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RNA Interference-mediated Silencing of the Fatty Acid Synthase Gene Attenuates Growth and Induces Morphological Changes and Apoptosis of LNCaP Prostate Cancer Cells[1]

Ellen De Schrijver, Koen Brusselmans, Walter Heyns, Guido Verhoeven, and Johannes V. Swinnen[2]

Laboratory for Experimental Medicine and Endocrinology, Department of Developmental Biology, Gasthuisberg, Catholic University of Leuven, B-3000 Leuven, Belgium

ABSTRACT

Fatty acid synthase (FASE), a key enzyme in the biosynthesis of fatty acids, is markedly overexpressed in many human epithelial cancers, rendering it an interesting target for antineoplastic therapy. Here, using the potent and highly sequence-specific mechanism of RNA interference (RNAi), we have silenced the expression of FASE in lymph node carcinoma of the prostate (LNCaP) cells. RNAi-mediated down-regulation of FASE expression resulted in a major decrease in the synthesis of triglycerides and phospholipids and induced marked morphological changes, including a reduction in cell volume, a loss of cell-cell contacts, and the formation of spider-like extrusions. Furthermore, silencing of the FASE gene by RNAi significantly inhibited LNCaP cell growth and ultimately resulted in induction of apoptosis. Importantly and in striking contrast with LNCaP cells, RNAi-mediated inhibition of FASE did not influence growth rate or viability of nonmalignant cultured skin fibroblasts. These data indicate that RNAi opens new avenues toward the study of the role of FASE overexpression in tumor cells and provides an interesting and selective alternative to chemical FASE inhibitors in the development of antineoplastic therapy.

INTRODUCTION

FASE[3] is a key enzyme catalyzing the terminal steps in the synthesis of saturated fatty acids (1). In most human tissues, FASE expression is low because the bulk of the required lipids are obtained from the diet (2). In contrast, expression of FASE is significantly elevated in a wide variety of human epithelial cancers, including cancer of the prostate, breast, endometrium, ovary, lung, colon, and stomach (Refs. 3–6 and references therein). Expression of FASE is already increased very early in cancer development and is additionally elevated in more advanced tumors, particularly those with a poor prognosis. Although the mechanisms underlying increased FASE expression in tumors are not fully understood, recent studies have shown that overexpression of FASE is part of a more general and coordinated activation of lipogenic gene expression, mediated at least in part by activation of the sterol regulatory element binding protein pathway (7–9). Activation of the latter pathway in tumors may be the result of steroid hormone action or alterations in growth factor production or signaling (8, 10–16).

Several studies using chemical inhibitors of FASE show that blockage of FASE activity severely attenuates growth and survival of tumor cells (3, 17–19). One of the best known and most studied inhibitors of FASE is cerulenin, a natural mycotoxin (3). Cerulenin inhibits growth, is cytotoxic for different human cancer cells *in vitro*, and slows down development of ovarian cancer xenografts in mice *in vivo* (3, 20–22). However, FASE inhibitors such as cerulenin have several shortcomings. Cerulenin harbors a very reactive epoxide group that may interact also with other proteins and may affect processes other than fatty acid synthesis. In this respect, cerulenin has been shown to suppress protein palmitoylation, a posttranslational modification allowing key signaling proteins to attach to the plasma membrane (23, 24). Analysis of a range of cerulenin analogues showed that inhibition of palmitoylation is independent of its effects on fatty acid synthesis and is more closely related to growth inhibition of cancer cells than inhibition of FASE activity (24). Moreover, cerulenin also suppresses cholesterol synthesis (25, 26) and inhibits proteolysis (27, 28). In addition, the use of cerulenin as a FASE inhibitor is limited because of its chemical instability (3). More stable FASE inhibitors such as C75 have recently become available (18), but the specificity of these compounds requires additional investigation.

In this article, we have used an entirely different approach to interfere with FASE activity and to definitely establish a role for FASE as a key target for antineoplastic therapy. This approach is based on selective gene silencing by RNAi. RNAi is a cellular process resulting in enzymatic cleavage and breakdown of mRNA, guided by sequence-specific double-stranded RNA oligonucleotides (siRNAs; Ref. 29). Exogenously added synthetic 21-nucleotide siRNA duplexes were shown to act as very potent and highly sequence-specific agents to silence homologous gene expression, thereby holding great potential for the analysis of gene function and for gene-specific therapeutic approaches (30). In the present work, we assessed the potential of siRNAs as alternative agents to molecularly target FASE and induce growth arrest and apoptosis in cancer cells.

MATERIALS AND METHODS

Cell Culture. The human prostatic cancer cell line LNCaP was obtained from the American Type Culture Collection (Manassas, VA). Cultures of nonmalignant skin fibroblasts, derived from a 19-month-old male infant, were kindly provided by Prof. Jean-Jacques Cassiman (Center for Human Genetics, Catholic University of Leuven, Leuven, Belgium). Cells were maintained at 37°C in a humidified incubator with a 5% CO_2/95% air atmosphere in RPMI 1640 supplemented with 10% FCS, 3 mM L-glutamine, 100 μg/ml streptomycin, and 100 units/ml penicillin (Invitrogen, Carlsbad, CA).

RNAi. Transfection of LNCaP cells or fibroblasts with siRNA-targeting FASE or luciferase was carried out as described previously (30, 31). Synthetic sense and antisense oligonucleotides were purchased from Dharmacon (Lafayette, CO). For design of siRNA oligos targeting FASE, a DNA sequence of the type AA(N_{19}) was selected (AACCCTGAGATCCCAGCGCTG) according to the manufacturer's protocol. This sequence corresponded to the nucleotides 1210–1231 located 3′ to the first nucleotide of the start codon of the human FASE cDNA. The DNA sequence was submitted to a BLAST search against the human genome sequence to ensure that only the *FASE* gene was targeted. As a nonspecific siRNA control, the GL2 luciferase siRNA duplex was used as described previously (30). The corresponding single-stranded sense and antisense siRNA oligos (20 μM) were annealed by incubation in

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[2] To whom requests for reprints should be addressed, at Laboratory for Experimental Medicine and Endocrinology, Gasthuisberg, O&N9, K. U. Leuven, Herestraat 49, B-3000, Leuven, Belgium. Phone: 32-16-34-59-70; Fax: 32-16-34-59-34; E-mail: johan.swinnen@med.kuleuven.ac.be.

[3] The abbreviations used are: FASE, fatty acid synthase; LNCaP, lymph node carcinoma of the prostate; RNAi, RNA interference, siRNA, small interfering RNA; EGFP, enhanced green fluorescent protein.

annealing buffer [100 mM potassium acetate, 30 mM HEPES-KOH (pH 7.4), and 2 mM magnesium acetate] for 1 min at 90°C, followed by 1 h at 37°C. Transfections were performed in 60-mm dishes at a density of 0.4×10^6 LNCaP cells/dish or 10^5 fibroblasts/dish using Oligofectamine (Invitrogen) and 0.33 nmol of siRNA duplex. The final concentration of siRNA in the 60-mm dishes was 166 nM. At the indicated time points after transfection, cells were used for cell proliferation/cytotoxicity assays, immunoblotting analysis, FASE activity assays, 2-^{14}C-labeled acetate incorporation assays, and Oil red O stainings. For Hoechst 33342 and Annexin V-EGFP/propidium iodide stainings, transfections were carried out in Lab-Tek II chamber slides (Nalge Nunc International, Naperville, IL; 1.2×10^5 LNCaP cells or 3×10^4 fibroblasts/chamber) using Oligofectamine (Invitrogen) and 0.088 nmol of siRNA duplex, resulting in a final siRNA concentration of 166 nM.

Immunoblot Analysis. At the indicated times, cells were washed and lysed in a reducing SDS buffer [62.5 mM Tris (pH 6.8), 2% SDS, 0.715 M 2-mercaptoethanol, and 8.7% glycerol]. Protein concentrations were determined on diluted samples using a bicinchoninic acid procedure (Pierce Biochemical Company, Rockford, IL). Equal amounts of protein were separated on NuPAGE Tris-acetate gels (Invitrogen), which were blotted onto polyvinylidene difluoride membranes (Roche, Mannheim, Germany). Membranes were blocked in a Tris-buffered saline solution with 5% nonfat dry milk and incubated with antibodies against cytokeratin 18 (Santa Cruz Biotechnology, Santa Cruz, CA) or FASE (13). Horseradish peroxidase-conjugated secondary antibodies (Dako, Carpinteria, CA) were used for detection of immunoreactive proteins by chemiluminescence (Renaissance; New England Nuclear, Dreiech, Germany).

Assay of FASE activity (*in Vitro*). At the indicated times, cells were washed with PBS, harvested by scraping in 500 μl of PBS, pelleted by centrifugation, and resuspended in 200 μl of a hypotonic buffer [1 mM DTT, 1 mM EDTA, and 20 mM Tris-HCl (pH 7.5)]. Equal amounts of protein (40 and 100 μg for LNCaP cells and fibroblasts, respectively) were used to measure FASE activity as described previously (10).

Incorporation of 2-^{14}C-Acetate into Cellular Lipids. Seventy-two h after transfection with siRNA, cells were refed, and 2-^{14}C-labeled acetate (57 mCi/mmol; 2 μCi/dish; Amersham International, Aylesbury, United Kingdom) was added to the culture medium. After 4 h of incubation at 37°C, cells were washed with PBS (culture medium and wash fluid were collected), trypsinized, and resuspended in 0.8 ml of PBS. Lipids were extracted using the Bligh Dyer method as previously described (32), and radioactivity was measured by scintillation counting. Measurements were performed in triplicate, and values were normalized for sample protein content. Acetate incorporation into specific lipids was analyzed after separation of lipids by TLC. Therefore, lipid extracts and appropriate lipid standards were spotted on silica gel G plates (Merck, Darmstadt, Germany). For separation of neutral lipids, plates were developed in hexane-diethyl ether-acetic acid (70:30:1, vol/vol/vol); development in chloroform-methanol-acetic acid (65:25:10, vol/vol/vol) was used for separation of phospholipids. Lipid samples and standards were visualized by autoradiography and iodine vapor, respectively. Lipid fractions were quantified using PhosphorImager screens (Molecular Dynamics, Sunnyvale, CA) and normalized for sample protein content.

Oil Red O Staining. Lipid accumulation was determined by Oil red O staining as described previously (32).

Proliferation/Cytotoxicity Assay. Growth and viability of LNCaP cells and fibroblasts (transfected with siRNA-targeting FASE or luciferase) was analyzed with the Trypan Blue Dye Exclusion assay. At the indicated times, cells were trypsinized and combined with the floating cells in the culture medium. Cells were pelleted by centrifugation at $200 \times g$ for 10 min, resuspended in a trypan blue solution, and counted using a hemocytometer. The cells with and without blue dye staining inside were recorded as dead and alive, respectively. Measurements were performed in triplicate.

Detection of Apoptosis by Fluorescence Microscopy. Cells were plated in chamber slides as described above, transfected with siRNA targeting FASE or luciferase, and analyzed for apoptosis 72 h after transfection. For analysis of changes in nuclear morphology during apoptosis, Hoechst dye 33342 (Sigma, Bornem, Belgium) was added to the culture medium. Fragmentation of the nucleus into oligonucleosomes and chromatin condensation was detected by fluorescence microscopy using a filter for Hoechst 33342 (365 nm). Apoptosis was also determined with an Annexin V-EGFP/propidium iodide Apoptosis Detection Kit (BD Biosciences, Palo Alto, CA) according to the manufacturer's protocol. Briefly, the cells were washed and subsequently incubated for 15 min at room temperature in the dark in 300 μl of 1× binding buffer containing 5 μl of Annexin V-EGFP and 10 μl of propidium iodide. Afterward, apoptosis was analyzed by fluorescence microscopy using a dual-filter set for EGFP (490 nm) and propidium iodide (560 nm).

Statistical Analysis. Comparison of values was performed using a nonparametric Mann-Whitney *U* test or a one-way ANOVA test. If significant differences were observed after ANOVA analysis, values were compared with a Tukey test. $P < 0.05$ was considered statistically significant. Data are

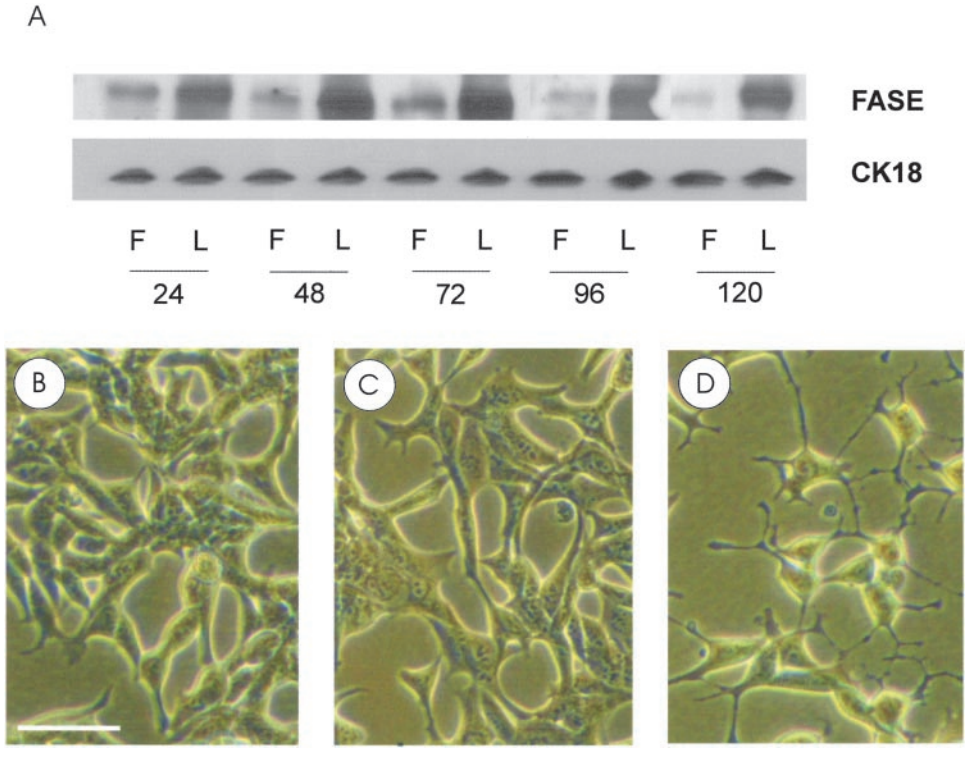

Fig. 1. Impact of FASE RNAi on FASE expression and cell morphology of LNCaP cells. *A*, FASE protein levels of LNCaP cells transfected with luciferase siRNA (L) or FASE siRNA (F) were determined by Western blot analysis at the indicated times (h) after transfection. Cytokeratin 18 (CK18) expression was used as internal control. *B–D*, phase contrast microscopic analysis of untransfected LNCaP cells (*B*) and LNCaP cells 72 h after transfection with luciferase siRNA (*C*) or FASE siRNA (*D*), illustrating the morphological changes at the cellular level induced by FASE RNAi. *Bar* indicates 50 μm.

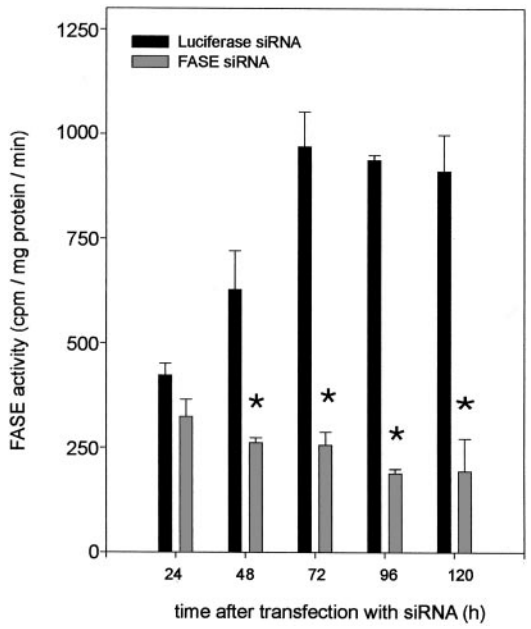

Fig. 2. FASE RNAi decreases FASE activity *in vitro*. LNCaP cells were transfected with siRNA-targeting luciferase or FASE. At the indicated time points, protein extracts were made, and FASE activity was measured by quantification of 2-^{14}C-labeled malonyl-CoA incorporation into fatty acids *in vitro*. *Columns* represent means of data (n = 4); *bars*, ±SD. ∗, significantly different from control (luciferase siRNA transfected cells) by Tukey test.

expressed as means ± SD. All observations were confirmed by three independent experiments.

RESULTS

FASE RNAi Severely Affects Expression of FASE and Induces Morphological Changes in LNCaP Cells. To specifically silence the *FASE* gene, LNCaP cells were transfected with siRNA-targeting FASE mRNA. As a control for specificity of RNAi, LNCaP cells were transfected with siRNA-targeting luciferase, which is not expressed by LNCaP cells. Western blot analysis demonstrated that FASE RNAi severely suppressed expression of FASE in LNCaP cells when compared with control cells transfected with luciferase siRNA (Fig. 1*A*). Silencing of the *FASE* gene was already visible 48 h after transfection, reached an optimum after 72 h, and lasted for at least another 48 h (through 120 h after transfection; Fig. 1*A*). No effects of RNAi were observed on the expression of cytokeratin 18, which was used as an internal control for specificity and loading.

Microscopic analysis revealed that FASE RNAi induced morphological changes in LNCaP cells, which became already visible at 24 h but were even more prominently present at 72, 96, and 120 h after transfection with FASE siRNA (Fig. 1, *B–D*). When compared with untransfected cells (Fig. 1*B*) or cells transfected with luciferase siRNAs (Fig. 1*C*), FASE siRNA-transfected cells grew less dense, were smaller in volume, made only poor cell-cell contacts, and showed an astrocyte-like phenotype with the presence of multiple extrusions (Fig. 1*D*).

FASE RNAi Decreases FASE Activity and Lipid Synthesis in LNCaP Cells. To analyze whether FASE RNAi resulted in decreased enzymatic activity, FASE activity of LNCaP cell protein extracts was measured by quantification of 2-^{14}C-labeled malonyl-CoA incorporation into fatty acids *in vitro*. At 48 h after transfection, FASE RNAi resulted in a significant decrease of FASE activity in LNCaP extracts (Fig. 2). The effect of RNAi on FASE activity was even more pronounced 72 h after transfection and lasted for at least 3 days (through 120 h after transfection).

To evaluate the impact of reduced FASE activity on endogenous lipid synthesis, LNCaP cells, at 72 h after transfection with siRNA-targeting FASE or luciferase, were exposed to 2-^{14}C-labeled acetate for 4 h. Thereafter, cellular lipids were extracted, and incorporation of 2-^{14}C-labeled acetate was measured. To avoid effects caused by differences in cell mass between FASE and luciferase siRNA-transfected LNCaP cells, scintillation counting measurements were normalized for sample protein content. In agreement with the previous findings, incorporation of 2-^{14}C-labeled acetate into cellular lipids of FASE siRNA-transfected LNCaP cells was reduced to 33 ± 3%

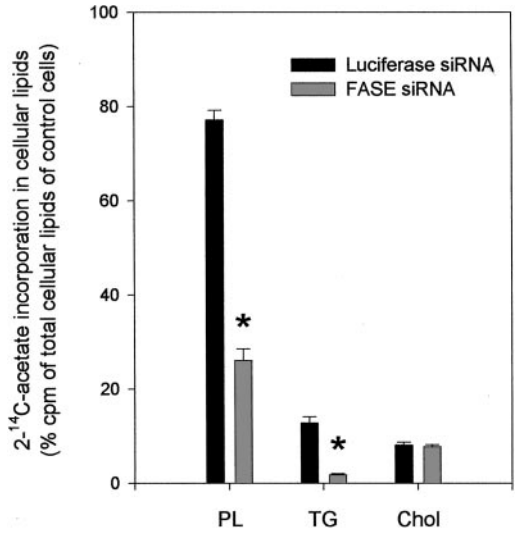

Fig. 3. Effect of FASE RNAi on lipid biosynthesis. LNCaP cells were transfected with siRNA-targeting luciferase or FASE, and after 72 h cells were treated with 2-^{14}C-labeled acetate for 4 h. Lipid extracts were prepared, different lipid species were separated by TLC, and ^{14}C-incorporation was quantitated. PL, phospholipids; TG, triglycerides; Chol, cholesterol. *Columns* represent means of data (n = 6); *bars*, ±SD. ∗, significantly different from control (luciferase siRNA-transfected cells) by Tukey test.

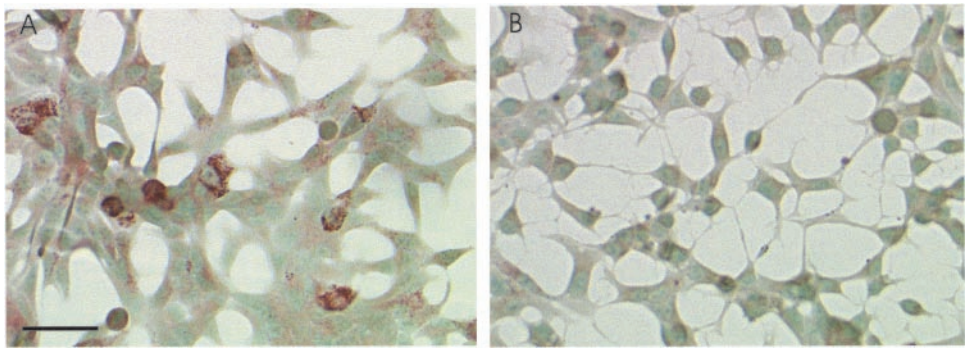

Fig. 4. FASE RNAi decreases accumulation of neutral lipids in LNCaP cells. Accumulation of neutral lipids was visualized by Oil red O staining at 72 h after transfection with luciferase siRNA (*A*) or FASE siRNA (*B*). *Bar* indicates 100 μm.

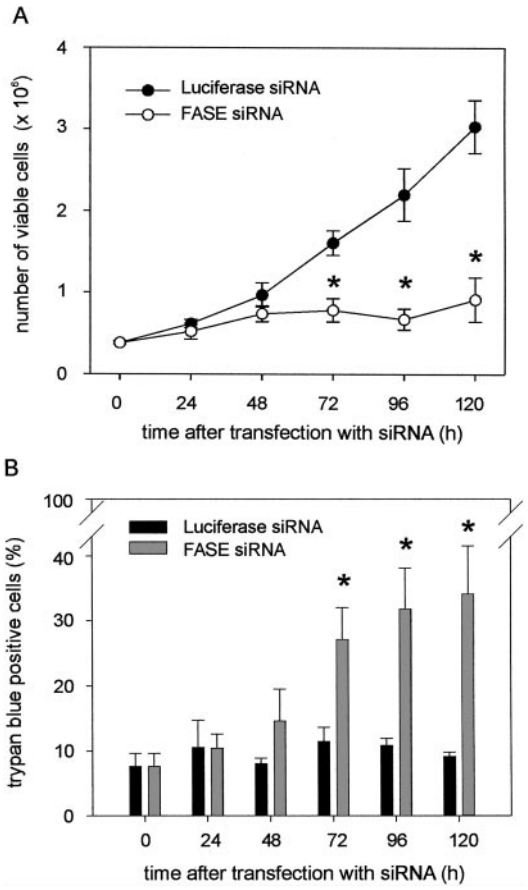

Fig. 5. Impact of FASE RNAi on LNCaP cell number and viability. LNCaP cells were transfected with siRNA-targeting luciferase or FASE. At the indicated time points, attached and detached cells were collected and combined and were stained with trypan blue. Viable (*A*) and dead cells (*B*) were counted. *Columns* represent means of data ($n = 6$); *bars*, ±SD. ∗, significantly different from control (luciferase siRNA-transfected cells) by Tukey test.

(mean ± SD; $n = 5$) of the levels in control cells transfected with luciferase siRNA.

To investigate the impact of RNAi-mediated *FASE* silencing on different lipid species, lipid extracts were analyzed by TLC. As shown in Fig. 3, the majority of ^{14}C label in control cells is incorporated into phospholipids, and FASE RNAi caused a 3-fold decrease in the labeling of these lipids. Smaller but substantial amounts of label were found in triglycerides (typical storage products of fatty acids, present in lipid droplets; Ref. 32) and in free cholesterol. FASE RNAi caused a 7-fold decrease in the synthesis of triglycerides (Fig. 3), resulting in the disappearance of lipid droplets, as evidenced by staining with Oil red O (Fig. 4). In support of the specificity of FASE RNAi, no effect was observed on the production of free cholesterol (Fig. 3).

Silencing of the *FASE* Gene by RNAi Inhibits *in Vitro* Growth of LNCaP Cells and Induces Apoptosis. To assess potential effects of RNAi-mediated *FASE* silencing on growth and cell survival, LNCaP cells (both attached and detached cells) were stained with trypan blue as a marker of cell death at different time points after transfection with siRNA-targeting FASE (or luciferase as a control). Counting the number of viable cells revealed that silencing of the *FASE* gene caused a stagnation of the number of viable cells, whereas control cells continued to proliferate (Fig. 5*A*). The number of trypan blue-positive cells increased from 8% in the control cells to up to 34% in the FASE siRNA-treated cells, indicating that *FASE* gene silencing also induced cell death (Fig. 5*B*). Staining of the nuclear DNA of FASE siRNA-transfected cells with Hoechst 33342 revealed nuclear fragmentation and chromatin condensation (Fig. 6*B*), typical hallmarks of apoptosis that were not detected in control cells transfected with luciferase siRNA (Fig. 6*A*). Additional evidence for the occurrence of apoptosis was obtained by double staining of the cultures with propidium iodide (which stains the nuclei of dead cells) and Annexin V-EGFP, a protein that binds with high affinity to phosphatidylserine, which is translocated from the inner to the outer membrane leaflet early in the apoptotic process. As shown in Fig. 6*C*, control cells stained negative for both propidium iodide and Annexin V-

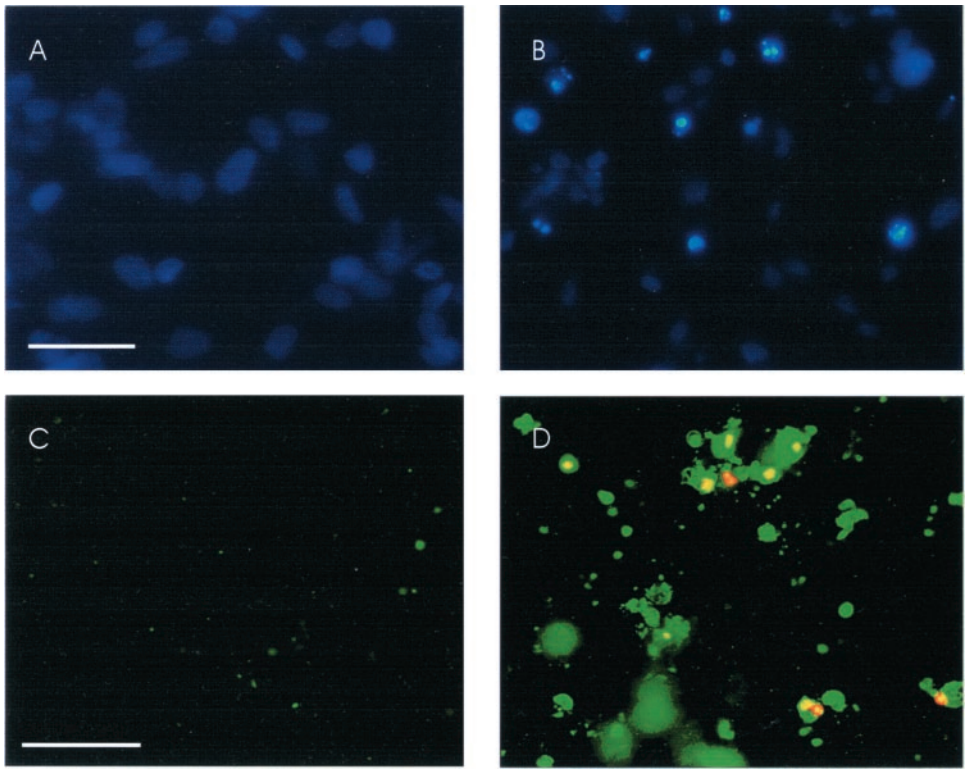

Fig. 6. FASE RNAi-induced apoptosis of LNCaP cells. LNCaP cells were transfected with siRNA-targeting luciferase (*A* and *C*) or FASE (*B* and *D*). At 72 h after transfection, cells were stained with Hoechst 33342 (*A* and *B*) or with Annexin V-EGFP and propidium iodide (*B* and *D*) and analyzed by fluorescence microscopy. *Bar* indicates 50 μm.

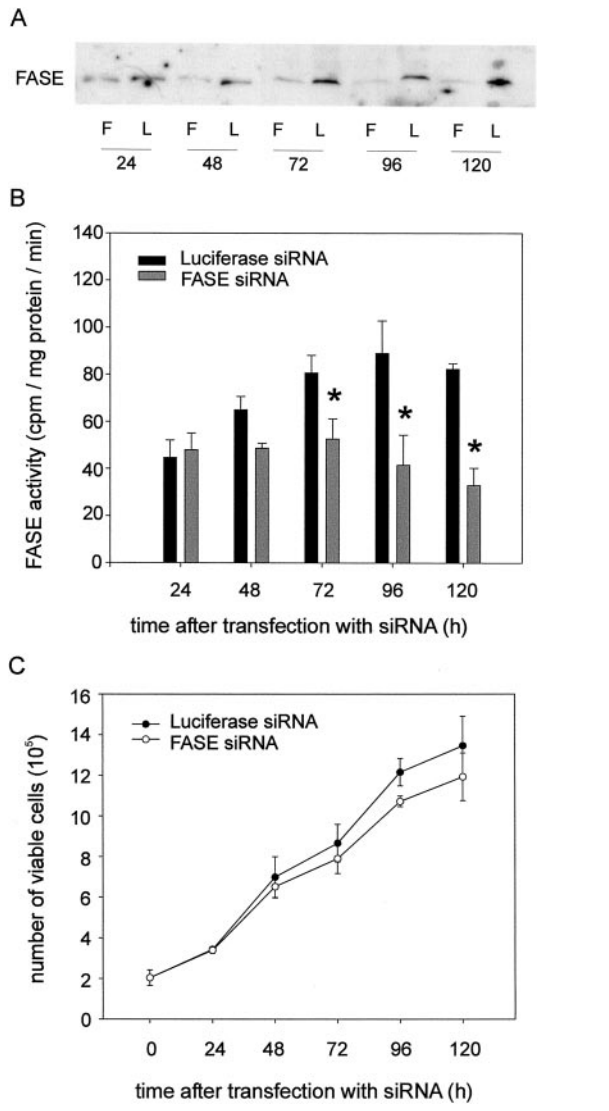

Fig. 7. Impact of FASE RNAi on normal human fibroblasts. *A*, FASE protein levels of fibroblasts transfected with luciferase siRNA (L) or FASE siRNA (F) were determined by Western blot analysis at the indicated time points (h) after transfection. *B*, FASE activity of extracts (collected at the indicated time points) from fibroblasts transfected with siRNA-targeting luciferase or FASE, as measured by quantification of 2-^{14}C-labeled malonyl-CoA incorporation into fatty acids *in vitro*. *Columns* represent means of data ($n = 4$); *bars*, ±SD. *, significantly different from control (luciferase siRNA-transfected cells) by Tukey test. *C*, proliferation of fibroblasts transfected with FASE or luciferase siRNA, as revealed by counting the number of viable cells using the Trypan Blue Dye Exclusion assay. *Columns* represent means of data ($n = 4$); *bars*, ±SD.

EGFP. FASE siRNA-transfected cultures, on the other hand, showed many annexin V-positive cells (Fig. 6*D*). The majority of the cells were negative for propidium iodide, indicating that they were at an early stage of apoptosis. The double positive staining of particular cells (Fig. 6*D*) revealed that these cells were at a late apoptotic (or necrotic) stage. Taken together, these findings provide strong evidence that RNAi-mediated silencing of *FASE* induced cell death through apoptosis.

RNAi-mediated FASE Inhibition Selectively Induces Apoptosis in LNCaP Prostate Cancer Cells but not in Normal Human Fibroblasts. To investigate the selectivity of apoptosis induced by FASE inhibition toward cancer cells compared with normal cells, we also analyzed the impact of RNAi-mediated gene silencing of *FASE* on nonmalignant cells. Because benign prostatic epithelial cells when cultured *in vitro* (*a*) showed high FASE expression and in no respect resembled normal epithelial cells, which in intact tissue *in vivo* show very low FASE expression, and (*b*) were resistant to various siRNA transfection techniques, we used nonmalignant human fibroblasts as a control of normal low FASE-expressing cells. Compared with LNCaP cells (Figs. 1*A* and 2), fibroblasts showed a 10-fold lower FASE expression and FASE activity (Fig. 7, *A* and *B*). Similar to the observations in LNCaP cancer cells, siRNA targeting FASE severely decreased the (already low) expression and activity of FASE in fibroblasts (Fig. 7, *A* and *B*). In contrast with LNCaP cells, silencing of the *FASE* gene by RNAi did not inhibit proliferation of fibroblasts, which showed comparable growth rates as control fibroblasts (transfected with luciferase siRNA; Fig. 7*C*). Furthermore, fibroblasts transfected with FASE siRNA did not reveal any signs of apoptosis but showed normal viability, similar to that of control fibroblasts, as evidenced by staining with trypan blue, Hoechst, or Annexin V-EGFP/propidium iodide (data not shown). These findings clearly demonstrate that RNAi-mediated *FASE* silencing selectively induces cell death in LNCaP cells but not in normal human fibroblasts, thereby lending additional support to the contention that FASE is a selective target for antineoplastic therapy.

DISCUSSION

Differences in gene expression between normal cells and cancer cells often provide interesting targets for antineoplastic therapy. Up to now, mainly chemical inhibitors have been used to exploit such differences and to cause growth disadvantage to cancer cells. Chemical inhibitors, however, have the intrinsic disadvantage that they often evoke nonspecific side effects. Sequence-based approaches such as conventional antisense technologies provide an attractive alternative but usually offer only a transient and partial suppression of the gene of interest. The development of 21-nucleotide siRNAs specifically recognizing particular mRNA sequences provides new powerful reagents to selectively down-regulate gene expression and holds great potential not only for the analysis of gene function but also for the development of gene-specific therapeutic agents.

In this article, we have used siRNA to target the *FASE* gene, which is markedly overexpressed in a wide variety of human cancers. Treatment of LNCaP cells with siRNA-targeting FASE caused a 4-fold reduction of FASE expression and of FASE activity. Moreover, a single transfection with siRNA caused a sustained down-regulation of FASE for at least 4 days. Suppression of FASE by RNAi resulted in a marked decrease of lipogenesis and more particularly in a reduced synthesis of phospholipids and triglycerides. In support of the selectivity of RNAi-mediated *FASE* gene silencing and in contrast with the chemical FASE inhibitor cerulenin, no effect was observed on cholesterol synthesis. As a result of FASE RNAi, LNCaP cells underwent striking morphological changes. The cells became smaller, made poor cell-cell contacts, and displayed multiple spider-like extensions. Suppression of FASE expression significantly inhibited the growth of LNCaP cells and ultimately resulted in apoptosis and cell death. To explore whether FASE RNAi causes a selective growth disadvantage to cancer cells expressing high levels of FASE, we wanted to evaluate the effects of FASE RNAi in a control line with low levels of FASE expression. Unfortunately, all tumor lines tested and even subcultures of normal prostate epithelial cells [as prepared in our laboratory (33) or as supplied by BioWhittaker] displayed high FASE activity. Subcultures from nonmalignant human fibroblasts, however, were found to have a level of FASE activity 10 times lower than that observed in LNCaP. Interestingly, in these fibroblasts, siRNA-targeting FASE still reduced FASE activity, but this reduction did not result in growth inhibition and apoptosis. These data support the contention that FASE RNAi-mediated growth inhibition, and apoptosis is selective for cancer cells with high levels of FASE expression.

The mechanisms by which FASE silencing selectively reduces growth and provokes apoptosis in tumor cells certainly merit additional investigation. The finding that the majority of the radiolabeled acetate incorporated in the lipid fraction goes to phospholipids and that suppression of FASE expression has major effects on the production of these phospholipids is compatible with a role of FASE in the synthesis of membranes required for proliferation. If membrane synthesis were the only site of action, however, growth reduction would also have been expected in fibroblast cultures, unless membrane synthesis in these cells is less dependent on FASE. The selective occurrence of growth inhibition and apoptosis in cell lines expressing high levels of FASE and the observation that in tumor cells FASE overexpression is part of a more general activation of lipogenic gene expression rather point to the alternative possibility that inhibition of FASE expression promotes accumulation of toxic intermediary metabolites such as malonyl-CoA as observed with chemical inhibitors of FASE activity (22, 34, 35).

Taken together, RNAi-mediated silencing of the *FASE* gene definitely establishes FASE as a potential target for antineoplastic therapy. Moreover, RNAi provides a novel, convenient, and selective way to interfere with FASE expression and to study the role of FASE in cancer cell biology. The feasibility of RNAi-mediated gene silencing as a novel tool to arrest tumor growth and to kill cancer cells is being tested in several laboratories, and initial results are certainly promising (36–39). Additional success will depend on the development of (cell-selective) vector systems able to deliver RNAi-inducing sequences in tumor cells.

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