Mechanism of Apoptosis Induced by the Inhibition of Fatty Acid Synthase in Breast Cancer Cells

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Abstract

Fatty acid synthase (FAS) has been found to be overexpressed in a wide range of epithelial tumors, including breast cancer. Pharmacologic inhibitors of FAS cause apoptosis of breast cancer cells and result in decreased tumor size in vivo. However, how the inhibition of FAS induces apoptosis in tumor cells remains largely unknown. To understand the apoptotic pathway resulting from direct inhibition of FAS, we treated breast tumor cells with or without FAS small interfering RNA (siRNA) followed by a microarray analysis. Our results indicated that the proapoptotic genes BNIP3, tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), and death-associated protein kinase 2 (DAPK2) were significantly up-regulated on direct inhibition of the FAS gene. We also found that the knockdown of FAS expression significantly increased ceramide level in the tumor cells, and this increase was abrogated by acetyl-CoA carboxylase inhibitor. In addition, carnitine palmitoyltransferase-1 (CPT-1) inhibitor up-regulated the ceramide and BNIP3 levels in these cells, whereas treatment of tumor cells with FAS siRNA in the presence of a ceramide synthase inhibitor abrogated the up-regulation of BNIP3 and inhibited apoptosis. Furthermore, we found that treatment of cells with BNIP3 siRNA significantly counteracted the effect of FAS siRNA-mediated apoptosis. Consistent with these results, a significant inverse correlation was observed in the expression of FAS and BNIP3 in clinical samples of human breast cancer. Collectively, our results indicate that inhibition of FAS in breast cancer cells causes accumulation of malonyl-CoA, which leads to inhibition of CPT-1 and up-regulation of ceramide and induction of the proapoptotic genes BNIP3, TRAIL, and DAPK2, resulting in apoptosis. (Cancer Res 2006; 66(11): 5934-40)

Introduction

Mammalian fatty acid synthase (FAS) is a complex multifunctional enzyme that contains seven catalytic domains and a phosphopantotheine prosthetic group on a single polypeptide and catalyzes the synthesis of palmitate from the substrates acetyl-CoA, malonyl-CoA, and NADPH (1). This enzyme also plays a pivotal role in energy homeostasis by converting excess carbon intake into fatty acids for storage, which, when necessary, provide energy via β-oxidation (1). The endogenous synthesis of fatty acid is usually minimal in cells because diet supplies most of the fatty acids, and, consequently, FAS is expressed at low or undetectable level in most normal human tissues, with the exception of lactating breast and cycling endometrium (1). In contrast, FAS is specifically overexpressed in a variety of human malignancies and therefore is considered as an ideal therapeutic target (1-4). For breast cancer, FAS has been reported to be overexpressed in tumor cells, correlate with peritumoral lymphatic vessel invasion and inversely correlate with disease-free survival (5-7). Moreover, treatment of tumor cells with pharmacologic inhibitors of FAS leads to cell growth arrest and apoptosis of breast tumor cells both in vitro and in vivo, indicating that the elevated level of FAS observed in tumor tissue actually reflects a causal role of the enzyme in tumorigenesis (8-10). However, how up-regulation of FAS promotes tumorigenesis and how inhibition of FAS leads to apoptosis in tumor cells remain unknown, although several possibilities have been speculated. It has been suggested that cell death resulting from the blockade of FAS may be metabolic in origin and occurs due to inhibition of fatty acid β-oxidation (10). Furthermore, malonyl-CoA, which accumulates after treatment of tumor cells with FAS inhibitors, has been implicated, at least in part, in mediating the cytotoxicity (10, 11). However, how the supraphysiologic level of malonyl-CoA leads to apoptosis is not yet known. In this report, we explored the mechanism of induction of apoptosis resulting from direct and specific inhibition of the FAS gene by small interfering RNA (siRNA). Our results indicate that apoptosis due to inhibition of FAS in breast tumor cells is mediated by up-regulation of ceramide following induction of the proapoptotic genes BNIP3, tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL), and death-associated protein kinase 2 (DAPK2).

Materials and Methods

Cell culture and reagents. Human breast carcinoma cell lines MCF-7, MDA-MB-231, and MDA-MB-435 were purchased from the American Type Culture Collection (Rockville, MD). The cells were cultured in RPMI 1640 supplemented with 10% FCS, 100 μg/mL streptomycin, 100 units/mL penicillin, and 250 nmol/L dexamethasone (Sigma Chemical Co., St. Louis, MO) and grown at 37°C in a 5% CO2 atmosphere. 5-( Tetradecyloxy)-2-furoic acid (TOFA; acetyl-CoA carboxylase inhibitor), fumonisin B1 (ceramide synthase inhibitor), etomoxir [carnitine palmitoyltransferase-1 (CPT-1) inhibitor], and C2-ceramide were purchased from Sigma.

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Figure 1. FAS siRNA inhibits expression of the FAS gene in breast cancer cells leading to apoptosis. A, four individual siRNAs against the FAS gene were combined into one pool, and the human breast cancer cell lines MCF-7, MDА-435, and MDА-231 were transfected with various amounts of the pooled FAS siRNA as indicated. Right, as a negative control, the cells were also transfected with GFP siRNA. Seventy-two hours after transfection, cells were collected, and the expressions of FAS and tubulin were examined by Western blot (WB). B, human breast cancer cell line MCF-7 was transfected with 300 nmol/L pooled FAS siRNA and the cells were collected at different time points after transfection as indicated and Western blot analysis for FAS and tubulin was done. C, MCF-7 cells were mock transfected (0) or transfected with three different doses (D) of 300 nmol/L (E) FAS siRNA and incubated for 72 hours (D) or for various lengths of time as indicated (E). The cells were then fixed and permeabilized, and TUNEL assay was done using the in situ cell death detection kit/TMR red. The percentage of apoptotic cells in each well was counted under confocal microscope. *, P < 0.05, statistically significant correlation.

experiments. The siRNA was transfected into the breast cancer cells using the trans-TKO transfection reagent (Mirus Corp., Madison, WI) according to the manufacturer's protocol.

Western blot. The cells were collected and resuspended in lysis buffer [50 mmol/L Tris-Cl (pH 7.4), 1% NP40, 0.25% sodium deoxycholate, 150 mmol/L NaCl, 1 mmol/L EDTA]. The lysates were boiled for 5 minutes, resolved by SDS-PAGE on a 10% polyacrylamide gel, and blotted onto nitrocellulose membrane. The membranes were treated with antibodies against FAS (0.2 μg/ml; Immuno-Biological Laboratories Co., Minneapolis, MN), tubulin (1:1,000; Upstate Biotechnology, Charlottesville, VA), BNIP3 (1:500; BioCarta, San Diego, CA), TRAIL (1:500; Stratagene, La Jolla, CA), and DAPK2 (1:500; Chemicon, Temecula, CA). The membranes were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized by enhanced chemiluminescence Plus system (Amersham Life Sciences, Piscataway, NJ).

In situ apoptosis assay. The cells were fixed with 4% paraformaldehyde in PBS followed by permeabilization with 0.2% Triton X-100/0.1% sodium citrate at 4°C. The cells were then washed extensively, and the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay was done using the in situ cell death detection kit/TMR red (Roche Applied Science, Indianapolis, IN). The reaction was stopped after 1 hour, and the percentage of apoptotic cells in each well was counted under confocal microscope.

Microarray analysis. The cells were collected, and total RNA was prepared using Qiagen (Valencia, CA) RNA isolation kit. The RNA was converted to cDNA and biotinylated following by hybridization to a human apoptosis and cell cycle-specific cDNA microarray (HS-603, SuperArray Bioscience Corp., Frederick, MD).

Real-time reverse transcription-PCR. Seventy two hours after transfection of the siRNA, total RNA was isolated from the cells and reverse transcribed. The cDNA was then amplified with a pair of forward and reverse primers for the following genes: BNIP3 (5′-CTCTGGTGAACTGCTACCT-CAGCAAT and 5′-TCATGACGCGTCTTCTCCTCAGT), TRAIL (5′-AGAGGCTCTCCAGAGATAG and 5′-TGTTCTAGTCTCATCAAGTG), DAPK2 (5′-CTTGTGCTTCAAGCCGAAAAC and 5′-CTCTGATTTCA-CAATTTCAAGG), TRAIL-B3 (5′-AAGTCTGACCATGAC and 5′-CCCTGATTCAGGTCATGAG), CD40 (5′-CAGGACAGAAACTGTTGAG and 5′-TAAAGACCAAGCAAGGAG), and β-actin (5′-TGGACCTTTCAACACCC-CAGCCATG and 5′-CGTATGGAGCCAGCTGTTG). PCRs were done using DNA Engine Opticon2 System (MJ Research, South San Francisco, CA) and the DyNaMo SYBR Green qPCR kit (Finnzyme Corp., Espoo, Finland). The thermal cycling conditions comprised an initial denaturation step at 95°C for 15 minutes followed by 30 cycles of PCR using the following profile: 94°C, 30 seconds; 57°C, 30 seconds; and 72°C, 30 seconds.

Ceramide quantitation assay. The ceramide content of the cells was assayed as described before (12). Briefly, total lipid was extracted from the cells, and the dried lipid was solubilized in 11.5 mmol/L Triton X-100 and 0.5 mmol/L cardiolipin by bath sonication and resuspended in a reaction mixture (180 μL) containing 20 mmol/L MOPS (pH 7.2), 50 mmol/L NaCl, 1 mmol/L DTt, 3 mmol/L CaCl2, 0.2 mmol/L diethylenetriaminepentaacetic acid, and 10 μg purified recombinant human ceramide kinase. The reaction was started by the addition of 11 μL ATP [1 μL (γ-32P)ATP (10 μCi/μL) plus 10 μL 20 mmol/L ATP in 100 mmol/L MgCl2] and continued at 37°C for 20 minutes. The reaction was terminated by extraction of lipids with 1.2 mL chloroform/methanol (1:1) and 0.3 mL of 1 mol/L NaCl (13). The organic phase was washed twice with 500 μL of 1 mol/L KCl in 20 mmol/L MOPS. Following extraction of the organic phase, ceramide 1-phosphate was resolved by TLC using chloroform/methanol/acetonic acid (65:15.5, v/v/v) as solvent and visualized by autoradiography (14).

Immunohistochemistry. Human breast cancer specimens were obtained from surgical pathology archives of the Akita Red Cross Hospital (Akita, Japan). All of the tissue sections were obtained by surgical resection. For immunohistochemical staining, 4-μm-thick sections were cut out from the formaldehyde-fixed and paraffin-embedded tissue specimens and mounted on charged glass slides. The sections were baked at 60°C for 1 hour, deparaffinized by two changes of xylene, and rehydrated in graded alcohol solutions. For antigen retrieval, the sections were either heated in 25 mmol/L sodium citrate (pH 9) at 85°C for 30 minutes (for FAS) or autoclaved in 10 mmol/L sodium citrate buffer (pH 6) for 10 minutes (for BNIP3). The slides were treated with 3% H2O2 to block endogenous peroxidase activity and then incubated overnight at 4°C with anti-FAS rabbit polyclonal antibody (1 μg/mL; Immuno-Biological Laboratories) or...
anti-BNIP3 rabbit polyclonal antibody (1:1,000; BioCarta). The sections were then incubated with HRP-conjugated anti-rabbit IgG for 30 minutes at room temperature, and 3,3′-diaminobenzidine substrate chromogen solution (EnVision Plus kit, Dako Corp., Carpinteria, CA) was applied. Finally, the sections were counterstained with hematoxylin. Results of the immunohistochemistry were judged based on the intensity of staining, comparing between the tumor cells and the normal glands on the same slide. Grading of the FAS and BNIP3 expression level was done by two independent persons without prior knowledge of the patient data. The cases were then divided into those that showed positive staining and those that showed reduced expression of the two genes.

Results

Specific inhibition of the FAS gene induces apoptosis in breast tumor cells in vitro. To examine the effect of direct inhibition of the expression of the FAS gene in breast cancer cells, a pool of four individual siRNAs against the FAS gene (FAS siRNA) was transfected into FAS-positive breast cancer cell lines MCF-7, MDA-231, and MDA-435. As shown in Fig. 1A, in each of the tested cell lines, the FAS siRNA inhibited expression of the FAS gene in a dose-dependent manner, whereas the GFP siRNA had no appreciable effect, indicating the specific effect of this set of siRNA in knocking down the expression of the FAS gene in human breast tumor cells. We also observed the time-dependent nature of this inhibition, with a significant effect being noted after 48 hours (Fig. 1B). Furthermore, as shown in Fig. 1C, each of the individual siRNA species within the pool was also able to knock down the expression of the FAS gene albeit with less efficiency. Next, to examine the end result of the direct inhibition of the FAS gene, MCF-7 cells were transfected with various amounts of FAS siRNA, and the extent of apoptosis was measured by assessing DNA fragmentation. As shown in Fig. 1D to E, FAS siRNA significantly augmented the degree of apoptosis in both dose and time-dependent manner (P < 0.05), whereas the GFP siRNA did not have any notable effect (data not shown). Our results therefore suggest that these siRNAs, specifically targeted to the FAS gene, lead to apoptosis of tumor cells and may have potential therapeutic utility.

Inhibition of FAS leads to up-regulation of proapoptotic genes. To understand the mechanism of apoptosis induced by inhibition of FAS, we treated MCF-7 cells with 300 nmol/L FAS siRNA or GFP siRNA for 72 hours, and their RNA was prepared. These RNAs were converted to cDNA that was then used for hybridization with the apoptosis/cell cycle-specific microarray, which contains 96 key apoptosis genes, 96 key cell cycle regulation genes, and 75 stress and toxicity genes. Among the genes whose expressions were significantly altered by FAS inhibition, the five most strongly up-regulated genes were BNIP3, TRAIL, DAPK2, TRAIL-R3, and CD40 (Fig. 2A). To confirm the result of the microarray analysis, we treated the MCF-7 cells with FAS siRNA in a similar manner and did real-time reverse transcription-PCR (RT-PCR) on the RNA samples. As shown in Fig. 2B, among these
Inhibition of FAS leads to up-regulation of ceramide synthesis. Malonyl-CoA, the substrate for FAS, has been known to inhibit CPT-1 (15). On the other hand, inhibition of CPT-1 was previously found to lead to accumulation of ceramide (16). Therefore, to explore a possibility that inhibition of FAS leads to apoptosis via up-regulation of ceramide, we treated the MCF-7...
breast tumor cells with 300 nmol/L FAS siRNA or GFP siRNA for 72 hours and measured the level of ceramide in the cells. As shown in Fig. 3A, treatment with FAS siRNA resulted in a significant up-regulation of ceramide synthesis in these cells compared with the treatment with GFP siRNA (P = 0.001). We observed a similar elevation of ceramide level in MDA-435 breast cancer cells following FAS siRNA treatment (data not shown). To clarify the involvement of malonyl-CoA in up-regulation of ceramide by FAS siRNA, we treated the MCF-7 cells with TOFA, an inhibitor of acetyl-CoA carboxylase that catalyzes synthesis of malonyl-CoA from acetyl-CoA. As shown in Fig. 3A, TOFA significantly inhibited ceramide accumulation in the presence of FAS siRNA (P = 0.002), suggesting the active role of malonyl-CoA in FAS siRNA-mediated ceramide up-regulation. Because malonyl-CoA is known to inhibit CPT-1, we next explored the effect of etomoxir, an inhibitor of CPT-1, on the MCF-7 cells. As shown in Fig. 3B, treatment with etomoxir resulted in a significant accumulation of ceramide in these cells (P < 0.0001), and the degree of accumulation of ceramide was found to be comparable with that observed by FAS siRNA treatment. Furthermore, as shown in Fig. 3C, treatment with etomoxir also induced a substantial degree of apoptosis in the MCF-7 cells (P < 0.0001). These results strongly suggest that FAS siRNA leads to accumulation of malonyl-CoA, which in turn leads to inhibition of CPT-1, resulting in accumulation of ceramide.

Because our microarray analysis indicated that FAS inhibition leads to up-regulation of BNIP3 expression, we examined the involvement of ceramide in overexpression of BNIP3 and apoptosis following FAS inhibition. Toward that end, we first tested the effect of exogenously added C2-ceramide on MCF-7 cells. As shown in Fig. 3D, 25 and 50 μmol/L C2-ceramide significantly up-regulated the BNIP3 mRNA level in these cells as tested by real-time RT-PCR (P = 0.004 and 0.002, respectively). In addition, we observed a significant degree of induction of apoptosis by 25 μmol/L C2-ceramide (P < 0.0001), which was further enhanced by the 50-μmol/L dose (P < 0.0001; Fig. 3E). To test whether up-regulation of ceramide is an essential step in FAS siRNA-mediated BNIP3 up-regulation, MCF-7 cells were treated with FAS siRNA or GFP siRNA with or without fumonisin B1 (a ceramide synthase inhibitor), and the level of BNIP3 expression was examined by quantitative RT-PCR. As shown in Fig. 3F, FAS siRNA significantly augmented the BNIP3 expression in these cells (P < 0.0001), and this effect was nullified by treatment with the ceramide inhibitor, indicating a positive involvement of ceramide in FAS siRNA-mediated up-regulation of BNIP3 expression. A ceramide quantitation assay was also done to confirm that fumonisin B1 indeed significantly abolished the ceramide level in these cells in the presence of FAS siRNA (P < 0.0001; Fig. 3G). In addition, we examined the effect of fumonisin B1 on FAS siRNA-mediated apoptosis and found that the MCF-7 cells underwent apoptosis when treated with FAS siRNA but not with GFP siRNA (P < 0.0001) as expected (Fig. 3H). However, apoptosis induced by FAS siRNA was significantly inhibited on treatment with fumonisin B1 (P < 0.0001). Furthermore, to corroborate the role of BNIP3 in FAS siRNA-mediated apoptosis, we treated the cells with FAS siRNA, BNIP3 siRNA, or a combination of both and found that BNIP3 siRNA significantly counteracted the effect of FAS siRNA (P < 0.0001; Fig. 3I). Taken together, these results strongly suggest that FAS siRNA causes apoptosis in breast tumor cells via up-regulation of the BNIP3 gene primarily through a ceramide-dependent pathway.

Expression of FAS and BNIP3 inversely correlates in human breast cancer. To examine whether our finding that inhibition of FAS leads to BNIP3 up-regulation is also reflected in the clinical setting, we examined the levels of FAS and BNIP3 proteins in a set of breast cancer samples. As shown in Fig. 4A, expression of FAS was almost undetectable in normal mammary ducts and glands, whereas the protein was strongly expressed in the poorly differentiated tumor cells in the same patient. On the other hand, BNIP3 was found to be abundantly expressed in the epithelial cells of normal ducts and glands, whereas expression of the protein was significantly reduced in tumor cells. Importantly, as shown in two representative fields in Fig. 4A, almost reverse staining pattern was observed when the same field was examined for FAS and BNIP3 expression. Statistical evaluation also revealed a significant inverse correlation between expression status of these two genes (P = 0.018, Fig. 4B). These results are consistent with our notion that inhibition of FAS leads to up-regulation of the proapoptotic gene BNIP3 in breast cancer cells and suggest a possibility that FAS protects the breast cancer cells through down-regulation of the BNIP3 gene.

Discussion

FAS is found to be overexpressed in various types of cancers and has been suggested that such up-regulation of FAS provides some selective advantage to the tumor cells by promoting proliferation and inhibiting apoptosis (1). Consistent with this idea, the chemical inhibitors of FAS have been shown to induce apoptosis in cancer cells in culture and decrease tumor size in animal models of various cancers (1). However, how inhibition of FAS promotes apoptosis in tumor cells remains elusive. In this report, to understand the mechanism of apoptosis induced by FAS inhibition, we used FAS siRNA to knock down FAS expression in a specific manner. We first showed the efficacy of the set of FAS siRNA to directly abrogate the expression of the FAS gene and trigger apoptosis, which is also consistent with the recent reports from our group and others, where FAS siRNA has been shown to cause apoptosis in various prostate cancer cells in vitro (17, 18).

FAS inhibition by the synthetic inhibitors, such as cerulenin or siRNA, has been observed to lead to a significant accumulation of malonyl-CoA in tumor cells, as would be expected from the fact that FAS uses malonyl-CoA as a substrate (10, 11, 19). We have also shown in this report that inhibition of malonyl-CoA synthesis significantly counteracts the effect of FAS siRNA, indicating an important role of malonyl-CoA accumulation in FAS siRNA-mediated apoptosis. Malonyl-CoA is also a physiologic inhibitor of the mitochondrial outer membrane enzyme CPT-1 that transesterifies long-chain acyl-CoAs to acylcarnitine, permitting their entry into the mitochondria for fatty acid oxidation (15). It is therefore plausible that inhibition of FAS that causes malonyl-CoA accumulation may lead to concomitant inhibition of CPT-1. Thupari et al. (10) have indeed observed that inhibition of FAS by cerulenin causes CPT-1 inhibition, and in agreement with this observation, we have found that inhibition of CPT-1 mimics the effect of FAS siRNA. Interestingly, inhibition of CPT-1 has been found to be significantly correlated with accumulation of ceramide, a sphingolipid that has been implicated in apoptotic response of cells to death inducers, such as Fas/Fas ligand, TNF-α, growth factor withdrawal, hypoxia, and DNA damage (16, 20). In this report, we showed that treatment of breast tumor cells with FAS siRNA significantly augmented the synthesis of ceramide and concomitant cell death. Furthermore, we provided evidence that exogenous ceramide mimicked the effect of FAS siRNA in these cells, whereas a ceramide synthase inhibitor significantly
counteracted the effect of FAS siRNA. Taken together, our results strongly indicate that direct inhibition of FAS by siRNA leads to accumulation of malonyl-CoA, which in turn inhibits CPT-1, resulting in up-regulation of ceramide and apoptotic cell death. Because FAS is found to be up-regulated in a variety of cancers, it will be interesting to test whether overexpression of FAS in vivo results in tumorigenesis via suppression of ceramide synthesis pathway.

The results of our microarray analysis indicated that FAS-mediated apoptotic pathway involved induction of the proapoptotic genes BNIP3, TRAIL, and DAPK2. BNIP3 is a mitochondria-associated cell death protein, originally identified as an adenovirus E1B 19-kDa-interacting protein (21). Consistent with the proapoptotic function of BNIP3, the gene is found to be significantly down-regulated in various types of cancers, including pancreatic, colorectal, gastric, and hematopoietic cancer cells, and this down-regulation occurs at least in part by hypermethylation of the promoter of the BNIP3 gene (22–24). BNIP3 not only induces apoptotic cell death but also is implicated in necrosis and autophagy (25, 26). Cell death induced by BNIP3 has been found to be caspase independent but is accompanied by rapid and profound mitochondrial dysfunction (25). Interestingly, C2-ceramide has been shown to up-regulate the expression of BNIP3, leading to autophagic cell death in malignant glioma cells (26). This result is consistent with our finding of increased ceramide synthesis and BNIP3 up-regulation following FAS inhibition by siRNA. Moreover, our observations that BNIP3 up-regulation and apoptosis induced by FAS siRNA is nullified by the ceramide synthase inhibitor and

![Diagram](Image)

**Figure 5.** A proposed apoptotic pathway induced by direct inhibition of the FAS gene.

that FAS siRNA-mediated apoptosis is significantly abrogated by BNIP3 siRNA strongly suggest that apoptotic cell death resulting from FAS inhibition occurs via up-regulation of ceramide synthesis following BNIP3 overexpression.

TRAIL is a proapoptotic gene of the TNF family that has been shown to induce apoptosis in a wide range of transformed cell lines (27). We found that FAS inhibition by siRNA that led to overexpression of ceramide was also associated with up-regulation of the expression of the TRAIL gene. Consistent with our result, Herr et al. (28) showed that C2-ceramide increased the expression of TRAIL in neuroblastoma cells. Furthermore, recently, in a microarray analysis of a set of prostate cancer patients, Rossi et al. (29) observed a significant inverse correlation between FAS and TRAIL expression, which is in good agreement with our finding that TRAIL is a downstream component of the apoptotic pathway initiated by FAS inhibition. DAPK2 is a proapoptotic gene encoding a protein that belongs to the serine/threonine protein kinase family (30, 31). This protein contains an NH2-terminal protein kinase domain followed by a conserved calmodulin-binding domain with significant similarity to that of DAPK1, which also is a positive regulator of programmed cell death (32). Interestingly, DAPK1 activity is critical for the apoptotic cascade involving C2-ceramide and C8-ceramide, although the direct involvement of DAPK2 in ceramide-mediated apoptotic pathway remains to be shown (33, 34).

Based on our results, we propose a model for the apoptotic pathway induced by FAS inhibition, whereby inhibition of FAS leads to accumulation of malonyl-CoA, which in turn inhibits CPT-1 resulting in up-regulation of ceramide followed by induction of the proapoptotic genes BNIP3, TRAIL, and DAPK2 (Fig. 5). It will be interesting to test the proposed pathway in an animal model, and understanding this pathway will provide new insights into cancer cell metabolism and aid in designing more specific anticancer drugs.

**Acknowledgments**

Received 9/6/2005; revised 3/7/2006; accepted 3/28/2006.

**Grant support:** NIH, Department of Defense, Illinois Department of Public Health, William McElroy Charitable Foundation, and American Lung Association, Illinois.

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