter. Before applying this method, we had done trypan blue exclusion and Annexin V staining to establish the apoptotic pathway. The prostate cancer cell line LNCaP previously described as sensitive to ACCα and FAS siRNA–induced apoptosis was used as a positive control (18, 19). In both MDA-MB-231 and HBL100 tumor cell lines, transfection with siRNA-targeting ACCα or FAS for 96 hours resulted in an increase in DiOC6-negative cells, from 1% to 3% in the control cells to up to 80% to 90% and 70% to 80% in the ACCα and FAS siRNA–treated cells, respectively (Fig. 2A, black columns). This effect is already significant at 72 hours posttransfection, with an increase in DiOC6-negative cells to up to 55% and 40% in the ACCα and FAS siRNA–treated cells, respectively (data not shown). Moreover, whatever the cell line and in both ACCα and FAS siRNA, ROS formation is restricted to DiOC6-negative cells (Fig. 2A, gray columns). This effect is less marked in MCF-7 and LNCaP cell lines, with apoptotic cells increasing from 5% in the control cells to up to 25% to 40% in cells treated by ACCα or FAS siRNA (data not shown). Finally, percentage of Hoechst low-intensity cells matched with the percentage of DiOC6-negative/ROS-positive cells (Fig. 2A, white columns). Counting the number of cells revealed that ACCα and FAS siRNA results in a stagnation of the number of cells (Table 1) whereas control cells continue to proliferate. Apparently, for both cell lines, ACCα or FAS silencing–induced apoptosis did not result in the accumulation of cells in a particular phase of the cell cycle (Table 1).

To address whether ROS production is the cause of HBL-100 and MDA-MB-231 cell death induced by ACCα and FAS siRNA, we next used the free radical–scavenging antioxidant α-tocopherol, the most biologically active form of vitamin E. For both siRNA transfections, we showed that α-tocopherol is able to block the ROS formation, as well as the loss of mitochondrial membrane potential and the occurrence of hypoploidy (Fig. 2A), without affecting cell cycle phase distribution (Table 1), except for the HBL-100 cell line in which α-tocopherol slightly increased the ratio of S-phase cells.

Our present data provide evidence that RNAi-mediated ACCα or FAS gene silencing inhibits cell growth and leads to apoptosis induced by ROS production, which is suppressed by the lipid-soluble radical-scavenging antioxidant α-tocopherol.

Effect of siRNA-targeting ACCα on de novo fatty acid synthesis and fatty acid composition in human breast cancer cells. To determine the metabolic consequences of RNAi treatment, de novo fatty acid synthesis was quantified by measuring deuterated acetate incorporation into palmitic acid using gas chromatography. As expected, deuterated acetate is incorporated into palmitic acid in control cells (Fig. 2B, histogram). In ACCα and FAS siRNA–transfected cells, incorporation of deuterated acetate into 16:0 was drastically reduced. The magnitude of this decrease ranged from ~91% with FAS siRNA to ~97% with ACCα siRNA.
relative to nontransfected cells or to control cells transfected with luciferase siRNA, showing that de novo fatty acid synthesis and palmitic acid content in human breast cancer cells. SiRNA-transfected tumor cells (A and B) and nonmalignant mammary epithelial cells H-MEC (C) in the presence or absence of vitamin E (vitE; 10 μmol/L) were analyzed by flow cytometry (A and C, left) and by fatty acid measurement (B and C, right). A and C, left; mitochondrial potential membrane was measured using DiOC_{6}, ROS was measured by fluorescent probe hydroethidium, and DNA content was measured using Hoechst. Black columns, percentage of DiOC_{6}-negative cells; gray columns, percentage of hydroethidium-positive cells; white columns, percentage of cells with DNA hypoploidy. Columns, mean of three independent experiments done in duplicate; bars, SD. B and C, right, cells were collected for fatty acid analysis 72 hours after transfection with siRNA-targeting ACCα and FAS in the presence or absence of vitamin E. Deuterated PL-14:0 was added as an internal standard just before lipid extraction. Thereafter, cellular lipids were extracted as described in Materials and Methods. Incorporation of deuterated acetate into palmitic acid (16:0) (histogram) along with absolute amount of 16:0 (graph) was determined by gas chromatography. Columns and points, mean of three independents experiments; bars, SD. *, significantly different from control (luciferase siRNA–transfected cells) by Mann-Whitney test.

Table 1. Effect of ACCα and FAS RNAi on cell number and cell cycle distribution with or without vitamin E supplementation

|              | Final no. cells (mean × 10^{3} ± SD) | Cell cycle         | Final no. cells (mean × 10^{3} ± SD) | Cell cycle         |
|--------------|--------------------------------------|--------------------|--------------------------------------|--------------------|
|              |                                      | G_{0}/G_{1} (%)    | S (%)                                | G_{2}/M (%)        |
|              |                                      |                    |                                      |                    |
| Control      | 86 ± 4.3                             | 65.8               | 20.6                                 | 13.6               |
| siRNA LUC    | 84.5 ± 3.3                           | 60.8               | 30.8                                 | 8.4                |
| siRNA ACCα   | 72.2 ± 2.4                           | 77.7               | 17.2                                 | 5.1                |
| siRNA FAS    | 19.7 ± 1.8                           | 72.8               | 20.1                                 | 7.1                |
| siRNA ACCα + vitamin E | 62.5 ± 3.1 | 60.9               | 24.2                                 | 14.9               |
| siRNA FAS + vitamin E | 59.8 ± 3.8 | 59.8               | 24.5                                 | 15.6               |
| vitamin E    | 69.5 ± 2.8                           | 66.3               | 24.0                                 | 9.7                |
|              |                                      |                    |                                      |                    |
|              |                                      | 100.2 ± 8.1        | 88.8                                 | 10.6               |
|              |                                      |                    |                                      | 0.6                |
|              |                                      | 90.5 ± 3.4         | 88.6                                 | 10.9               |
|              |                                      |                    | 26.7 ± 2.6                           | 11.9               |
|              |                                      |                    | 22.7 ± 2.2                           | 7.8                |
|              |                                      |                    | 52.8 ± 2.6                           | 10.9               |
|              |                                      |                    | 51.4 ± 2.6                           | 10.2               |
|              |                                      |                    | 73.3 ± 2.9                           | 9.5                |
|              |                                      |                    |                                      | 0.8                |

NOTE: Transfections were done at a density of 1 × 10^{3} tumor cells using a final siRNA concentration of 100 nmol/L. Twenty-four hours after transfection, 10 μmol/L vitamin E was added to the medium. Cells were counted with a cell counter 96 hours after transfection, stained with Hoechst, and then analyzed for DNA content by flow cytometry. Results were provided from three independent experiments done in duplicate.
(Fig. 2C, right); this decrease did not affect proliferation nor viability of these normal epithelial cells (Fig. 2C, left).

Rescue of ACCα knockdown–induced apoptosis by overexpressing a siRNA-resistant ACCα mutant. To confirm the specificity of ACCα-RNAi, synthetic genes expressing either wild-type ACCα (ACCα-wt) or siRNA-resistant ACCα mutant (ACCα-m) were constructed and transfected into cells 24 hours before ACCα siRNA transfection. The ectopically expressed proteins were detected with an anti-myc antibody. As a negative control, both vectors expressing wild-type ACCα-wt and ACCα-m were cotransfected with FAS siRNA. The overexpression of the ACCα-m in the ACCα-siRNA–treated cells completely reversed the siRNA phenotype, preventing cell death (Fig. 3A) and restoring cell viability (compared the final number of cells in Tables 1 and 2). As predicted, overexpression of the ACCα-wt fails to prevent apoptosis induced by either ACCα or FAS knockdown (Fig. 3A). Palmitic acid content and de novo fatty acid synthesis were assessed in the siRNA-treated cells cotransfected with the ACCα-wt and ACCα-m synthetic genes. As shown in Fig. 3B (histogram), the drastic decrease of 16:0 induced by ACCα siRNA is blocked by cotransfection with the ACCα-m (95% versus 34%). Moreover, the pool of palmitic acid is fully restored by ACCα-m overexpression in MDA-MB-231 (Fig. 3B, right graph). In contrast, ACCα-m is incapable of restoring the levels of 16:0 decreased by FAS siRNA (90% versus 86%). Similarly, overexpression of ACCα-wt fails to restore 16:0 level in both ACCα and FAS siRNA–treated cells (95% versus 70.5% and 90% versus 85%, respectively).

Rescue of ACCα and FAS knockdown–induced apoptosis by supplementation with palmitic acid 16:0. To determine whether ACCα silencing–induced apoptosis is triggered by palmitic acid depletion, we tested the effect of supplementation with 100 μmol/L 16:0 on siRNA-transfected cell lines by three-color flow cytometry. As shown in Fig. 4A (histogram), 16:0 prevents ROS production, loss of mitochondrial membrane potential, as well as hypoploidy caused by ACCα knockdown, showing that 16:0 is sufficient to block the occurrence of siRNA-induced apoptosis. A dose-response assay reveals that the lower dose used (100 nmol/L 16:0), which is 10-fold higher than the background levels of palmitic acid in the medium, is also sufficient to prevent apoptosis (data not shown). Palmitic acid supplementation also rescues cells from apoptosis induced by FAS RNAi (Fig. 4A). De novo lipogenesis is not restored by palmitic acid supplementation of either ACCα- or FAS-silenced cells, as expected (Fig. 4B). Cell cycle progression and final cell numbers of siRNA-transfected cells as well as nontransfected cells are not affected by 16:0 supplementation (data not shown).

Taken together, a critical concentration of palmitic acid or metabolic derivatives of palmitic acid, resulting from either de novo lipogenesis or from circulating lipids, is essential for the survival of breast cancer cells.

Discussion

The present study was undertaken to examine the role of ACCα activity in breast cancer cell survival. Previous studies showed that FAS inhibitors, cerulenin and C75, and RNAi-silencing FAS are proapoptotic for breast cancer cells (11–13, 15–18, 23). On the other hand, the specific ACCα inhibitor 5-(tetradecyloxy)-2-furoic acid had no effect on cell proliferation and cell apoptosis, and preincubation of cells with 5-(tetradecyloxy)-2-furoic acid protected them against FAS inhibitor cytotoxicity (14, 17). Here, we show that, in contrast to the chemical inhibition by 5-(tetradecyloxy)-2-furoic acid, the inhibition of ACCα activity by RNAi results in a marked decrease of lipogenesis, leading to induction of cell apoptosis. This observation on breast cancer cells corroborates recent data on the prostate cancer cell line LNCaP that showed that cytotoxic mechanism of FAS inhibition is unrelated to malonyl-CoA accumulation (19). The ACCα silencing by RNAi is specific as the restoration of cellular palmitic acid content by palmitic acid supplementation or ACCα overexpression

Figure 3. Rescue of ACCα knockdown–induced apoptosis and restoration of ACCα enzymatic function by overexpressing a siRNA-resistant ACCα mutant. Synthetic genes expressing either wild-type ACCα (ACCα-wt) or siRNA-resistant ACCα mutant (ACCα-m) were transfected to cells before ACCα and FAS siRNA transfection. A, the effect of cotransfection on apoptosis and ROS was determined by flow cytometry after staining with DiOC6 (black columns) and hydroethidium (gray columns). Columns, mean of three experiments done in duplicate; bars, SD. Moreover, in HBL-100, Western blot showed the level of expressed proteins detected with an anti-myc antibody. B, the effect of cotransfection on ACCα enzymatic function was followed by analyzing total palmitic acid content (graph) and incorporation of deuterated acetate into palmitic acid (histogram). Columns and points, mean of three independent experiments; bars, SD. *, significantly different from control (luciferase siRNA–transfected cells) by Mann-Whitney test.
fully restores cell viability. The specific suppression of ACCα as well as the activity of FAS results in oxidative stress associated with mitochondrial impairment; these are prevented by the lipid-soluble antioxidant vitamin E. The discrepancy between the effect of ACCα gene silencing by specific RNAi and the effect of 5-(tetradecyloxy)-2-furoic acid evokes a nonspecific pleiotropic effect of 5-acyl-2-furoic acid, affecting pathways other than fatty acid synthesis, which, in turn, might counteract the induction of oxidative stress and apoptosis. Thus, our data showed that, together with FAS, ACCα could be considered as a target for breast cancer therapy.

Exogenous palmitic acid intake counteracts the beneficial effect of ACCα and FAS inhibition against cancer cell survival. Thus, the potential of using the lipogenesis pathway as a target for antineoplastic therapy based on dietary intervention with palmitic acid depends on the daily intake and uptake of this fatty acid. Quantification of palmitate in healthy women sera reveals an average content of 1,200 μmol/L, 3,12-fold higher than the dose of 100 μmol/L used in our study. Even if equivalence between human serum and cell-culture medium is unknown, the potential of using ACCα as a target for antineoplastic therapy based on dietary intervention with palmitic acid seemed to be unlikely. In this respect, additional investigations, such as dietary interventions (i.e., ω-3 fatty acids or conjugated linoleic acid; ref. 24), are required to improve and to confirm the anticancer potential of inhibition of lipogenesis.

Interestingly, dietary palmitic acid has been described as an allosteric inhibitor of fatty acid synthesis in lactating mammary gland (25), likely through the allosteric inhibition of ACCα. It is obvious that, in this study, breast tumor cells lack the feedback regulation of fatty acid synthesis by dietary long-chain saturated palmitic acid independently to ACCα level expression. We hypothesize that this feedback inhibition deficiency of fatty acid synthesis is one of the features of premalignant cell survival strategy, maybe to adjust the insufficient supply of dietary fatty acids. The loss of feedback inhibition of ACCα may occur at numerous levels of transcriptional or posttranslational regulation, as ACCα is subject to global and local regulation (i.e., dietary and hormonal transcriptional regulation, adenosine monophosphate–activated protein kinase–dependent phosphorylation, and intracellular abundance of citrate or palmitoyl-CoA).

Although there is strong evidence that cancer cell proliferation and survival are dependent on de novo fatty acid synthesis, the physiologic significance of this pathway is largely unknown. It is reasonable to propose that, in dividing tumor cells, synthesis of fatty acids might provide a large pool of available lipids required for the newly synthesized cellular membrane. Thus, as we have shown in this study, lipogenesis may be not essential for high cell proliferation when a sufficient amount of exogenous fatty acids is present in the microenvironment. Fatty acid is likely essential to the control of membrane potential to maintain mitochondria integrity as lipid depletion per se induces oxidative stress by impairing mitochondrial function.

On the other hand, according to the extremely attractive theory recently proposed by Hochachka et al. (26), it is also conceivable that enhanced lipogenesis in tumor cells represents an adaptive response to control redox imbalance caused by glycolytic metabolism. It is well known that cancer cells have an unusual tolerance to limiting O2 availability with an unusual carbohydrate metabolism exhibiting excessive lactate production by aerobic glycolysis. In both normal and malignant prostate cells, three major pathways—lactate dehydrogenase, the respiratory chain, and lipid synthesis—are involved in the regulation of the redox balance. Interestingly, in prostate cancer cells, because intracellular O2 supplies decline (i.e., hypoxic condition), the respiratory chain cannot fully oxidize the reducing equivalents being formed and, in this metabolic context, increased lipogenesis has been proposed to be the main physiologic function to maintain redox balance through oxidation of NADPH during fatty acid synthesis. This hypothesis envisaged fatty acid synthesis as a means to mop up excess reducing potential in cancer cells. Finally, the three above speculations—roles of lipogenesis in proliferation, mitochondria function, and hypoxia defense—are not exclusive.

### Table 2. Effect of the ACCα synthetic gene overexpression on cell number and cell cycle distribution in siRNA-treated cells

|                | HBL-100     | MDA-MB-231  |
|----------------|-------------|-------------|
|                | Final no. cells (mean × 10^4 ± SD) | Cell cycle | Final no. cells (mean × 10^4 ± SD) | Cell cycle |
| Control        | 86 ± 4.3    | 65.8        | 20.6 | 13.6 | 100.2 ± 8.1     | 88.8 | 10.6 | 0.6 |
| pcDNA          | 87 ± 3.8    | 63.1        | 31.9 | 5.0 | 88.9 ± 6.5     | 82.8 | 16.6 | 0.6 |
| ACCα-m         | 87.2 ± 4.4  | 53.0        | 34.9 | 12.1 | 70.7 ± 5.7     | 87.1 | 12.3 | 0.6 |
| siRNA ACC + ACCα-m | 82.1 ± 4.9  | 46.5        | 33.2 | 20.3 | 72.8 ± 3.6     | 81.8 | 17.7 | 0.5 |
| ACCα-wt        | 88.2 ± 7.1  | 50.5        | 35.9 | 13.6 | 85.9 ± 5.2     | 83.6 | 15.5 | 0.9 |
| siRNA ACC + ACCα-wt | 44.1 ± 3.5  | 59.6        | 26.2 | 14.2 | 45.9 ± 3.7     | 93.4 | 6.6  | 0  |
| siRNA FAS + ACCα-m | 32.9 ± 2.3  | 58.0        | 28.2 | 13.8 | 31.0 ± 2.2     | 88.5 | 11.0 | 0.5 |
| siRNA FAS + ACCα-wt | 32.1 ± 2.0  | 70.8        | 20.4 | 8.8 | 34.8 ± 1.7     | 86.7 | 13.1 | 0.2 |

NOTE: Synthetic genes expressing either wild-type ACCα (ACCα-wt) or siRNA-resistant ACCα mutant (ACCα-m) were transfected to 1 × 10^5 tumor cells 24 hours before ACCα and FAS siRNA transfections using a final siRNA concentration of 100 nmol/L. Cells were counted with a cell counter 96 hours after transfection, stained with Hoechst, and then analyzed for DNA content by flow cytometry. Results were provided from three independent experiments done in duplicate.

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3 Personal communication.
A recent publication has just strengthened the link between glycolysis, lipogenesis, and tumor cell proliferation (27). Hatzivassiliou and colleagues have shown that down-regulation of ATP citrate lyase, a key enzyme linking glucose metabolism to lipid synthesis via the conversion of citrate to acetyl-CoA, affects glucose-dependent de novo lipid synthesis in tumor cells, which leads to an inhibition of tumor cell proliferation. Cancer cells displaying high rates of glucose metabolism are strongly affected whereas those displaying a low rate of aerobic glycolysis are unaffected. Those data reinforce our analysis of the importance of lipogenesis for tumor cell proliferation.

According to experimental studies, α-tocopherol seems to exhibit a dual and opposite role on mammary carcinogenesis considering its preventive or curative effect. If tocopherol may be chemopreventive to breast cancer (28), it could enhance tumor growth and metastasis in animal model developing breast tumor (29–31). The fact that α-tocopherol blocks apoptosis induced by ACC or FAS deficiency is in agreement with a stimulatory effect of antioxidants in tumor growth.

Our previous epidemiologic findings involving biological measurements of dietary fat intake support a metabolic role of lipids in mammary carcinogenesis. In humans, dietary saturated palmitic acid seems to be associated with an increased risk of breast cancer (32, 33), an observation which fits well with our experimental results on effects of palmitic acid supplementation in breast cancer cells. Moreover, the fatty acid composition in serum seems to influence the fatty acid composition of cell membranes, showing that dietary fatty acids influence cellular fatty acid composition (34). In already established breast cancer, membrane-saturated fatty acid composition is profoundly altered compared with the surrounding healthy tissue (2). Such an alteration, from both metabolic and dietary origins, can have profound effects on many cellular processes, such as hormonal response. Although there is good evidence that cellular lipid content is essential in mammary carcinogenesis, the question of how fatty acids could enhance mammary carcinogenesis still needs to be clarified.

Taken together, RNAi-mediated silencing of ACCα gene establishes ACCα as a potential target for anticancer therapy and would allow to define the role of ACCα, and consequently saturated palmitic acid, in cancer cell biology. However, dietary intake of lipids, such as palmitate and vitamin E, might be taken into account to fully estimate efficacy of anticancer therapy based on inhibition of lipogenesis.

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