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FAS expression inversely correlates with PTEN level in prostate cancer and a PI 3-kinase inhibitor synergizes with FAS siRNA to induce apoptosis

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Fatty acid synthase (FAS), a key enzyme of the fatty acid biosynthetic pathway, has been shown to be overexpressed in various types of human cancer and is, therefore, considered to be an attractive target for anticancer therapy. However, the exact mechanism of overexpression of the *FAS* gene in tumor cells is not well understood. In this report, we demonstrate that the expression of the tumor suppressor gene PTEN has a significant inverse correlation with FAS expression in the case of prostate cancer in the clinical setting, and inhibition of the *PTEN* gene leads to the overexpression of FAS *in vitro*. We also found that the combination of the expression status of these two genes is a better prognostic marker than either gene alone. Furthermore, our results indicate that the specific inhibition of *FAS* gene by siRNA leads to apoptosis of prostate tumor cells, and inhibition of PI 3-kinase pathway synergizes with FAS siRNA to enhance tumor cell death. These results provide a strong rationale for exploring the therapeutic use of an inhibitor of the PTEN signaling pathway in conjunction with the FAS siRNA to inhibit prostate tumor growth.
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Fatty acid is an essential factor for diverse cellular functions including energy storage, membrane formation, signal transduction and protein acylation (Kuhajda, 2000). Animals derive fatty acids from diet as well as *de novo* synthesis, which is catalysed by the enzyme fatty acid synthase (FAS). FAS is a 250–270 kDa cytosolic protein that forms a homodimer and catalyses the synthesis of palmitate from the condensation of malonyl CoA and acetyl CoA. This enzyme also plays an important role in energy homeostasis by converting excess carbon intake into fatty acids for storage, which, when necessary, provide energy via β-oxidation (Kuhajda, 2000). Since diet supplies most of the fatty acids, the endogenous synthesis of fatty acid is usually minimal in cells. Consequently, FAS is expressed at low or undetectable level in most normal human tissues, with the exception of lactating breast and cycling endometrium (Kuhajda, 2000). In contrast, FAS is overexpressed in a variety of human malignancies including the prostate, breast, ovarian, endometrial, lung, colorectal, stomach and skin cancer (Epstein *et al*., 1995; Alo' *et al*., 1996; Shurbaji *et al*., 1996; Gansler *et al*., 1997; Milgraum *et al*., 1997; Rashid *et al*., 1997; Piyathilake *et al*., 2000; Kusakabe *et al*., 2002; Swinnen *et al*., 2002; Innocenzi *et al*., 2003; Sebastiani *et al*., 2004). In these cancer cells, FAS expression is found to be elevated despite the high level of ambient fatty acids and is, seemingly, independent of the regulatory signals that downregulate fatty acid synthesis in normal cells. Although the exact role of the *FAS* gene in tumorigenesis is yet to be elucidated, treatment of tumor cells with pharmacological inhibitors of FAS leads to cell cycle arrest and apoptosis (Kuhajda, 2000). These observations suggest that FAS overexpression confers a selective advantage to tumor cells by inhibiting apoptosis and promoting cell cycle progression. As FAS is downregulated in most normal human tissues and is overexpressed in a variety of tumors, FAS is considered to be an attractive target for anticancer therapy. However, relatively little is known about the regulatory mechanisms controlling *FAS* gene expression in human tumors, and understanding these mechanisms is of paramount interest to design intervention of the FAS enzymatic pathway.

Thus far, several factors including androgen, epidermal growth factor (EGF), PTEN and p53 have been demonstrated to modulate the expression of the *FAS* gene *in vitro*. Recent functional analyses of the promoter of the *FAS* gene suggest that sterol regulatory element binding proteins (SREBP) and insulin play major role in the regulation of *FAS* gene expression (Magana and Osborne, 1996; Sul *et al*., 2000). Indeed, androgen and EGF have been found to elevate the expression of the *FAS* gene in prostate cancer cells *in vitro* through the

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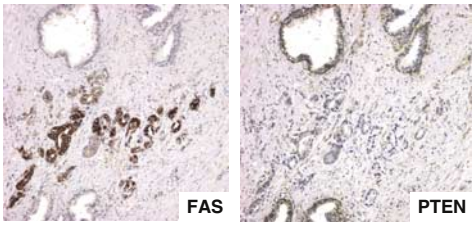
action of the SREBPs (Swinnen *et al*., 2000; Heemers *et al*., 2001). FAS has also been shown to be regulated by the tumor suppressor gene *PTEN in vitro* (Van de Sande *et al*., 2002). This regulation is thought to be mediated by a PI 3-kinase/Akt-dependent pathway, since overexpression of Akt-1 enhanced the expression of the *FAS* gene, while treatment of the cells with a PI 3-kinase inhibitor reduced FAS expression (Van de Sande *et al*., 2002; Yang *et al*., 2002). Another tumor suppressor gene *p53* has also been suggested to be involved in the regulation of FAS in a colon carcinoma cell line *in vitro* (Li *et al*., 2001). Thus, the *FAS* gene appears to be regulated by multitude of factors in malignant cells at least in culture. However, to develop a viable therapeutic intervention of the FAS activity via any of these pathways, it is essential to validate the involvement of these factors at the clinical setting.

In order to examine the relationship between different clinicopathological factors and FAS expression levels in prostate cancer, an immunohistochemical analysis was performed on 78 prostate tumor specimens, randomly selected from surgical pathology archives of the Akita Red Cross Hospital (Akita, Japan), dating from 1988 to 2001. The prostate cancer specimens included 34 cases of needle biopsy, 12 cases of transurethral resection and 32 cases of radical prostatectomy. The ages of the patients ranged from 53 to 89 years, with a mean of 72 years. Each patient sample was assigned two separate Gleason grades, corresponding to the two predominant histological patterns. The nuclear grades were determined according to the WHO criteria, and the degree of differentiation was classified into 'well', 'moderate' or 'poor' by pathologists. Complete 5-year follow-up data were available for 43 patients, and those who died of other causes were eliminated from the study. As shown in Figure 1a, FAS expression was found to be highly elevated in prostatic tumor cells compared to the expression level in the epithelial cells of normal ducts and glands. Less than 10% of the cells in benign prostatic hyperplasia expressed FAS, while the stroma, endothelial cells and nerve bundles did not frequently ($<5\%$) express the *FAS* gene. In the cancerous cells, FAS expression was mostly cytoplasmic and nuclear stain was observed in only $<1\%$ of the cells. Notably, expression of the *FAS* gene exhibited a significant correlation with the degree of differentiation as well as Gleason grade, which is also consistent with previous observations (Epstein *et al*., 1995; Shurbaji *et al*., 1996; Swinnen *et al*., 2002). However, the degree of metastasis had no significant correlation with FAS expression, which suggests that overexpression of the *FAS* gene is a relatively early event in human prostate cancer. We then examined the relationship of FAS expression with several potential regulatory factors, including androgen, EGF, PTEN and p53 by analysing the same set of prostate cancer specimens. We found that, among these factors, PTEN was expressed at a high level in normal prostatic glands and ducts, while the poorly differentiated tumor cells in the same specimen had significantly reduced level of PTEN expression (Figure 1a). In fact, as shown in a representative field in Figure 1a, almost exactly reverse staining pattern was observed when each cell in the same field was examined for PTEN and

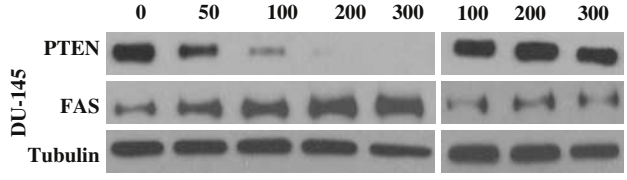
a



b **Relation of FAS expression with other clinicopathological factors**

		FAS expression		P value
	All (78)	negative (36)	positive (42)	
Gleason grade				
≤7	38	29 (76.32%)	9 (23.68%)	
>7	40	17 (42.5%)	23 (57.5%)	0.005*
Differentiation				
Well	17	12 (70.59%)	5 (29.41%)	
Moderate	25	18 (72.0%)	7 (28%)	
Poor	36	16 (44.44%)	20 (55.56%)	0.05*
Nuclear grade				
I	38	24(63.16%)	14(36.84%)	
II/III	40	22(55.0%)	18(45.0%)	0.62
PTEN				
Positive	50	34(68.0%)	16(32.0%)	
Reduced	28	12(42.86%)	16(57.14%)	0.05*
P53				
Wild type	75	44(58.67%)	31(41.33%)	
Mutant	3	2(66.67%)	1(33.33%)	1.0
EGFR				
Positive	42	28(66.67%)	14(33.33%)	
Reduced	36	18(50.0%)	18(50.0%)	0.21
AR				
Positive	59	34(57.63%)	25(42.37%)	
Negative	19	12(63.16%)	7(36.84%)	0.87
Metastasis status				
Organ confined	48	33(68.75%)	15(31.25%)	
Lymph node metastasis	25	11(44.0%)	14(56.0%)	0.07
Bone metastasis	27	11(40.74%)	16(59.26%)	0.08

c



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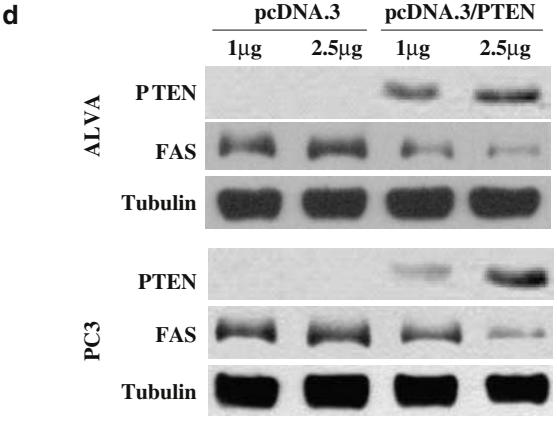
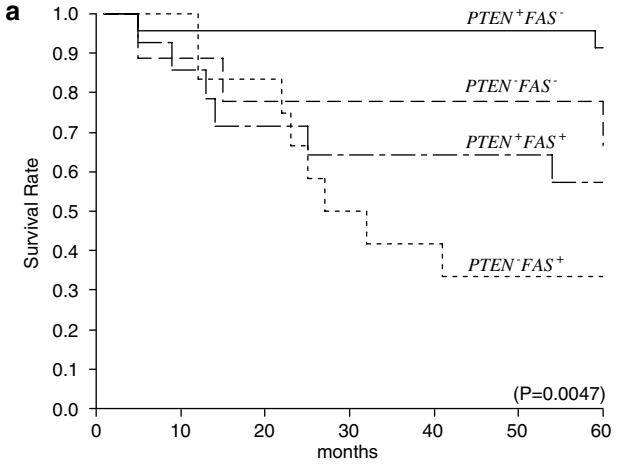


FAS expression. The results of the PTEN and FAS immunohistochemistry were quantitatively judged based on the intensity of staining on a scale of 0–3. Statistical evaluation revealed a strong inverse correlation ($P = 0.05$) between PTEN and FAS expression status (Figure 1b). Out of 50 patients who were positive for PTEN, 34 (68%) exhibited negative FAS expression, and among 28 patients with reduced PTEN expression, 16 (57.14%) had positive FAS level. These results of the immunohistochemical analysis are consistent with the notion that PTEN negatively regulates the expression of the *FAS* gene. On the other hand, expression of AR, p53 or EGFR did not show any significant correlation with FAS expression, although FAS has been previously shown to be regulated by these factors *in vitro* (Figure 1b). Therefore, expression of the *FAS* gene, for the most part, appears to be independent of these factors in human prostate cancer, although the possibility of involvement of some downstream pathways regulated by these factors cannot be ruled out.

Since the result of IHC analysis indicated that the loss of PTEN significantly correlated with the overexpression of FAS in prostate cancer, we examined whether abrogation of the PTEN expression indeed elevated the expression of the *FAS* gene in tumor cells. To knock down the PTEN expression, four individual siRNAs directed against the *PTEN* gene were pooled and transfected into PTEN-positive prostate cancer cell line, DU-145. As shown in Figure 1c, we found that abrogation of PTEN expression by 100, 200 and 300 nM siRNA was followed by a significant increase in the expression of the *FAS* gene, while the PTEN expression was notably suppressed. This effect was mediated specifically by PTEN siRNA, since equivalent amount of GFP siRNA did not affect the expression of *PTEN* or *FAS* gene (Figure 1c, right panels). We observed similar effect of PTEN inhibition on the expression of the *FAS* gene in the PTEN-positive breast carcinoma cell lines, MDA-435 and MCF-7 (Supplementary Figure 1). As a complementary approach, we overexpressed the *PTEN* gene in PTEN-negative prostate carcinoma cells, PC3 and ALVA, and found that PTEN downregulated the expression of the *FAS* gene in a dose-dependent manner in these cells (Figure 1d). These results corroborate the notion that PTEN controls the expression of the *FAS* gene, and suggests that the loss of PTEN expression, which is frequently observed in the case of human prostate cancer, leads to the upregulation of FAS expression.

To further clarify the roles of *PTEN* and *FAS* gene expression in prostate cancer, we next evaluated the prognostic importance of the combination of these two markers. Univariate survival analysis was performed using the Kaplan–Meier method in the prostate cancer cases with a 5-year follow-up. As shown in Figure 2a, patients with negative expression of PTEN and elevated expression of FAS ($PTEN^-$ FAS^+) had significantly worse ($P = 0.0047$) overall survival rate than the other three patient groups ($PTEN^+FAS^+$, $PTEN^+FAS^-$ and $PTEN^-FAS^-$). Importantly, Cox regression analysis (Figure 2b) revealed that the combination of *PTEN* and *FAS* gene expression was an independent prognostic marker for prostate cancer. The hazard ratio of FAS (positive expression) and PTEN (negative expression) as individual marker was 4.454 and 2.819, respectively. When both markers were combined, however, the hazard ratio was 11.525, meaning that the death risk of a patient with positive expression of FAS and negative PTEN expression ($PTEN^-FAS^+$) was 11.525

Figure 1 Expression of FAS is negatively regulated by PTEN. (**a** and **b**) Correlation of FAS expression with PTEN and other clinical parameters. Sections (4 μm thick) were cut from the paraffin blocks of prostate tumors and mounted onto charged glass slides. The sections were deparaffinized, rehydrated and antigen retrieval was performed by treatment with 25 mM sodium citrate buffer, pH 9 (for FAS and p53), 10 mM sodium citrate buffer, pH 6 (for PTEN and androgen receptor) or pepsin treatment for 15 min at 37°C (for EGFR). The slides were incubated overnight at 4°C with the following antibodies: anti-FAS rabbit polyclonal antibody (1 : 100, Immuno-biological Laboratories Co., Japan), anti-PTEN rabbit polyclonal antibody (1 : 200, Upstate Biotechnology, MA, USA), anti-p53 mouse monoclonal antibody (1 : 100, Clone DO-7, Dako Corp, Carpentaria, CA, USA), anti-androgen receptor (AR) rabbit polyclonal antibody (1 : 100, Zymed Corp) and anti-EGFR mouse monoclonal antibody (1 : 70, Zymed Laboratories). The sections were incubated with the corresponding HRP-conjugated secondary antibodies, and DAB substrate chromagen solution (Envision-plus kit, DAKO Corp, CA, USA) was applied followed by counter-staining with hematoxylin. A representative field with immunos-taining for FAS and PTEN is shown in (**a**). The association between FAS and other prognostic markers was calculated by χ^2 analysis, using SPSS software (**b**). (**c**) Inhibition of the PTEN gene attenuates FAS expression. Human prostate cancer cell line DU-145 was obtained from ATCC, and cultured in RPMI 1640 medium supplemented with 10% FBS, streptomycin (100 μg/ml), penicillin (100 U/ml) and 250 nM dexamethasone, at 37°C in a 5% CO_2 atmosphere. Four individual siRNAs against the PTEN gene, combined into one pool, were designed and synthesized from Dharmacon Inc. (Lafayette, CO, USA). The PTEN siRNA pool was transfected into the PTEN-positive prostate cancer cell line, DU-145, at indicated amounts (left panels), using the Trans-TKO transfection reagent (Mirus Corp.) according to the manufacturer's protocol. As a negative control (right panels), the DU-145 cells were also transfected with siRNA duplex targeting the GFP (green florescent protein) gene (Dharmacon Inc.). After 72 h post-transfection, cells were collected and lysed with mammalian cell lysis buffer (50 mM Tris-Cl (pH 7.4), 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA). The lysates were subjected to SDS–PAGE followed by Western blot analysis using antibodies against PTEN (1 : 1000, Upstate Biotechnology), FAS (1 : 1000, Immuno-biological Laboratories Co., Japan) or tubulin (1 : 1000, Upstate Biotechnology). The membranes were incubated with HRP-conjugated secondary antibodies and visualized by ECL Plus system (Amersham Life Sciences). (**d**) PTEN downregulates the expression of the FAS gene. Human prostate cancer cell line, ALVA, was kindly provided by Dr W Rosner (Columbia University, New York, USA), and PC3 was purchased from ATCC. The cells were cultured as described in (**c**) legend. To create a mammalian expression plasmid of PTEN, total human placental RNA (Clontech) was reverse-transcribed and the cDNA was PCR amplified with a pair of forward and reverse primers (5′GCAAG CTTGCCACCATGACAGCCATCATCAAAGAGATCG and 5′GCGGATCCTCAGACTTTTGTAATTTGTGTATGC). The PCR product was cloned into the mammalian expression vector pcDNA3 (Invitrogen, CA, USA) and the resultant clone was designated as pcDNA3/PTEN. The empty pcDNA3 vector or the pcDNA3/PTEN expression plasmid was transiently transfected into the prostate cancer cells, and 48 h after post-transfection, cells were lysed and Western blot was performed as described in (**c**) legend



b Cox regression analysis

Variables	reference level	β	SE	Wald's x^2	Hazard ratio	95% CI	P
FAS status	reduced expression	1.494	0.524	8.133	4.454	1.595-12.433	0.004
PTEN status	positive expression	1.0336	0.466	4.939	2.819	1.130-7.030	0.026
Combination of FAS PTEN status	reduced FAS/ PTEN positive expression	2.445	0.800	9.344	11.525	2.404-55.25	0.002

Figure 2 Combination of PTEN and FAS expression significantly correlates with survival. (**a**) Overall survival rate over a period of 5 years was calculated in 43 patients with prostate cancer in relation to the expression of the PTEN and FAS genes by Kaplan–Meier method using the SPSS program. The patients were grouped into four categories: PTEN-positive and reduced FAS ($PTEN^+FAS^-$); PTEN-positive and increased FAS ($PTEN^+FAS^+$); PTEN-negative and reduced FAS ($PTEN^-FAS^-$) and PTEN-negative and increased FAS ($PTEN^-FAS^+$). The *P*-value was determined by log-rank test. (**b**) Cox regression analysis was conducted to assess the contribution of the indicated variables to disease prognosis

times as compared to patients of other three categories ($PTEN^+FAS^+$, $PTEN^+FAS^-$ and $PTEN^-FAS^-$). These data underscore the prognostic importance of combination of the *FAS* and *PTEN* genes, and also point toward

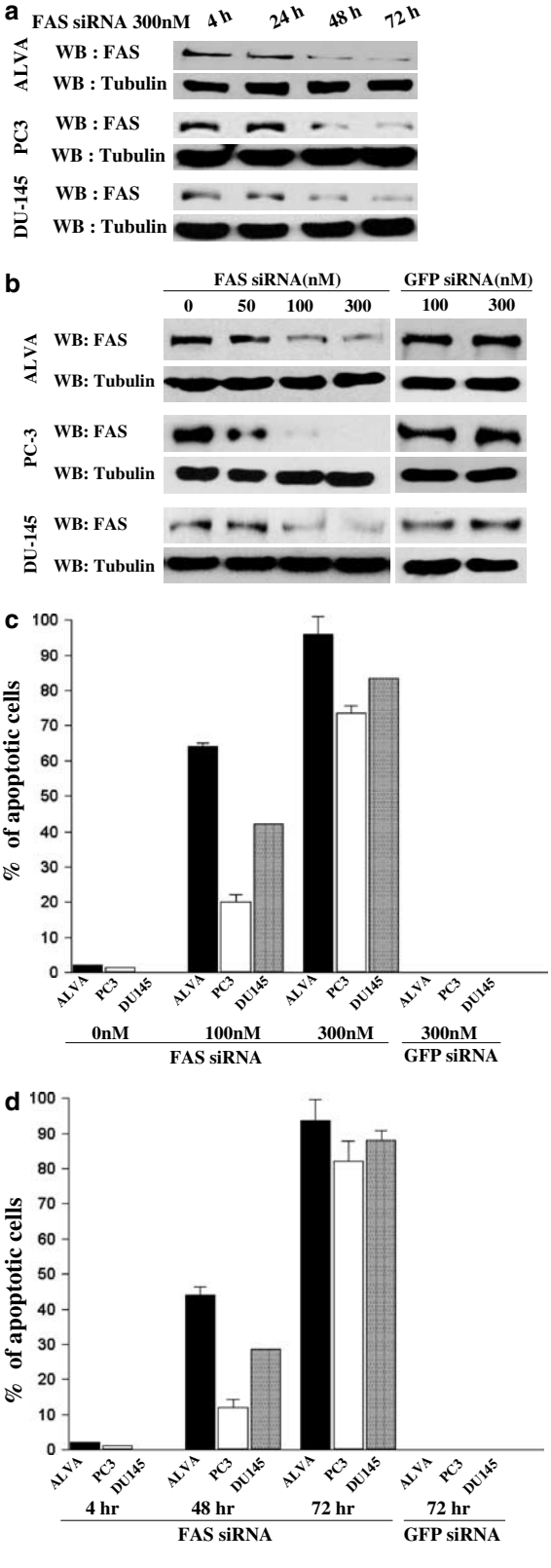


Figure 3 Inhibition of FAS expression in prostate cancer cells leads to apoptosis. (**a**) Time course of inhibition of the FAS gene by siRNA. Four individual siRNAs against the FAS gene (sense strand sequences: GAGCGUAUCUGUGAGAAAC, GACGA GAGCACCUUUGAUG, UGACAUCGUCCAUUCGUUU, CC AUGGAGCGUAUCUGUGA) were designed and synthesized by Dharmacon Inc. The individual siRNAs combined into one pool were transfected into the prostate cancer cells, ALVA, PC3 and DU-145, using Trans-TKO transfection reagent. At different time points after transfection, cells were collected, and Western blot analysis for FAS and tubulin was performed as described in Figure 1c legend. (**b**) Dose-dependent inhibition of the *FAS* gene by siRNA. FAS siRNA, as described above, was transfected into ALVA, PC3 and DU-145 cells in various amounts. As a negative control (right panels), the cells were also transfected with GFP siRNA. After 72 h post-transfection, cells were collected and the expression of FAS and tubulin was examined by Western blot as described above. (**c**) Apoptotic effect of FAS siRNA on prostate cancer cells. Prostate cancer cell lines, ALVA, PC3 and DU-145, were transfected with various amounts (left panels) or 300 nM (right panels) of FAS siRNA or GFP siRNA and incubated at 37°C for 72 h (left panels) or different lengths of time (right panels), as indicated. The cells were then fixed with 4% paraformaldehyde in PBS followed by permeabilization with 0.1% Triton X-100/0.1% sodium citrate at 4°C. The cells were washed extensively and the TUNEL assay was performed using the *In Situ* Cell Death Detection kit/TMR Red (Roche Applied Science). The reaction was stopped after 1 h, and the percentage of apoptotic cells in each well was counted under confocal microscope

the clinical relevance of the PTEN-FAS pathway in prostate tumorigenesis.

It has been suggested that the upregulation of FAS observed in various tumor cells is due to the selective advantage that the *FAS* gene confers by promoting proliferation and inhibiting apoptosis in these cells. Therefore, the specific inhibition of *FAS* gene is expected to result in the blockade of tumor cell growth. To examine the effect of direct inhibition of the expression of the *FAS* gene in prostate cancer cells, four individual siRNAs against the *FAS* gene, in combination, were transfected into the FAS-positive prostate cancer cell lines, ALVA, PC3 and DU-145. As shown in Figure 3a, 300 nM of the siRNA abrogated the expression of the *FAS* gene in a time-dependent manner in all the cell lines tested, with a significant effect being observed 48 h post-transfection. Furthermore, when various amounts of the FAS siRNA were transfected into these cells, the siRNA dose dependently inhibited the expression of the *FAS* gene, indicating the efficacy of this set of siRNA in knocking down FAS expression (Figure 3b). In order to examine the effect of the direct inhibition of the *FAS* gene, the prostate cancer cells were transfected with various amounts of FAS siRNA or equivalent amounts of GFP siRNA, and the extent of apoptosis was measured by assessing the DNA fragmentation. As shown in Figure 3c, in each case, FAS siRNA significantly augmented the degree of apoptosis in both dose- and time-dependent manner. This effect was mediated specifically by the inhibition of the *FAS* gene, since equivalent amounts of GFP siRNA did not have any such effect (Figure 3c). These results suggest that this set of siRNAs, specifically targeted to the *FAS* gene, leads to apoptosis of tumor cells and, therefore, is potentially useful for therapeutic application.

As described above, our data suggest that PTEN controls the expression of the *FAS* gene both *in vitro* and *in vivo*. PTEN is a dual specificity phosphatase that inhibits PI 3-kinase-dependent activation of Akt, and deletion or inactivation of PTEN results in constitutive Akt activation (Cantley and Neel, 1999). Notably, Akt has also been shown to modulate the expression of the

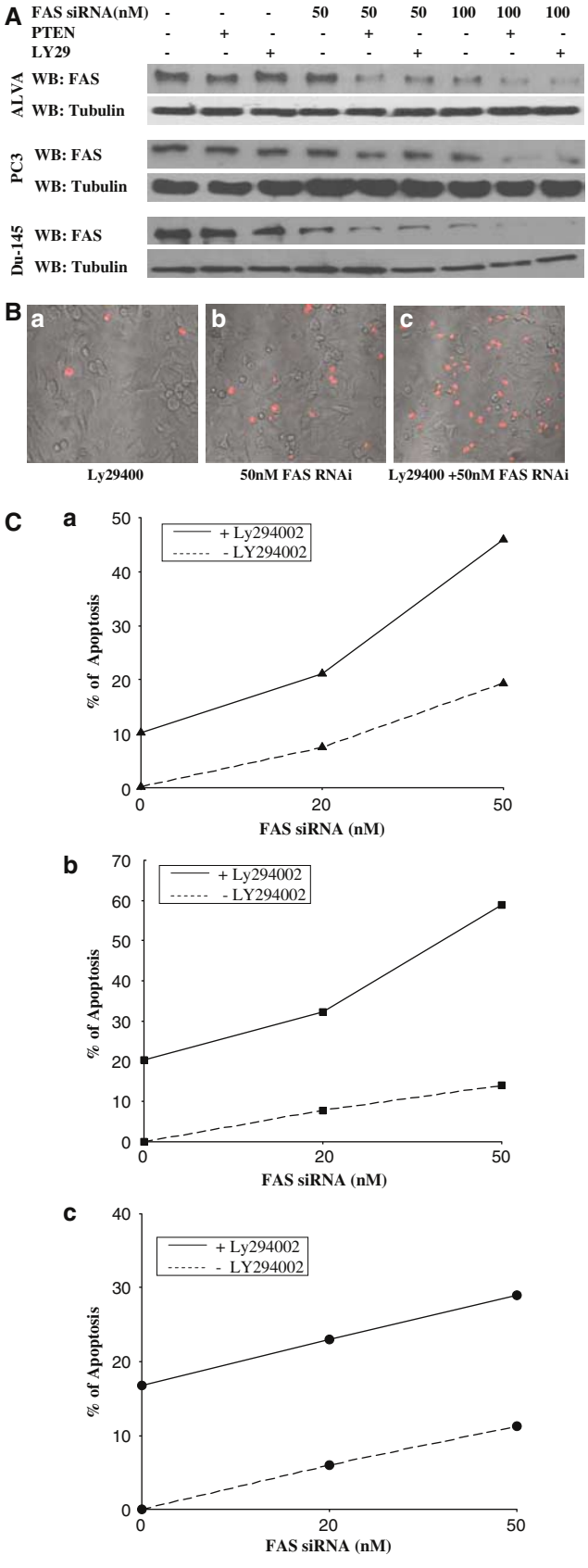


Figure 4 FAS siRNA cooperates with the PEN signaling pathway to inhibit FAS expression and trigger apoptosis. (**A**) Effect of combination of FAS siRNA and PTEN pathway components on FAS expression. PTEN expression plasmid, Ly294002 (PI 3-kinase inhibitor), FAS siRNA or a combination of these agents, as indicated, was transfected/added to the prostate cancer cells, ALVA, PC3 and DU-145. After 72 h post-transfection, cells were collected and the levels of FAS and tubulin were examined by Western blot analysis. (**B** and **C**) Effect of combination of FAS siRNA and PTEN pathway components on apoptosis. FAS siRNA (20 and 50 nM), Ly294002 (100 nM) or a combination of these agents were transfected/added to the above prostate cancer cells. After 72 h, an *in situ* apoptosis assay was performed as described in Figure 3c legend. Confocal micrographic images of representative fields from wells treated with LY294002 (a), 50 nM FAS siRNA (b) and a combination of both (c) are shown in (**B**). (**C**) The percentage of apoptotic cells in all the experimental groups described in (**B**) was calculated in the case of ALVA (a), PC3 (b) and DU-145 (c) cells. Each experiment was carried out in duplicate. The interactive effect of Ly294002 and various doses of FAS siRNA on apoptosis was examined by two-way factorial ANOVA using the SPSS program

FAS gene *in vitro* (Van de Sande *et al*., 2002; Yang *et al*., 2002). Therefore, to explore the possibility that inhibition of PI 3-kinase/Akt pathway could cooperate with the gene-specific siRNA to block the expression of the *FAS* gene, we treated the prostate cancer cells with the FAS siRNA and PTEN expression vector or PI 3-kinase inhibitor, Ly294002. As shown in Figure 4A, in prostate cancer cell lines, ALVA, PC3 and DU-145, low amount of PTEN expression plasmid, 100 nM LY294002 or 50 nM FAS siRNA individually did not have any significant effect on the expression of the *FAS* gene. The same dose of PTEN expression vector or LY294002, however, cooperated with different doses of FAS siRNA and significantly abrogated the FAS expression. This result is consistent with the notion that PTEN downregulates the expression of the *FAS* gene and raises an attractive possibility to inhibit FAS in prostate tumor cells using a combination of FAS siRNA and an inhibitor of PI 3-kinase/Akt pathway. To further explore this possibility, we treated the prostate cancer cells with 100 nM Ly294002, various doses of FAS siRNA or a combination of both, and examined the apoptotic index. As shown in Figure 4C (a), in the case of prostate cancer cell line, ALVA, while 100 nM Ly294002 or 50 nM FAS siRNA alone induced apoptosis in only 10.2 and 19.4% of cells, respectively, the extent of apoptosis was strikingly higher (45.9%) when a combination of both the agents was used. Thus, the apoptotic effect of the combination of PI 3-kinase inhibitor and FAS siRNA significantly exceeded the sum of either agent administered alone, indicating a significant synergistic interaction between these two agents ($P = 0.006$). It should be noted that such synergistic effect was caused specifically by the FAS siRNA, since 50 nM GFP siRNA alone or in combination with 100 nM Ly294002, induced apoptosis in <1 and 11.3% cells, respectively. Similarly, in PC3 cells, 100 nM Ly294002, 50 nM FAS siRNA or 50 nM GFP siRNA caused apoptosis in 20.4, 14 and <1% cells, respectively, while the combination of Ly294002 and FAS siRNA or Ly294002 and GFP siRNA induced apoptosis in 58.8 and 20% cells, indicating a specific and significant ($P = 0.004$) synergy between Ly294002 and FAS siRNA (Figure 4C (b)). In the case of DU-145, 100 nM Ly294002, 50 nM FAS siRNA or 50 nM GFP siRNA caused apoptosis in 16.8, 11.3 and <1% cells, respectively. However, the combination of Ly294002 and FAS siRNA or Ly294002 and GFP siRNA induced apoptosis in 29 and 15% cells, indicating an additive but not synergistic effect of the combination of Ly294002 and FAS siRNA (Figure 4C (c)). The lack of synergistic interaction between these two agents in DU-145 may be at least partially explained by the high endogenous level of PTEN in these cells. These results provide a strong rationale for exploring the therapeutic use of an inhibitor of the PI 3-kinase pathway in conjunction with the FAS siRNA, particularly in patients lacking the PTEN activity, which is a common occurrence in human prostate cancer.

PTEN signaling pathway is strongly implicated in the development of prostate cancer. It has been demonstrated that mice with prostate-specific biallelic deletion of the *PTEN* gene spontaneously develop PIN (prostatic intraepithelial neoplasia) lesions followed by invasive adenocarcinoma, and more than 50% of the animals develop pulmonary metastasis by 29 weeks of age (Wang *et al*., 2003). PTEN deletions are found in 30% of primary prostate cancers and 63% of metastatic prostate tissue samples, placing PTEN mutation among the most common genetic alterations in human prostate cancer (Suzuki *et al*., 1998; Dahia, 2000). On the other hand, FAS, which plays a central role in *de novo* lipogenesis, is known to be overexpressed in various types of tumors including prostate cancer. These observations raised a possibility of a functional relationship between these two genes; however, the expression of *FAS* and *PTEN* genes has not been previously examined in a comparative manner in clinical samples of cancer patients. In this report, we have demonstrated that the expression of the *PTEN* gene has a significant inverse correlation with FAS expression level in the case of prostate cancer. Furthermore, mimicking the situation of loss of PTEN expression in human prostate cancer, we knocked down the expression of the *PTEN* gene, which led to the upregulation of the FAS expression. These results strongly implicate that loss of the *PTEN* gene leads to elevation of FAS expression in human prostate cancer. We have also demonstrated that combination of PTEN and FAS expression can be an independent prognostic factor in the case of human prostate cancer, which also indicates the significance of the PTEN-FAS regulatory pathway at the clinical setting.

FAS, which is selectively overexpressed in the cancer cells, is an attractive target for anticancer therapy. The pharmacological inhibitors of FAS such as cerulenin, C75 and Orlistat have been shown to significantly suppress the cellular FAS level and also induce apoptosis in a variety of human cancer cells including the prostate, breast and ovarian cancer (Pizer *et al*., 1996, 2001; Pflug *et al*., 2003; Kridel *et al*., 2004). However, the specificity of action of these inhibitors is a major concern, as they are also known to inhibit protein palmitoylation, protein acylation and cholesterol synthesis, and to cause undesirable side effects such as anorexia and weight loss (Malvoisin and Wild, 1990; Jochen *et al*., 1995; Clegg *et al*., 2002; Takahashi *et al*., 2004). It is therefore desirable to develop a strategy that will specifically inhibit the expression of the *FAS* gene. In this report, we demonstrated the efficacy of a set of FAS siRNA to abrogate directly the expression of the *FAS* gene and trigger apoptosis, which is also consistent with a recent report, where FAS siRNA has been shown to cause apoptosis in LnCaP cells *in vitro* (De Schrijver *et al*., 2003). While siRNA is a powerful tool to downregulate a particular gene, there are also several limitations for its practical usage such as problems with stability and delivery (Woessmann *et al*., 2003). Therefore, combinatorial approach with highly effective multiple agents are warranted. Based on our clinical observations and *in vitro* data that PTEN inhibits the expression of the *FAS* gene, we explored the possibility

of inhibiting the PTEN signaling pathway and FAS in conjunction, and found that the FAS siRNA indeed synergized with the PI 3-kinase inhibitor to trigger apoptosis in prostate cancer cells. Together, the data presented in this paper define PTEN as a regulatory factor for FAS expression *in vivo* and provide a strong rationale for use of the inhibitor of PTEN signaling pathway with direct inhibition of the *FAS* gene as an anticancer therapeutic approach.

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