Nelfinavir Induces Liposarcoma Apoptosis through Inhibition of Regulated Intramembrane Proteolysis of SREBP-1 and ATF6

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Abstract

**Purpose:** We previously reported that nelfinavir (NFV) induces G1 cell-cycle block and apoptosis selectively in liposarcoma cell lines due to increased SREBP-1 (sterol regulatory element binding protein-1) expression in the absence of increased transcription. We postulate that NFV interferes with regulated intramembrane proteolysis of SREBP-1 and ATF6 (activating transcription factor 6).

**Experimental Design:** Time-lapse, confocal microscopic studies show that NFV inhibits the nuclear translocation of full-length SREBP-1–EGFP and ATF6–EGFP fusion proteins. siRNA-mediated knockdown of site-1 protease (S1P) and/or site-2 protease (S2P) leads to inhibition of SREBP-1 intracellular trafficking to the nucleus and reduces liposarcoma cell proliferation. Treatment of LiSa-2 liposarcoma cells with 3,4-dichloroisocoumarin, a serine protease inhibitor of S1P, did not affect SREBP-1 processing. In contrast, 1,10-phenanthroline, an S2P-specific inhibitor, reproduces the molecular and biological phenotypes observed in NFV-treated cells, which implicates S2P as a target of NFV.

**Results:** NFV-induced upregulation of SREBP-1 and ATF6 results from inhibition of S2P, which together with S1P mediates regulated intramembrane proteolysis from their precursor to their transcriptionally active forms. The resulting endoplasmic reticulum (ER) stress and concurrent inhibition of the unfolded protein response induce caspase-mediated apoptosis.

**Conclusions:** These results provide new insight into the mechanism of NFV-mediated induction of ER stress and cell death in liposarcomas and are the first to report targeting S2P for cancer therapy.

In vivo evaluation of NFV in a murine liposarcoma xenograft model leads to inhibition of tumor growth without significant toxicity.

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Introduction

The HIV protease inhibitor (PI) nelfinavir (NFV; Viread) has shown promising anticancer activity via the induction of apoptotic cell death, induction of endoplasmic reticulum (ER) stress and autophagy, inhibition of epidermal growth factor receptor– and insulin-like growth factor receptor–mediated PI3K (phosphoinositide 3-kinase)/Akt activation (1–4), and induction of chemo- and radiosensitization (5). NFV also decreases VEGF/hypoxia-inducible factor-1α expression, tumor hypoxia, and angiogenesis (6). In addition, NFV inhibits STAT3 and extracellular signal-regulated kinase 1/2 (7). Such pleiotropic activities show that NFV has multiple off-target effects.

Liposarcomas arise from adipocytes or their precursors (8). Chemotherapy for recurrent or metastatic liposarcomas is generally palliative (9). We hypothesized that NFV would inhibit liposarcoma growth because HIV PI use is linked to a clinical syndrome of peripheral lipodystrophy known as “HIV protease-induced lipodystrophy syndrome” (10). In this syndrome, adipocyte apoptosis is observed and associated with alteration of sterol regulatory element binding protein-1 (SREBP-1) expression (11–13).

SREBPs are synthesized as inactive, membrane-bound precursors in the ER, tethered at their carboxy-terminal domain by SREBP cleavage-activating protein (SCAP; refs. 14, 15). In the presence of cholesterol, SCAP binds Insig-1 or Insig-2 (16). On cholesterol depletion, SCAP and Insig fail to interact, the SREBP–SCAP complex is transported to the Golgi apparatus, where it is processed in 2 sequential proteolytic cleavage steps by site-1 protease (S1P), a subtilisin-like serine protease, and site-2 protease (S2P), a metalloprotease protease, to release the transcriptionally active amino-terminal fragment to the nucleus in an evolutionarily conserved process, regulated...
intramembrane proteolysis (RIP; refs. 16–18). SREBP-1 is a key transcription factor necessary for adipogenesis, cholesterol biosynthesis, and adipocyte differentiation (19). Its target genes include genes related to cholesterol synthesis such as fatty acid (FA) synthase (FASN; ref. 20) and anti-proliferative and proapoptotic genes such as p21WAF1/CIP1, Fas, and Bax (21, 22).

Most proteins fold and mature in the ER lumen. An imbalance between the load of unfolded proteins that enter the ER (ER stress) and the capacity of the cellular machinery to handle this load [unfolded protein response (UPR)] eventually triggers apoptosis (23, 24). Activating transcription factor 6 (ATF6) is a precursor ER-bound transcription factor that activates UPR genes. When unfolded proteins accumulate in the ER, ATF6 is processed by RIP by using intramembrane proteolysis through inhibition of site-2 protease activity. These results in overwhelming endoplasmic reticulum stress and an impaired unfolded protein response. The liposarcoma cells respond by undergoing apoptosis. This novel approach to cancer therapeutics is heretofore unreported. A clinical trial for NFV in liposarcoma supported by an FDA Orphan Products Development grant (R01 FD003006) is currently ongoing, which has shown early activity (NCT00233948: A Phase I/II Study of Nelfinavir in Liposarcoma; ref. 34).

Cell culture
SW872 dedifferentiated liposarcoma was purchased from the American Type Culture Collection (25). LiSa-2 pleomorphic liposarcoma cell line was a kind gift from Dr. Silke Brüderlein (University of Ulm, Ulm, Germany; ref. 26) and maintained in Iscove's modified Dulbecco’s medium/RPMI in a 4:1 ratio supplemented with 10% FBS, 2 mmol/L L-glutamine, and 0.1 mg/mL gentamicin. The cells were DNA fingerprinted to confirm identity as previously described (27).

Materials and Methods

Cell culture
SW872 cells were treated with 10 μmol/L NFV or DMSO for 4 hours, followed by cycloheximide (100 μmol/L), for the indicated time. Cell lysates were harvested to detect SREBP-1 or ATF6 by Western blotting. FASN and GRP78 were detected in lysates prepared from LiSa-2 cells treated with NFV for 24 hours.

Plasmid and quantitative RT-PCR
pTK-HSV-BP1a encoding human SREBP-1a and pCGN-ATF6 encoding human ATF6 were kindly provided by Dr. Guosheng Liang (UT Southwestern Medical School, Dallas, TX) and Dr. Amy Lee (University of Southern California, Los Angeles, CA). cDNA encoding SREBP-1a and ATF6 were amplified from pTK-HSV-BP1a and pCGN-ATF6 by PCR (Supplementary Data). The PCR fragments were inserted into pEGFP-C2 (Clontech) or Annexin V detection (Santa Cruz Biotechnology, Inc.) by flow cytometry. Clonogenic assay was carried out in NFV-treated LiSa-2 cells, according to the protocol previously described (3). A fluorescence-based digital image microscopy system (DIMSCAN; Bioimaging Solutions Inc.) was used to quantify the number of viable NFV- or M8-treated LiSa-2 cells as previously described (28). Briefly, 1,000 cells were seeded in a 96-well plate in quadruplicate from Abcam, Inc.

Apoptosis, clonogenicity, and proliferation assays
LiSa-2 cells were treated with NFV for 24 hours for caspase 9/6 assay according to the manufacturer’s protocol (Clontech) or Annexin V detection (Santa Cruz Biotechnology, Inc.) by flow cytometry. Clonogenic assay was carried out in NFV-treated LiSa-2 cells, according to the protocol previously described (3). A fluorescence-based digital image microscopy system (DIMSCAN; Bioimaging Solutions Inc.) was used to quantify the number of viable NFV- or M8-treated LiSa-2 cells as previously described (28). Briefly, 1,000 cells were seeded in a 96-well plate in quadruplicate from Abcam, Inc.
Biotechnology, Inc.) were transfected into cells with Lipofectamine (Invitrogen Corp.). After 48-hour incubation, the transfected cells were collected for qRT-PCR of S1P and S2P or Western blot of SREBP-1 or subjected to the DIMSCAN assay.

The DIMSCAN assay was used to quantify the number of viable siRNA-treated LiSa-2 cells. A total of 2,000 cells were seeded in a 96-well plate in multiples of 10 wells, followed by transfection with S1P, S2P, or S1P and S2P or scrambled siRNA. Twenty-four hours later, the cells were transfected with pSREBP-1–EGFP. At 48 and 72 hours after siRNAs transfection, cells were stained and scanned.

**Confocal microscopy time-lapse imaging**

On day 0, LiSa-2 cells were plated in a 2-well chamber slide at 3 × 10^5 cells per well. On day 2, cells were transfected with pSREBP-1–EGFP or pATF6–EGFP. On day 3, transfected cells were stained with Hoechst 33322 (Invitrogen) at final concentration of 1 μg/mL for 5 minutes and transferred to the Microscopy Core Lab. Cells were maintained in the live cell chamber at 37°C in 5% CO2 during confocal microscopy. To start the time-lapse experiment, DMSO or 10 μmol/L NFV was added to the pre-treated wells. The first image (t = 0 hour) was recorded 5 minutes later. The N-terminal EGFP-labeled SREBP-1 or ATF6 was visualized with a Zeiss LSM510 META NLO Axiom microscope, using a 488 argon laser (green) and chameleon 2P laser (blue) and the objective lens (LD-Achroplan 40×/0.6NA with correction collar). Digital images were captured every 2 hours for 12 hours with no significant loss of signal.

In the siRNA knockdown study, S1P, S2P, or equal amounts of S1P and S2P siRNA were transfected on day 2. On day 3, cells were transfected with pSREBP-1–EGFP. On day 4, nucleus was stained for time-lapse imaging as described. The first image (t = 0 hour) was recorded 24 hours after pSREBP-1–EGFP transfection (48 hours after siRNA transfection) and recorded every 2 hours for 12 hours. Images were generated using Multi-Time Macro in the LSM 510 software and was analyzed using LSM Image Browser and Concatenation Macro software.

**In vivo evaluation of NFV in a murine xenograft liposarcoma model**

A heterotopic murine liposarcoma model was established with human LiSa-2 cells in severe-combined immunodeficient (SCID) mice. Log-phase growth cells were harvested and resuspended in PBS at 1 × 10^7 cells/mL. One hundred microliters of cells was injected subcutaneously into the flanks of 6- to 8-week-old female SCID mice. Tumors that developed were surgically removed at week 5, cut into 2-mm^3 pieces, and placed into a surgically prepared subcutaneous pocket in the recipient mice. The recipient mice were allowed to recover 7 days before treatment with NFV (n = 7) or control (n = 7). The average starting weight of the mice was 20 gm and tumor volume (V) was determined by the equation: $V = \frac{1}{2}(l \times w^2)$.

Given our previous experience, prolonged twice-daily oral gavage was not considered feasible. NFV 625 mg tablets were purchased from the City of Hope Pharmacy and crushed into a fine powder. NFV powder was admixed with Transgenic Dough Diet (Bio-Serv) to dose at 500 mg/kg/d. Mouse feed was replaced daily with 4 g of Transgenic Dough Diet alone (control) or admixed with NFV. Tumors were bidimensionally measured, and the mice were weighed 3 times weekly.

**NFV pharmacokinetics in SCID mice**

NFV powder was resuspended in 1% carboxymethylcellulose to 100 mg/mL. Groups of three 8- to 10-week-old female SCID mice underwent oral gavage with 500 mg/kg of NFV in carboxymethylcellulose and then euthanized at 0, 1, 2, 4, 8, and 24 hours after oral gavage. Blood was collected and plasma was separated for analysis of NFV concentration with an UPLC-tandem mass spectrometric assay, developed, and validated in the Analytical Pharmacology Core Facility at the City of Hope (Supplementary Data).

**Measurement of ER stress**

S1P, S2P, or equal amounts of S1P and S2P siRNA were transfected on day 1. Cell lysates for Western blot detection of GRP78 were prepared from the transfected cells on day 3. NFV-treated LiSa-2 cells were collected at 24 hours for Western blot detection of GRP78. Thapsigargin (TG, 0.5 μmol/L) served as a positive control for ER stress induction.

**Statistical analysis**

Data are presented as the mean ± SD of 3 independent experiments. Group comparisons for continuous data were done with Student’s t test for independent means or 2-way ANOVA.

**Results**

**NFV induces caspase-dependent apoptosis**

Caspase activation was analyzed in NFV-treated LiSa-2 and SW872 cells. As shown in Figure 1A, activity level of caspase 9/6 increases in a dose-dependent fashion and reaches a maximum of 4 and 5 fold over baseline activity at the highest dose (20 μmol/L). Clonogenic assays in LiSa-2 cells (Fig. 1B) confirm NFV inhibits clonogenicity in a dose-dependent manner.

**M8, the primary NFV metabolite, induces dose-independent cell death**

NFV is metabolized in vivo to its hydroxyl-β-butylamide metabolite, M8, which possesses similar in vitro antiviral activity as its parent compound. Figure 1C reveals similar (day 2) or slightly higher survival (day 6) in M8-treated LiSa-2 cells than NFV at the same doses. This shows that M8 possesses comparable antitumor activity as NFV.

**NFV increases SREBP-1 protein half-life**

Our previous data showed NFV leads to significantly elevated expression of precursor SREBP-1 (125 kDa) in
the absence of increased transcription (3). Consequently, protein stability of SREBP-1 was evaluated in the presence of NFV. DMSO-treated SW872 cells show degradation of precursor SREBP-1 (125 kDa) by 1 hour and its complete absence by 4 hours (Fig. 2A), along with increased detection of processed SREBP-1 (68 kDa) up to 24 hours. In contrast, NFV leads to accumulation of precursor SREBP-1 and minimal detection of its processed form through 24 hours (Fig. 2A). The results are shown quantitatively in Figure 2A, with the half-life ($T_{1/2}$) of 2 hours for control and 13 hours for NFV-treated cells. Similarly, ATF6 (Fig. 2B) was also tested in LiSa-2 cells and shows a similar dose-dependent increase of precursor ATF6 (90 kDa) by NFV and prolonged $T_{1/2}$ from 6 to 9 hours (Supplementary Data). Importantly, NFV did not alter SREBP-1 ubiquitination or acetylation (Supplementary Data).

**NFV inhibits intracellular trafficking of SREBP-1 and ATF6**

To explore whether the increased $T_{1/2}$ of precursor SREBP-1 or ATF6 results from NFV-mediated inhibition of RIP, plasmids encoding the full-length SREBP-1 or ATF6 gene fused in frame at their amino terminus with EGFP were constructed and transfected into LiSa-2 cells to monitor the trafficking of their mature forms from the cytoplasm (ER-Golgi) to the nucleus. As shown in Figure 2C and D, control cells show intense green fluorescence throughout the nucleus by 12 hours, which reflects normal RIP of the precursor SREBP-1–EGFP fusion protein to its transcriptionally active form. In contrast, the NFV-treated cells show retention of the fusion protein in the cytoplasm and minimal movement into the nucleus at 12 hours. Similarly, inhibition of RIP of the precursor ATF6 was observed, as shown in Figure 2D. These data show that NFV inhibits intracellular trafficking of SREBP-1 and ATF6 by inhibiting RIP.

**NFV inhibits FASN expression**

FASN is a transcriptional target of SREBP-1 (29). Accordingly, FASN was evaluated in NFV-treated LiSa-2 cells to evaluate the effect of inhibition of SREBP-1 production on downstream targets. Figure 2B shows NFV dose-dependently inhibits FASN expression (confirmed by FASN qRT-PCR gene expression; Supplementary Data), consistent with the hypothesis that NFV interferes with SREBP-1 processing.
siRNA-mediated inhibition of S1P or S2P blocks intracellular trafficking of SREBP-1 and reduces liposarcoma proliferation

Both SREBP-1 and ATF6 are cleaved by S1P and S2P during RIP. To further explore the mechanism of NFV-induced accumulation of unprocessed SREBP-1 and ATF6, siRNAs targeting S1P, S2P, or S1P plus S2P were transfected into LiSa-2 cells. siRNA reduced target S1P or S2P RNA level by more than 90% (Fig. 3A). Western blot confirms an accumulation of precursor SREBP-1 (Fig. 3A) in S1P and/or S2P siRNA-transfected cells. Notably, time-lapse microscopy shows the absence of nuclear green fluorescent protein in both S1P and S2P siRNA-transfected cells whereas control cells show diffuse nuclear fluorescence (Fig. 3B and C). These results indicate that inhibition of S1P and S2P results in potent inhibition of SREBP-1 trafficking to the nucleus. Significantly, combined S1P and S2P knockdown leads to the greatest inhibition of SREBP-1 movement, as shown by nearly complete exclusion of fluorescence in the nucleus (Fig. 3D). To determine the consequence of S1P and S2P knockdown on cell proliferation, a fluorescence-based assay, DIMSCAN, was carried out. Figure 4A shows that S1P or S2P siRNA reduces cell survival to 85% of control at 72 hours.

Figure 2. Nelfinavir increases SREBP-1 protein half-life (T_{1/2}) and inhibits intracellular trafficking of SREBP-1 and ATF6. A, SREBP-1 T_{1/2} determination. Lysate from SW872 cells pretreated with 10 mmol/L NFV or DMSO for 4 hours followed by cycloheximide (100 mmol/L) for the indicated time points was analyzed by Western blot. Relative SREBP-1 expression was quantitated by Image Quant (Molecular Dynamics). B, dose-dependent induction of precursor ATF6 and FASN reduction by NFV. LiSa-2 cell was treated with NFV at the indicated dose for 24 hours and lysate was harvested for Western blot analysis. C, visualization of SREBP-EGFP transport from cytoplasm to nucleus in NFV-treated cells. A total of 3 x 10^6 LiSa-2 cells/well were seeded in glass-bottomed 2-well chamber slide for overnight incubation and transfected with pSREBP-1-EGFP, cells were then cultured 24 hours to allow transgene expression before staining with Hoechst 33322 1 μg/ml for 5 minutes. The live cell image was captured and recorded at indicated time points using a confocal microscopy (t = 0 hour, 5 minutes after addition of 10 μmol/L nelfinavir or DMSO). D, visualization of transport of ATF6-EGFP from cytoplasm to nucleus in NFV-treated LiSa-2 cells. Cells were transfected with pATF6-EGFP for time-lapse imaging. Bar, 20 μm.

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siRNAs reduce survival by 50%. These results show that inhibition of S1P- and/or S2P-mediated RIP of SREBP-1 leads to cell death, which supports the notion that S1P and/or S2P may be potential targets of NFV.

**Blocking S2P function with a small molecule inhibitor reproduces the NFV-treated phenotype**

To more closely examine whether the activity observed for NFV was attributable to the inhibition of S1P or S2P, small molecule inhibitors were utilized. DCI is a potent serine PI of recombinant S1P (30). It does not alter the precursor SREBP-1 or ATF6 accumulation (Fig. 4B). In contrast, treatment of LiSa-2 cells with 1,10-phenanthroline, a metalloprotease-specific S2P inhibitor, leads to both dose- and time-dependent accumulation of precursor SREBP-1 and ATF6 (Fig. 4C), similar to the phenotype observed for NFV (31). Likewise, 1,10-phenanthroline leads to dose-dependent apoptosis (Fig. 4D), which highly suggests that NFV inhibits the proteolytic activity of S2P.

**NFV inhibits liposarcoma growth in a murine model**

To determine whether NFV possesses *in vivo* anticancer activity, a heterotopic SCID murine liposarcoma model was established and the mice were treated with NFV 500
mg/kg/d orally for 6 weeks. The results shown in Figure 5A indicate that by day 24, the tumor growth curve of the NFV-treated mice begin to diverge from the control mice and continue to do so throughout the entire experimental period (P < 0.05), which suggest that the in vitro observations for NFV may be equally applicable in vivo.

Clinical use of NFV in the treatment of HIV infection is commonly associated with diarrhea but rarely leads to hepatotoxicity and/or myelosuppression (32). To evaluate toxicities associated with NFV in SCID mice, after 2 weeks of continuous twice-daily dosing with vehicle only (carboxymethylcellulose), NFV 1 gm/kg/d, or 2 gm/kg/d by oral gavage, the mice were euthanized and subjected to necropsy. Figure 5B shows minimal or no weight loss associated with NFV with either dose level (P < 0.05). Daily cage examination failed to show diarrhea or its predetermined surrogate, 10% weight loss. Histologic evaluation of liver and bone marrow showed no microscopic difference in liver architecture or levels of myeloid or erythroid precursors between control and NFV-treated mice (data not shown).

To determine the pharmacokinetics of oral NFV in vivo, cohorts of 3 mice underwent a single, oral gavage with 500 mg/kg of NFV and the mice were euthanized for cardiac puncture at the indicated time points. The results show that at a dose of 500 mg/kg, a peak plasma concentration of NFV that approximates the biologically active dose (~10 μmol/L) is reached in 1 hour and remains steady for 8 hours (Fig. 5C). These data suggest that twice-daily dosing is feasible for long-term NFV dosing.

NFV and S2P siRNA induce ER stress

NFV induces ER stress (4). To determine whether S2P inhibition also induces ER stress, siRNAs targeting S1P, S2P, or S1P plus S2P were transfected into LiSa-2 cells and expression of the ER stress response protein GRP78 was
evaluated. TG served as a positive control for ER stress induction. Figure 6A shows that TG, high-dose NFV, and S2P or S1P plus S2P siRNA strongly induce expression of GRP78, whereas control and S1P siRNA alone does not. These results are consistent with the hypothesis that NFV inhibits S2P activity, which induces ER stress.

Discussion

NFV is a PI that was originally developed for therapy against HIV. NFV may inhibit cancer growth via multiple pathways (1–6). Recently, a study in ovarian cancer suggested a novel additional mechanism that NFV may target cancer stem cells (33). These reports have led support for the use of NFV alone or in combination with radiation and/or chemotherapy for advanced cancers in several clinical trials, including our ongoing trial in liposarcomas (5, 34).

Liposarcomas are one of the most common soft tissue sarcomas, and outcomes for recurrence remains poor. They may be particularly sensitive to NFV because it interferes with maturation of SREBP-1, an adipocytic transcription factor (19). Our data strongly suggest that NFV inhibits liposarcoma proliferation and promotes apoptosis by inhibiting RIP of SREBP-1 and ATF6. This results in accumulation of unfolded SREBP-1 and ATF6 precursor proteins (ER stress) and inhibition of the UPR because ER stress-induced activation of ATF6 is neutralized.

Regulation of SREBP-1 and ATF6 is similar (Fig. 6B). Both transcription factors are synthesized as ER transmembrane proteins (precursor) and transported to the Golgi where RIP occurs. Luminal S1P cleavage occurs first, followed by intramembrane S2P cleavage, which liberates the transcriptionally active amino-terminal segments of SREBP-1 and ATF6 to migrate to the nucleus in order to transactivate sterol biosynthesis or UPR activation genes.

Our previous data showed that precursor and processed forms of SREBP-1 were consistently upregulated by NFV in various liposarcoma cell lines (3). Our present results show that NFV induces similar upregulation of ATF6 (Fig. 2).
Confocal, time-lapse microscopy (Fig. 3) shows that SREBP-1 and ATF6 processing is inhibited by NFV, with retention of precursor SREBP-1 and ATF6 in the cytoplasm. This results in SREBP-1–mediated downregulation of FASN. Accumulation of unprocessed SREBP-1 and ATF6 induces ER stress, which leads to caspase-dependent apoptosis (dotted arrow). Our current model illustrated in Figure 6B shows that NFV-induced upregulation of precursor SREBP-1 and ATF6 results from NFV-mediated inhibition of S2P, which leads to inhibition of RIP. Confirmatory support remains pending our current efforts to purify S2P protein for enzymatic cleavage assays. The precursor proteins are retained in the cytoplasm, their transport to the nucleus is halted, and transcriptional activation of target genes is blocked. Accumulation of unprocessed SREBP and ATF6 induces ER stress, and a deficient UPR leads to caspase-dependent apoptosis.

Although our data indicate that NFV inhibits S2P activity, partial S1P inhibition cannot be excluded. RIP of SREBP-1 and ATF6 requires sequential S1P and S2P cleavage. Prywes and colleagues previously noted that the bulky ATF6 luminal domain blocks S2P cleavage and the primary role of S1P is to reduce the size of the luminal domain to prepare ATF6 to be an optimal S2P substrate (37). An analogous model for SREBP-1 exists, with removal of its second transmembrane domain by S1P, which allows for the movement of the substrate site within the membrane (38). This may explain why knockdown of S1P by siRNA still leads to accumulation of unprocessed precursor SREBP-1 in the cytoplasm in the absence of precursor SREBP-1 accumulation in DCI-treated cells. However,
Pore N, Gupta AK, Cerniglia GJ, Maity A. HIV protease inhibitors.”

Our previous data showed that conditional expression of SREBP-1 in SW872 liposarcoma cells reduces cellular proliferation and induces antiproliferative and proapoptotic genes such as P21WAPF/GPIP, Fas, and Bax. SREBP-1 acts as a proapoptotic gene in pancreatic β-islet cells, and caspases 2 and 7 are activated by SREBP-1 and SREBP-2 in statin-induced apoptotic gastric cancer cells (39, 40). ATF6-mediated apoptosis resulting from ER stress has been elucidated (41). Accumulation of misfolded proteins in the ER induces the UPR to promote cell survival by adjusting ER protein–folding capacity. However, if homeostasis cannot be reestablished, apoptosis is induced through ATF6-mediated activation of CHOP/GADD153 (42).

A notable target gene of SREBP is FASN. One of the hallmarks of cancer is increased de novo FA synthesis, referred to as the “lipogenic phenotype” (43, 44). FAs are essential constituents of all biological membrane lipids and are important substrates for energy metabolism (44). FASN catalyzes the terminal steps of long-chain FA synthesis (44). FASN is overexpressed in many cancers (43, 44).

References


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