

**Angiogenesis, Metastasis, and the Cellular Microenvironment****HO-3867, a Synthetic Compound, Inhibits the Migration and Invasion of Ovarian Carcinoma Cells through Downregulation of Fatty Acid Synthase and Focal Adhesion Kinase**Karuppaiyah Selvendiran<sup>1</sup>, Shabnam Ahmed<sup>1</sup>, Alex Dayton<sup>1</sup>, Yazhini Ravi<sup>1</sup>, M. Lakshmi Kuppusamy<sup>1</sup>, Anna Bratasz<sup>1</sup>, Brian K. Rivera<sup>1</sup>, Tamás Kálai<sup>2</sup>, Kálmán Hideg<sup>2</sup>, and Periannan Kuppusamy<sup>1</sup>**Abstract**

Fatty acid synthase (FAS) and focal adhesion kinase (FAK), which are overexpressed in a variety of human epithelial tumors, play a key role in the migration and invasion of cancer cells. Hence, strategies targeted at inhibiting the FAS/FAK proteins may have therapeutic potential for cancer treatment. The goal of the present study was to determine the effect of HO-3867, a synthetic compound, on the migratory ability of ovarian cancer cells and to understand the mechanistic pathways including the involvement of FAS, FAK, and associated signaling proteins. The study was done using two established human ovarian cancer cell lines, A2780 and SKOV3. Incubation with 10  $\mu\text{mol/L}$  HO-3867 for 24 hours significantly inhibited the native as well as the vascular endothelial growth factor (VEGF)-mediated migration and invasion of the cells. HO-3867 significantly attenuated FAS and FAK protein levels apparently through accelerated ubiquitin-dependent degradation, as shown by a clear downregulation of isopeptidase USP2a. Exposure of cells to HO-3867 also significantly inhibited FAS activity and mRNA levels and a number of downstream proteins, including phospho-extracellular signal-regulated kinase 1/2, phospho-human epidermal growth factor receptor 1, sterol regulatory element binding protein 1, VEGF, and matrix metalloproteinase 2. Western blot and immunohistochemical analyses of A2780 xenograft tumors in mice treated with HO-3867 showed significant reduction in FAS, FAK, VEGF, and downstream protein levels when compared with the untreated control. Collectively, the results showed that HO-3867 suppressed the migration and invasion of ovarian cancer cells by inhibiting the expression or activity of FAS and FAK proteins. The study suggests that molecular targeting of FAS and FAK by HO-3867 may be a potential strategy for ovarian cancer therapy. *Mol Cancer Res*; 8(9): 1188–97. ©2010 AACR.

**Introduction**

Tumor progression is a complex process that includes malignant transformation, proliferation, invasion, and metastasis of cancer cells. Particularly, cancer cell invasion and metastasis are the critical processes that define the aggressive phenotype of human cancers and pose major impediments to treatment (1, 2). Whereas the development of anticancer therapy is traditionally focused on the inhibition of cancer cell proliferation, therapeutic strategies targeted toward inhibiting the spread of cancer cells from a primary tumor to secondary sites can be

valuable in the treatment of aggressive malignancies (1). Tumor cell migration requires the concerted effort of a number of molecules such as integrins, cell adhesion molecules, soluble cytokines and growth factors, matrix-degrading proteases, and Rho GTPases (3). The migration process involves the assembly and disassembly of focal adhesions and is stimulated extracellularly and initiated by integrins and intracellular signaling proteins located in focal adhesions (4). Focal adhesion kinase (FAK), a tyrosine receptor kinase, is activated in focal adhesions and is important in cell-extracellular matrix interactions that affect cell migration, proliferation, and survival (5). Many malignant human tumors exhibit increased FAK expression and tyrosine phosphorylation (6), which are correlated with the acquisition of an invasive cell phenotype and increased metastasis (7).

Ovarian carcinoma remains the most lethal among gynecologic cancers due to a lack of early detection methods and effective treatments for late-stage malignancies (8). As found in many other types of human tumors, overexpression or hyperactivation of FAK and fatty acid synthase (FAS) has recently been found in most ovarian cancers,

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in which it is highly associated with high aggressiveness and poor patient survival (4, 9-11). Increased expression of FAS occurs very early in cancer development and becomes more distinct as the tumor progresses toward a more advanced stage. FAS enzyme is responsible for the *de novo* synthesis of fatty acids, and it has emerged as a potential therapeutic target for human cancer (10). High levels of FAS expression have been found in ovarian cancer (12) and in most human solid tumors (13). FAS plays a significant role in the synthesis of phospholipids partitioning into detergent-resistant membrane microdomains. These are raft aggregates implicated in key cellular processes including signal transduction, intracellular trafficking, cell polarization, and cell migration. Inhibition of FAS activity is selectively cytotoxic to human cancer cells *in vitro* and *in vivo* (9, 10), including human ovarian cancer xenografts (14). However, the mechanisms linking the inhibition of FAS activity to induction of cancer cell death and inhibition of cancer cell migration remain an active area of investigation.

We recently reported that HO-3867, a diarylidene-piperidone-based synthetic compound with an interesting antioxidant appendage, exhibited significant growth arrest and apoptosis in a number of human cancer cell lines including breast, colon, head and neck, liver, lung, ovarian, and prostate cancers with no apparent toxicity to noncancerous cells (15, 16). We observed that the anticancer activity of HO-3867 in ovarian cancer was mediated by inhibition of signal transducer and activator of transcription-3 (STAT3) phosphorylation at Tyr705 and Ser727 residues and induction of the apoptotic markers cleaved caspase-3 and poly(ADP-ribose) polymerase. The protective activity of HO-3867 toward noncancerous cells was shown to be mediated by the ability of the compound to confer selective antioxidant protection to the healthy cells. In a subsequent *in vivo* study, we further showed that HO-3867 significantly inhibited the growth of ovarian xenografted tumors (A2780) in a dose-dependent manner (17). Western blot analyses of the xenograft tumor tissues confirmed that HO-3867 inhibited pSTAT3 (Tyr705 and Ser727) and pJAK1 and increased the apoptotic markers cleaved caspase-3 and poly(ADP-ribose) polymerase.

Although our previous studies clearly showed the potential of HO-3867 as a safe and effective anticancer agent for ovarian cancer therapy, the possible effect and mechanism of the compound on tumor cell migration and invasion have not been established. Accordingly, the goal of the present study was to determine the effect of HO-3867 on the migratory ability of ovarian cancer cells and to understand the mechanistic pathways including the involvement of FAS, FAK, and associated signaling proteins. The study was done using two established human ovarian cancer cell lines, namely, A2780 and SKOV3, under *in vitro* as well as *in vivo* conditions on xenografted tumor in mice. The results clearly showed that HO-3867 suppressed the migration and invasion of the ovarian cancer cells by inhibiting the expression/activity of FAS and FAK proteins.

The study suggested that molecular targeting of FAS and FAK by HO-3867 might be a potential strategy for ovarian cancer therapy.

## Materials and Methods

### Materials

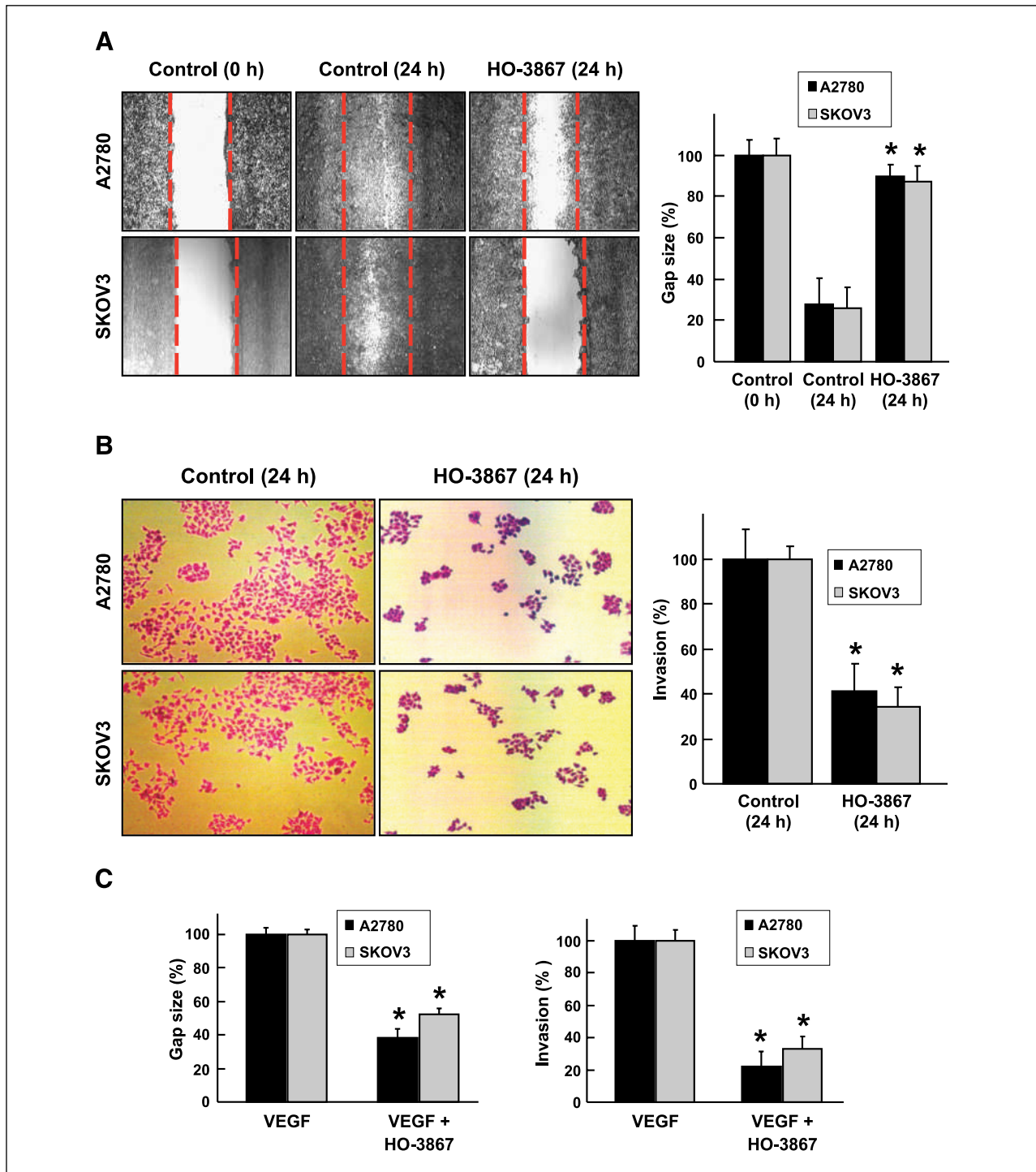
Cell culture medium (RPMI 1640) and DMEM, fetal bovine serum, antibiotics, sodium pyruvate, trypsin, and PBS were purchased from Life Technologies, Inc. Polyvinylidene fluoride membrane and molecular weight markers were obtained from Bio-Rad. Antibodies against phospho-human epidermal growth factor receptor 1 (pHER1), HER1, FAS, phospho-extracellular signal-regulated kinase 1/2 (pERK1/2), ERK1/2, actin, and USP2a were purchased from Cell Signaling Technology. Antibodies specific for sterol regulatory element binding protein 1 (SREBP1), FAK, matrix metalloproteinase (MMP)-2, vascular endothelial growth factor (VEGF), USP2a, and ubiquitin were purchased from Santa Cruz Biotechnology. Enhanced chemiluminescence reagents were obtained from Amersham Pharmacia Biotech (GE Healthcare). HO-3867 was synthesized in the laboratory (18). Stock solutions of the compounds were freshly prepared in DMSO. All other reagents of analytic grade or higher were purchased from Sigma-Aldrich.

### Cell lines and cultures

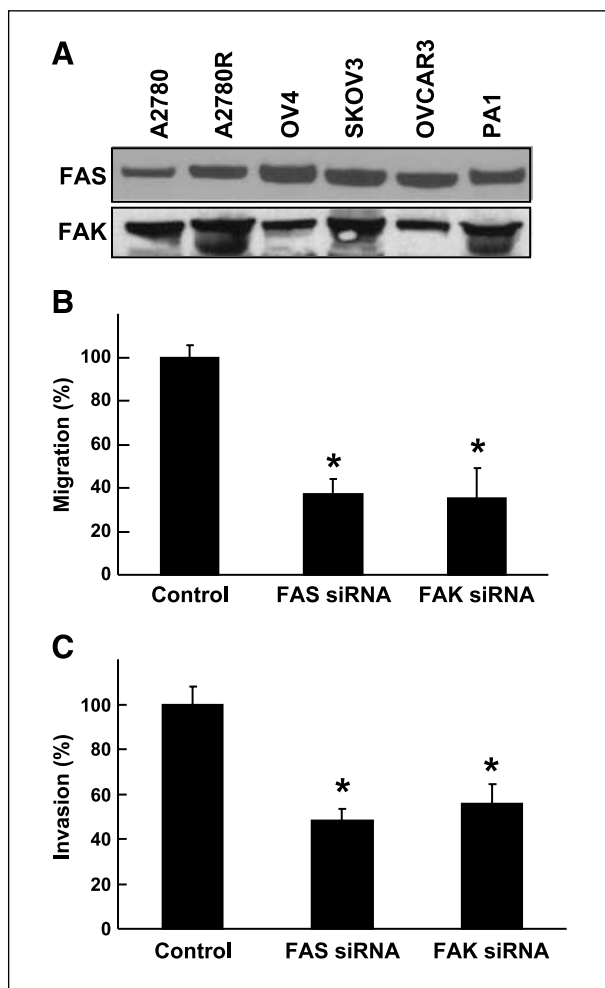
A2780 and SKOV3 human epithelial ovarian cancer cell lines were used in the study. The cells were grown in RPMI 1640 and DMEM supplemented with 10% fetal bovine serum, 2% sodium pyruvate, 1% penicillin, and 1% streptomycin. Cells were grown in a 75-mm flask to 70% confluence at 37°C in an atmosphere of 5% CO<sub>2</sub> and 95% air. Cells were routinely trypsinized (0.05% trypsin/EDTA) and counted using an automated counter (NucleoCounter, New Brunswick Scientific).

### Cell migration and invasion assays

Cell migration assay was done using the wound-healing method (1). Cells were plated at equal density and grown to 90% confluence. Wounds were created using a sterile pipette tip. Cells were then rinsed with medium and replaced with fresh medium and incubated with HO-3867 (10 μmol/L). Areas of wound were marked and photographed at various time points with a phase-contrast microscope. Cell-invasive assay was measured by an *in vitro* Boyden chamber assay (19). Briefly, 1 × 10<sup>5</sup> cells in 0.5 mL of serum-free RPMI 1640 were added to the wells of 8-μm-diameter pore membrane Boyden chambers, either coated with (BD Biosciences) or without (Corning) Matrigel. Cells were allowed to invade for 24 hours. Cells that had not penetrated the filters were removed by scrubbing with cotton swabs. Chambers were fixed in 100% methanol for 2 minutes, stained with 0.5% crystal violet for 2 minutes, rinsed in PBS, and examined under a bright-field microscope. Values for invasion were obtained by counting five fields per membrane and represented as



**FIGURE 1.** HO-3867 inhibits cancer cell migration and invasion. Cell migration (wound healing) assay and Transwell cell invasion assay were done using A2780 and SKOV3 cancer cells at 0 and 24 h, in the presence of HO-3867 (10  $\mu\text{mol/L}$ ) at 24 h. A, a representative image of six experiments is shown for each group. Gap size and cell invasion were quantified in the regions flanked by dotted lines. The residual gap between the migrating cells from the opposing edges is expressed as a percentage of the initial, scraped area. Columns, mean ( $n = 6$ ); bars, SE. \*,  $P < 0.05$ , versus control (24 h). The migration results show that HO-3867 significantly inhibits the reduction in gap size caused by cell migration. B, inhibition of A2780 and SKOV3 cell invasion by HO-3867 (10  $\mu\text{mol/L}$ ) at 24 h using a Boyden chamber migration assay. Representative images selected from six experiments are shown for each group. Quantification of cell invasion expressed as a percent of control. Columns, mean ( $n = 6$ ); bars, SE. \*,  $P < 0.05$ , versus control (24 h). The invasion results show that HO-3867 significantly inhibits cell invasion. C, quantitation of the effect of HO-3867 (10  $\mu\text{mol/L}$ ; 24-h incubation) on gap size and cell invasion of VEGF-induced cell migration and invasion in A2780 and SKOV3 cells. Columns, mean ( $n = 6$ ); bars, SE. \*,  $P < 0.05$ , versus the VEGF group. The results show that HO-3867 significantly inhibits the effect of VEGF on cell migration and invasion.



**FIGURE 2.** FAS and FAK are involved in cancer cell migration and invasion. A, basal levels of FAS and FAK expression in human ovarian cancer cell lines. B, effect of silencing of FAS and FAK in A2780 cells by transfection of FAS siRNA and FAK siRNA, respectively, on cell migration expressed as a percent of control. Columns, mean ( $n = 6$ ); bars, SE. \*,  $P < 0.05$ , versus control. C, effect of silencing of FAS and FAK in A2780 cells by transfection of FAS siRNA and FAK siRNA, respectively, on cell invasion expressed as a percent of control. Columns, mean ( $n = 6$ ); bars, SE. \*,  $P < 0.05$ , versus control. The results show that silencing of FAS and FAK significantly inhibits cell migration and invasion.

the average of three independent experiments done over multiple days.

#### FAS activity assay

The FAS activity was determined spectrophotometrically at 37°C in particle-free supernatants by measuring the decrease of absorption at 340 nm due to oxidation of NADPH.

#### Immunoblot analysis

Cells in RPMI 1640 were treated with DMSO (control) or HO-3867 (10  $\mu\text{mol/L}$ ) for 24 hours. Equal volumes of DMSO (0.1%, v/v) were present in each treatment.

Following treatment, the cell lysates were prepared in non-denaturing lysis buffer containing 10 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1% Triton X-100, 1 mmol/L EDTA, 1 mmol/L EGTA, 0.3 mmol/L phenylmethylsulfonyl fluoride, 0.2 mmol/L sodium orthovanadate, 0.5% NP40, 1  $\mu\text{g/mL}$  aprotinin, and 1  $\mu\text{g/mL}$  leupeptin. The lysates were centrifuged at  $10,000 \times g$  for 20 minutes at 4°C, and the supernatant was separated. The protein concentration in the lysates was determined using a Pierce detergent-compatible protein assay kit. For Western blotting, 25 to 50  $\mu\text{g}$  of protein lysate per sample were denatured in 2 $\times$  SDS-PAGE sample buffer and subjected to SDS-PAGE on a 10% Tris-glycine gel. The separated proteins were transferred onto a polyvinylidene fluoride membrane and blocked with 5% nonfat milk powder (w/v) in TBST (10 mmol/L Tris, 10 mmol/L NaCl, 0.1% Tween 20) for 1 hour at room temperature or overnight at 4°C. The membranes were then incubated with the primary antibodies. The bound antibodies were detected with horseradish peroxidase (HRP)-labeled sheep anti-mouse IgG or HRP-labeled donkey anti-rabbit IgG using an enhanced chemiluminescence detection system (ECL Advance Kit). Protein expressions were determined using Image Gauge version 3.45.

#### Reverse transcription-PCR

Total RNA isolated from ovarian tumor tissue was prepared with TRIzol (Life Technologies) according to the manufacturer's instructions. RNA quantification was done using spectrophotometry. Reverse transcription-PCR analysis for the mRNA expressions of *FAS*, *FAK*, *VEGF*, *p21*, and the internal control *GAPDH* was carried out using a GeneAmp PCR System Veriti thermocycler (Applied Biosystems) under the following conditions: initial denaturation at 94°C for 2 minutes, 35 cycles of amplification (denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds), and extension at 72°C for 5 minutes. The PCR products were electrophoresed on 1.5% agarose gel and stained with ethidium bromide.

#### Ovarian cancer tumor xenografts in mice

A2780 cells ( $5 \times 10^6$  in 60  $\mu\text{L}$  of PBS) were s.c. injected into the back of 6-week-old BALB/c nude mice from the National Cancer Institute. On the 5th day, when the tumor size reached approximately 2 to 4 mm, the control group was supplemented a normal diet (no treatment) whereas the experimental groups were treated with diarylidenylpiperidone compounds mixed with the animal feed (Harlan Teklad) at two different levels (500 and 100 ppm). The doses were chosen based on an initial dose-response study optimized to produce an observable effect on tumor growth. The tumor tissues were then subjected to immunoblotting and immunohistochemistry.

#### Immunohistochemistry

Tumor tissues were fixed in formalin and embedded in paraffin. Sections (6  $\mu\text{m}$  thick) were obtained and used for

H&E staining. For immunofluorescence staining, the tissue sections (8  $\mu\text{m}$  thick) were serially rehydrated in 100%, 95%, and 80% ethanol after deparaffinization with xylene. Slides were kept in steam for 30 minutes and then washed in PBS (pH 7.4) three times for 5 minutes each. Tissue sections were incubated with 2% goat serum and 5% bovine serum albumin in PBS to reduce nonspecific binding. The sections were then incubated for 4 hours with an antimouse anti-FAS or anti-VEGF. The sections were then incubated with secondary antibodies (1:1,000 dilutions) conjugated to HRP-labeled sheep anti-mouse IgG or HRP-labeled donkey anti-rabbit IgG (Amersham Pharmacia Biotech). The tissue slides were visualized using a Nikon fluorescence microscope.

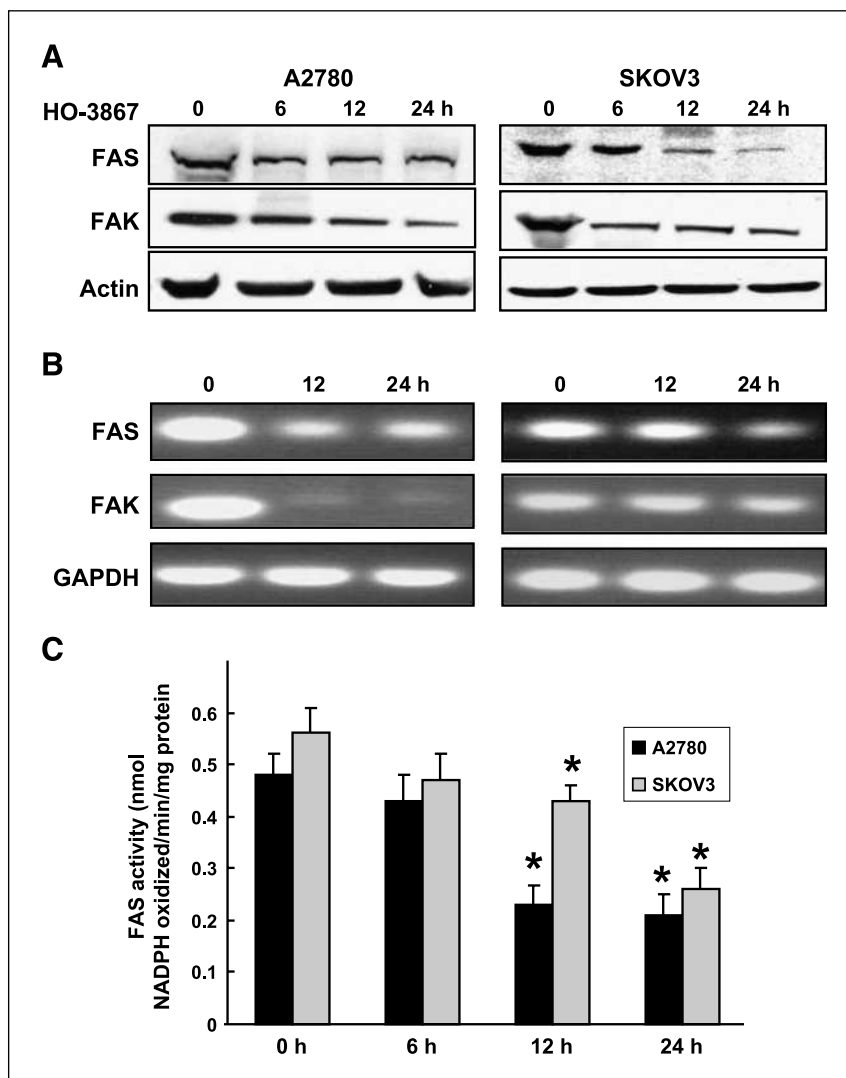
### Data analysis

The statistical significance of the results was evaluated using Student's *t* test.  $P < 0.05$  was considered significant.

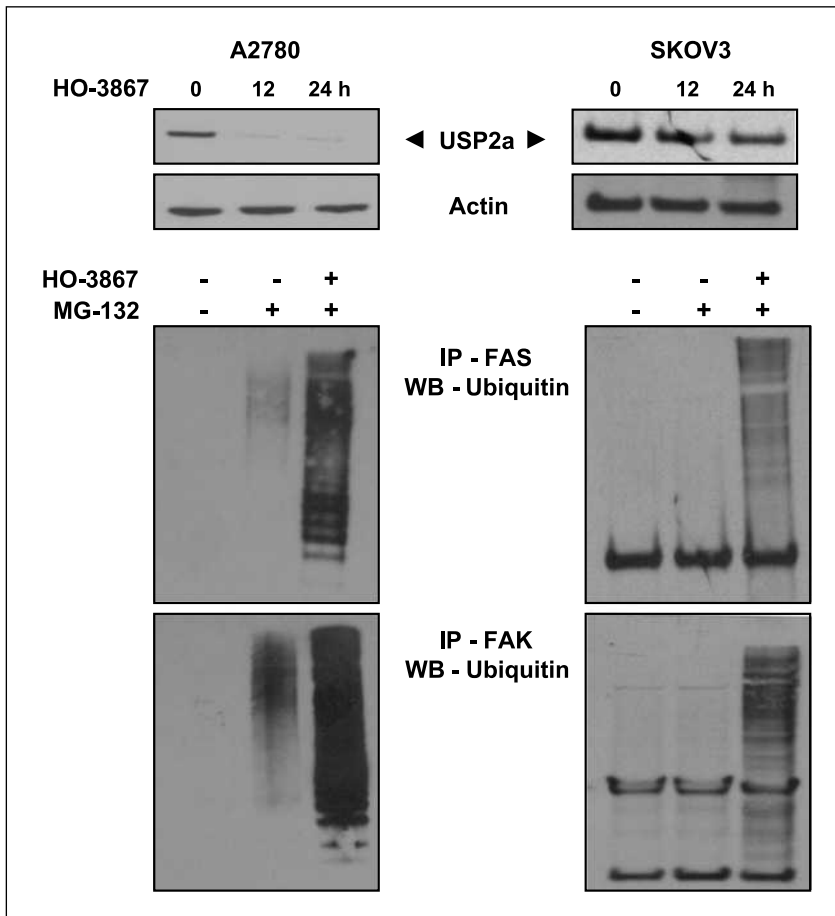
## Results

### Effect of HO-3867 on ovarian cancer cell migration and invasion

The effect of HO-3867 on the motility of ovarian cancer cells was measured by wound-healing migration and Transwell cell invasion assays. Incubation of A2780 or SKOV3 cells with HO-3867 (10  $\mu\text{mol/L}$ ) for 24 hours showed significant inhibition of cell migration (Fig. 1A) and invasion (Fig. 1B) when compared with untreated cells. Because VEGF-induced angiogenesis is initiated by cell migration and invasion, we next determined whether HO-3867 could inhibit the cell motility-promoting effect of VEGF. We observed that HO-3867 significantly inhibited the VEGF-induced migration and invasion of both ovarian cancer cell lines tested (Fig. 1C). The results suggested that HO-3867 could not only inhibit ovarian cancer cell migration and invasion but could also block VEGF-induced angiogenesis.



**FIGURE 3.** HO-3867 inhibits FAS and FAK expression in ovarian cancer cells. A, representative Western blots showing a time-dependent inhibition of FAS and FAK levels in A2780 and SKOV3 cells treated with HO-3867 (10  $\mu\text{mol/L}$ ). B, expression levels of FAS and FAK mRNA following HO-3867 exposure (10  $\mu\text{mol/L}$  for 24 h). C, FAS activity measured in A2780 and SKOV3 cells using NADPH by spectrophotometry. \*,  $P < 0.05$ , versus the respective control (0 h). The results show that HO-3867 significantly inhibits FAS and FAK expression in cancer cells.



**FIGURE 4.** HO-3867 downregulates FAS and FAK levels via proteasome pathways. To catch any ubiquitinated proteins in the cell lysates, agarose beads coated with domains having affinity to ubiquitin were incubated with the lysates at 4°C for 2 h. After washing the beads, the ubiquitinated proteins were subjected to immunoblot for FAS and FAK and blotted with ubiquitin antibody. A predominant ubiquitination of FAS and FAK is seen in the HO-3867-treated A2780 and SKOV3 cells under proteasomal inhibition using MG-132 (50  $\mu$ mol/L). The results show that HO-3867 clearly downregulates the FAS stability protein USP2a in a time-dependent manner.

#### Effect of FAS and FAK on ovarian cancer cell migration and invasion

We observed that FAS and FAK proteins were significantly expressed in all six human ovarian cancer cell lines tested, including the cisplatin-resistant cancer cell line A2780R (Fig. 2A). We next determined the effect of FAS and FAK inhibition by FAS siRNA and FAK siRNA transfection on the migration and invasion of A2780 cells. Cells transfected with FAS siRNA or FAK siRNA exhibited significant reduction in migration and invasion when compared with control cells. The results suggest that both FAS and FAK are involved in ovarian cancer cell migration and invasion.

#### Effect of HO-3867 on FAS and FAK expression in ovarian cancer cells

We next determined whether the inhibitory effect of HO-3867 on ovarian cancer cell migration and invasion was due to its regulation of FAS and FAK expression levels. Incubation of A2780 or SKOV3 cells with HO-3867 (10  $\mu$ mol/L) resulted in an incubation time-dependent inhibition of FAS and FAK, both at the protein and mRNA levels (Fig. 3A and B). We further checked the activity of FAS in cells treated with HO-3867 for 6, 12, and 24 hours.

The FAS activity was significantly reduced in both cell lines on incubation with HO-3867 for 12 or 24 hours (Fig. 3C). The results suggested that HO-3867 inhibited the expression of FAS and FAK in the ovarian cancer cells.

#### Effect of HO-3867 on FAS and FAK downregulation by proteasome pathways

It is known that the intracellular amount of rapid-turn-over proteins, such as FAS and FAK, is tightly regulated by the ubiquitin-dependent proteolytic pathway (20). The fast and significant decrease in FAS and FAK expression observed in HO-3867-treated cells prompted us to check any involvement of a ubiquitin-dependent degradation mechanism. The isopeptidase USP2a has been shown to regulate the stability of FAS in prostate cancer (20). We analyzed the USP2a level in HO-3867-treated ovarian cancer cells and observed that it was clearly downregulated by HO-3867 (Fig. 4). We further observed an enhanced polyubiquitination on FAS and FAK in HO-3867-treated cancer cells on coinubation with MG-132, a proteasome inhibitor. The results strongly suggested that HO-3867 inhibited FAS/FAK through ubiquitination and inhibited FAS stability through USP2a.

### Effect of HO-3867 on FAS/FAK regulatory genes

We determined the effect of HO-3867 on the FAS and FAK regulatory cell migration and invasion genes. HO-3867 inhibited pHER2, pERK1/2, SREBP1, MMP-2, and VEGF in A2780 and SKOV3 cells (Fig. 5). The results suggested that HO-3867 not only inhibited FAS and FAK expression but also blocked their regulating genes in the two ovarian cancer cell lines tested.

### Effect of HO-3867 on FAS/FAK levels in tumor tissues

Finally, we analyzed the expression levels of FAS, FAK, and their regulatory proteins in an *in vivo* xenograft mouse model of ovarian cancer. We observed, in concert with the decreased expression of both FAS and FAK, a clear downregulation of the target gene products of FAS and FAK, namely, SREBP1, MMP-2, and VEGF, in the xenografted tumor tissues of HO-3867-treated mice in a dose-dependent manner (Fig. 6A and B). We further determined the FAS and VEGF levels by immunocytochemistry. HO-3867 treatment significantly inhibited the protein levels of FAS and VEGF in tumor-bearing mice (Fig. 5C). The *in vivo* results suggest that administration of HO-3867 inhibits tumor growth through inhibition of migration and invasion regulatory genes, such as FAS and FAK, in ovarian cancer xenografts in mice.

## Discussion

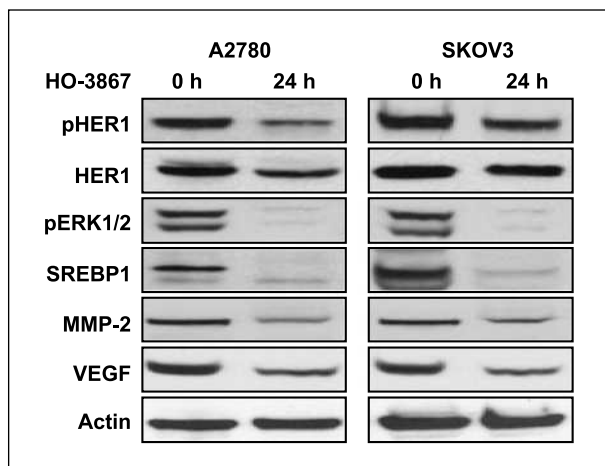
The results of the present study have shown for the first time that HO-3867 attenuates cancer cell migration and invasion through inhibition of FAS and FAK expression in ovarian cancer cell lines as well as in ovarian tumor xenografts in mice. The results confirm that FAS and FAK are indeed necessary for the motility of ovarian

cancer cells and that HO-3867 suppresses the levels of both FAS and FAK by promoting the ubiquitination-dependent degradation process and inhibiting the FAS-stabilizing gene *USP2a*. HO-3867 also significantly inhibited FAS activity and mRNA levels and the downstream proteins pERK1/2, pHER1, SREBP1, VEGF, and MMP-2. The study further confirmed the inhibition of FAS, FAK, and downstream proteins in tumor tissues obtained from A2780 xenograft tumor-bearing mice treated with HO-3867. Collectively, the study establishes that HO-3867 is capable of suppressing the migration and invasion of ovarian cancer cells by inhibiting the expression or activity of FAS and FAK proteins.

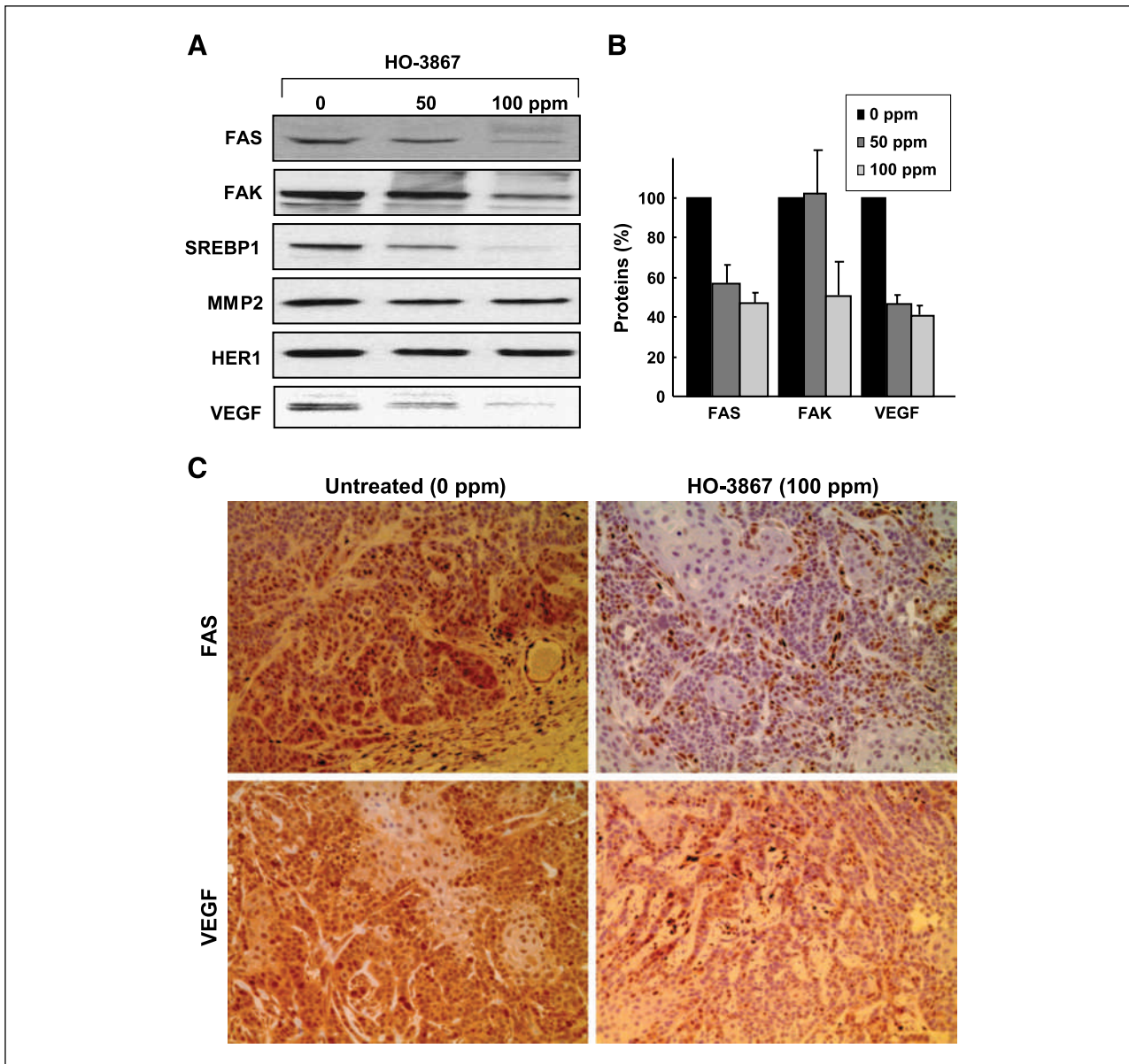
Cell migration and invasion are vital elements involved in numerous physiologic and pathologic processes, including angiogenesis and metastasis (2, 3). The high mortality rate among ovarian cancer patients is attributed not only to a lack of early detection and treatment but also to the highly invasive (metastatic) nature of the disease (8). The poor prognosis associated with the treatment of ovarian cancer is mainly due to the late stage of disease with metastasis at presentation. Particularly, malignant ovarian surface epithelial cells primarily spread to adjacent organs by local invasion. The significant failure rate of chemotherapy in ovarian cancer patients with advanced stage of metastatic disease is also a main concern, suggesting cell motility as a potential therapeutic target for ovarian cancer treatment. In the present study, for the first time, we show that HO-3867 acts as an effective blocker of ovarian cancer cell migration and invasion through inhibition of motility-promoting proteins including FAS, FAK, and VEGF.

FAS is a metabolic enzyme involved in the synthesis of long-chain saturated fatty acids that are essential for membrane synthesis in proliferating cells. FAS is overexpressed in many human cancers including the carcinomas of the breast (21), prostate (22), stomach (23), lung (24), ovary (25, 26), and mesothelioma (27). The fact that overexpression of FAS is more pronounced in clinically aggressive cancers (26) suggests a functional role for FAS in the progression of malignant cancer (9, 12). Inhibition of FAS activity preferentially attenuates tumor cell growth by inducing apoptosis through inactivation of pAkt and dephosphorylation of Bad in ovarian cancer (12, 28, 29). Because FAS seems to provide a selective advantage to tumor progression, FAS has become a promising target for anticancer drug development. Several studies have shown that blocking of FAS activity using pharmacologic inhibitors of FAS such as cerulenin and C75 attenuated carcinomas of the breast (29, 30), renal (31), colon (32), and liver (33). The present study shows that HO-3867 is capable of inhibiting both the expression and activity of FAS in A2780 and SKOV3 cells, resulting in the attenuation of their ability to migrate and invade.

FAK is a focal adhesion-associated protein kinase involved in cellular adhesion and spreading processes. It serves as a key protein in the regulation of focal adhesion dynamics (34). FAK is a critical mediator of integrin adhesion turnover that promotes cell migration (35). Several



**FIGURE 5.** HO-3867 downregulates FAS/FAK regulatory genes. A2780 and SKOV3 cells were treated with HO-3867 (10  $\mu$ mol/L, 24 h), followed by blotting of FAS/FAK-regulating proteins HER1, SREBP1, ERK1/2, MMP-2, and VEGF. The results show that HO-3867 at 24-h incubation clearly downregulates the FAS/FAK-regulating proteins in both of the ovarian cancer cell lines.



**FIGURE 6.** HO-3867 suppresses FAS/FAK and VEGF levels in tumor tissues. A, tissue lysates containing 50-mg protein of A2780 xenograft tumors from mice treated with 50 or 100 ppm HO-3867, containing 50  $\mu$ g protein each, were subjected to immunoblot analyses. Decreased FAS, FAK, and VEGF expression levels are noted in the HO-3867–treated tumor lysates in a dose-dependent manner. B, quantitation of FAS, FAK, and VEGF bands by densitometric analysis. Columns, mean ( $n = 3$ ); bars, SE. \*,  $P < 0.05$ , versus the untreated (0 ppm) group. C, immunohistochemistry showing decreased expression levels of FAS and VEGF in the tumor tissues. The results show that HO-3867 treatment of mice suppresses FAS, FAK, and VEGF expression in the tumor.

studies have shown the requirement of FAK signaling in promoting the invasiveness of cancer cells (36, 37). As in the case of FAS, overexpression of FAK has also been found in most ovarian tumors, in which it is shown to be associated with high aggressiveness and poor patient survival (4, 11). A recent report has highlighted a possible inverse correlation between FAK expression and clinical outcome (5). Therefore, FAK is an attractive target for ovarian cancer therapeutics and prevention. In the present

study, we observed that HO-3867 was capable of inhibiting FAK expression in the ovarian cancer cell lines tested.

Although the precise mechanism of FAS degradation has not yet been fully understood, it is evident that ubiquitination is involved in the HO-3867–mediated proteasomal degradation of FAS. A recent report showed that the isopeptidase USP2a is a preproteasomal, androgen-regulated isopeptidase and is a key regulator of prostate cancer cell survival through the stabilization of FAS (20). FAS has



been shown to colocalize and physically interact with USP2a in cancer cells, suggesting that this isopeptidase rescues FAS from degradation and thereby prevents apoptosis (38). Of interest, the rapid downregulation of FAS was consistent with accelerated ubiquitin-dependent degradation. This effect was further confirmed by the observation that the proteasome inhibitor MG-132 blocked the HO-3867-induced FAK degradation. There are also several lines of evidence in published reports showing that FAK is tightly regulated by ubiquitin pathways (39, 40). In addition, we observed that HO-3867 significantly suppressed the expression of FAS-regulating genes, such as pHER, SREBP1, pERK1/2, VEGF, and MMP-2. Similar to our results, a coordinated regulation of SREBP1 and FAS has been observed in clinical breast cancer (41).

The present results clearly indicated an increased degradation and a decreased transcription of FAS/FAK on treatment with HO-3867. Whereas degradation of FAS/FAK proteins occurred in 6 to 12 hours of treatment (6-hour data not shown), inhibition of the FAS/FAK-regulating genes HER1, SREBP1, and ERK1/2 was observed only at 24 hours, suggesting that HO-3867 works by inducing the degradation of FAS/FAK proteins as well as by inhibiting FAS/FAK expression, but at different time points.

A recent study from our laboratory has shown that HO-3867 inhibits the STAT3/JAK pathway in a wide range of human cancer cells, including ovarian cancer (17, 42). Activated STAT3 has a significant role in the metastatic progression of ovarian cancer and has important roles in promoting cell proliferation, cell survival, migration, and invasion in human cancer (43). Inhibition of STAT3/JAK has been shown to be involved in colorectal cancer cell growth, survival, invasion, and migration through regulation of the expression of genes such as Bcl-2, p21, VEGF, and MMPs (44). The FAS inhibitor C75 has been shown to induce suppression of the invasiveness and migration of renal carcinoma cells through concurrent inhibition of FAS and STAT3 (31). Interestingly, HO-3867 also showed a similar inhibitory effect toward FAS/STAT3 in human ovarian cancer cells. However, further investigation is necessary to determine the exact mechanisms involved in the anticancer activity of HO-3867.

The FAS and the fatty acid synthesis pathways have been explored as potential drug targets for cancer therapy (9). Cerulenin, a natural product, was the first specific inhibitor of FAS to be studied (45), whereas C75, a synthetic compound, was shown to be a more potent inhibitor of FAS (46, 47). Both of the inhibitors have been evaluated in a variety of human cancer cell lines and xenograft tumors (14, 27, 47). Despite its measurable effect on a human cancer xenograft, cerulenin was chemically unstable, thus precluding its use as a systemic anticancer drug. Further, cerulenin induced reversible weight loss in mice (14, 48). C75, too, was found to induce substantial weight loss through the induction of anorexia (eating disorder) in mice (47, 48). Recently, C93, a rationally designed molecule that inhibits FAS activity without affecting fatty acid oxidation, has been reported to inhibit FAS in chemically induced lung tumor and in preclinical models of lung cancer (12, 49, 50). Very recently, we have evaluated the efficacy of HO-3867 in an *in vivo* model of human ovarian tumor xenograft in mice (17). We observed a significant reduction in tumor growth volume without any adverse effect on body weight or diet consumption, suggesting that the *in vivo* antitumor efficacy of HO-3867 against ovarian cancer is without any apparent signs of toxicity.

The present study provides the first evidence that HO-3867 inhibits the migration and invasion of ovarian cancer cells through downregulation of FAS and FAK. The study suggests that molecular targeting of FAS and FAK by HO-3867 is a potential strategy for ovarian cancer therapy.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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#### References

- Eccles SA, Box C, Court W. Cell migration/invasion assays and their application in cancer drug discovery. *Biotechnol Annu Rev* 2005;11:391–421.
- Yamaguchi H, Wyckoff J, Condeelis J. Cell migration in tumors. *Curr Opin Cell Biol* 2005;17:559–64.
- Lauffenburger DA, Horwitz AF. Cell migration: a physically integrated molecular process. *Cell* 1996;84:359–69.
- Golubovskaya VM, Kweh FA, Cance WG. Focal adhesion kinase and cancer. *Histol Histopathol* 2009;24:503–10.
- Luo M, Guan JL. Focal adhesion kinase: a prominent determinant in breast cancer initiation, progression and metastasis. *Cancer Lett* 2009;289:127–39.
- Owens LV, Xu L, Craven RJ, et al. Overexpression of the focal adhesion kinase (p125FAK) in invasive human tumors. *Cancer Res* 1995;55:2752–5.
- Kornberg LJ. Focal adhesion kinase and its potential involvement in tumor invasion and metastasis. *Head Neck* 1998;20:745–52.
- Yap TA, Carden CP, Kaye SB. Beyond chemotherapy: targeted therapies in ovarian cancer. *Nat Rev Cancer* 2009;9:167–81.
- Kuhajda FP. Fatty acid synthase and cancer: new application of an old pathway. *Cancer Res* 2006;66:5977–80.
- Kuhajda FP. Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. *Nutrition* 2000;16:202–8.
- Halder J, Lin YG, Merritt WM, et al. Therapeutic efficacy of a novel focal adhesion kinase inhibitor TAE226 in ovarian carcinoma. *Cancer Res* 2007;67:10976–83.
- Zhou W, Han WF, Landree LE, et al. Fatty acid synthase inhibition activates AMP-activated protein kinase in SKOV3 human ovarian cancer cells. *Cancer Res* 2007;67:2964–71.
- Menendez JA, Decker JP, Lupu R. In support of fatty acid synthase

- (FAS) as a metabolic oncogene: extracellular acidosis acts in an epigenetic fashion activating FAS gene expression in cancer cells. *J Cell Biochem* 2005;94:1–4.
14. Pizer ES, Wood FD, Heine HS, Romantsev FE, Pasternack GR, Kuhajda FP. Inhibition of fatty acid synthase delays disease progression in a xenograft model of ovarian cancer. *Cancer Res* 1996;56:1189–93.
  15. Selvendiran K, Tong L, Vishwanath S, et al. EF24 induces G<sub>2</sub>/M arrest and apoptosis in cisplatin-resistant human ovarian cancer cells by increasing PTEN expression. *J Biol Chem* 2007;282:28609–18.
  16. Selvendiran K, Kuppusamy ML, Bratasz A, et al. Inhibition of vascular smooth-muscle cell proliferation and arterial restenosis by HO-3867, a novel synthetic curcuminoid, through up-regulation of PTEN expression. *J Pharmacol Exp Ther* 2009;329:959–66.
  17. Selvendiran K, Tong L, Bratasz A, et al. Anticancer efficacy of a difluorodiarylidene piperidone (HO-3867) in human ovarian cancer cells and tumor xenografts. *Mol Cancer Ther* 2010;9:1169–79.
  18. Kalai T, Kuppusamy P, Hideg K. Synthesis, characterization and structure-activity studies with a novel class of diarylidene piperidones. *Bioorg Med Chem*. In press.
  19. Yap LF, Jenei V, Robinson CM, et al. Upregulation of Eps8 in oral squamous cell carcinoma promotes cell migration and invasion through integrin-dependent Rac1 activation. *Oncogene* 2009;28:2524–34.
  20. Graner E, Tang D, Rossi S, et al. The isopeptidase USP2a regulates the stability of fatty acid synthase in prostate cancer. *Cancer Cell* 2004;5:253–61.
  21. Alo PL, Visca P, Marci A, Mangoni A, Botti C, Di Tondo U. Expression of fatty acid synthase (FAS) as a predictor of recurrence in stage I breast carcinoma patients. *Cancer* 1996;77:474–82.
  22. Epstein JI, Carmichael M, Partin AW. OA-519 (fatty acid synthase) as an independent predictor of pathologic state in adenocarcinoma of the prostate. *Urology* 1995;45:81–6.
  23. Kusakabe T, Nashimoto A, Honma K, Suzuki T. Fatty acid synthase is highly expressed in carcinoma, adenoma and in regenerative epithelium and intestinal metaplasia of the stomach. *Histopathology* 2002;40:71–9.
  24. Piyathilake CJ, Frost AR, Manne U, et al. The expression of fatty acid synthase (FASE) is an early event in the development and progression of squamous cell carcinoma of the lung. *Hum Pathol* 2000;31:1068–73.
  25. Alo PL, Visca P, Framarino ML, et al. Immunohistochemical study of fatty acid synthase in ovarian neoplasms. *Oncol Rep* 2000;7:1383–8.
  26. Gansler TS, Hardman W III, Hunt DA, Schaffel S, Hennigar RA. Increased expression of fatty acid synthase (OA-519) in ovarian neoplasms predicts shorter survival. *Hum Pathol* 1997;28:686–92.
  27. Gabrielson EW, Pinn ML, Testa JR, Kuhajda FP. Increased fatty acid synthase is a therapeutic target in mesothelioma. *Clin Cancer Res* 2001;7:153–7.
  28. Zhou W, Simpson PJ, McFadden JM, et al. Fatty acid synthase inhibition triggers apoptosis during S phase in human cancer cells. *Cancer Res* 2003;63:7330–7.
  29. Menendez JA, Vellon L, Mehmi I, et al. Inhibition of fatty acid synthase (FAS) suppresses HER2/neu (erbB-2) oncogene overexpression in cancer cells. *Proc Natl Acad Sci U S A* 2004;101:10715–20.
  30. Alli PM, Pinn ML, Jaffee EM, McFadden JM, Kuhajda FP. Fatty acid synthase inhibitors are chemopreventive for mammary cancer in neu-N transgenic mice. *Oncogene* 2005;24:39–46.
  31. Horiguchi A, Asano T, Asano T, Ito K, Sumitomo M, Hayakawa M. Pharmacological inhibitor of fatty acid synthase suppresses growth and invasiveness of renal cancer cells. *J Urol* 2008;180:729–36.
  32. Li JN, Gorospe M, Chrest FJ, et al. Pharmacological inhibition of fatty acid synthase activity produces both cytostatic and cytotoxic effects modulated by p53. *Cancer Res* 2001;61:1493–9.
  33. Gao Y, Lin LP, Zhu CH, Chen Y, Hou YT, Ding J. Growth arrest induced by C75, A fatty acid synthase inhibitor, was partially modulated by p38 MAPK but not by p53 in human hepatocellular carcinoma. *Cancer Biol Ther* 2006;5:978–85.
  34. Li S, Hua ZC. FAK expression regulation and therapeutic potential. *Adv Cancer Res* 2008;101:45–61.
  35. Chu PY, Huang LY, Hsu CH, et al. Tyrosine phosphorylation of growth factor receptor-bound protein-7 by focal adhesion kinase in the regulation of cell migration, proliferation, and tumorigenesis. *J Biol Chem* 2009;284:20215–26.
  36. Poulikakos PI, Xiao GH, Gallagher R, Jablonski S, Jhanwar SC, Testa JR. Re-expression of the tumor suppressor NF2/merlin inhibits invasiveness in mesothelioma cells and negatively regulates FAK. *Oncogene* 2006;25:5960–8.
  37. van Nimwegen MJ, van de Water B. Focal adhesion kinase: a potential target in cancer therapy. *Biochem Pharmacol* 2007;73:597–609.
  38. Priolo C, Tang D, Brahamandan M, et al. The isopeptidase USP2a protects human prostate cancer from apoptosis. *Cancer Res* 2006;66:8625–32.
  39. Kim B, van Golen CM, Feldman EL. Degradation and dephosphorylation of focal adhesion kinase during okadaic acid-induced apoptosis in human neuroblastoma cells. *Neoplasia* 2003;5:405–16.
  40. Lim ST, Chen XL, Lim Y, et al. Nuclear FAK promotes cell proliferation and survival through FERM-enhanced p53 degradation. *Mol Cell* 2008;29:9–22.
  41. Yang YA, Morin PJ, Han WF, et al. Regulation of fatty acid synthase expression in breast cancer by sterol regulatory element binding protein-1c. *Exp Cell Res* 2003;282:132–7.
  42. Selvendiran K, Ahmed S, Dayton A, et al. Safe and targeted anticancer efficacy of a novel class of antioxidant-conjugated difluorodiarylidene piperidones: differential cytotoxicity in healthy and cancer cells. *Free Radic Biol Med* 2010;48:1228–35.
  43. Devarajan E, Huang S. STAT3 as a central regulator of tumor metastases. *Curr Mol Med* 2009;9:626–33.
  44. Xiong H, Zhang ZG, Tian XQ, et al. Inhibition of JAK1,2/STAT3 signaling induces apoptosis, cell cycle arrest, and reduces tumor cell invasion in colorectal cancer cells. *Neoplasia* 2008;10:287–97.
  45. Kuhajda FP, Jenner K, Wood FD, et al. Fatty acid synthesis: a potential selective target for antineoplastic therapy. *Proc Natl Acad Sci U S A* 1994;91:6379–83.
  46. Kuhajda FP, Pizer ES, Li JN, Mani NS, Frehywot GL, Townsend CA. Synthesis and antitumor activity of an inhibitor of fatty acid synthase. *Proc Natl Acad Sci U S A* 2000;97:3450–4.
  47. Pizer ES, Thupari J, Han WF, et al. Malonyl-coenzyme-A is a potential mediator of cytotoxicity induced by fatty-acid synthase inhibition in human breast cancer cells and xenografts. *Cancer Res* 2000;60:213–8.
  48. Loftus TM, Jaworsky DE, Frehywot GL, et al. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* 2000;288:2379–81.
  49. Orita H, Coulter J, Lemmon C, et al. Selective inhibition of fatty acid synthase for lung cancer treatment. *Clin Cancer Res* 2007;13:7139–45.
  50. Orita H, Coulter J, Tully E, Kuhajda FP, Gabrielson E. Inhibiting fatty acid synthase for chemoprevention of chemically induced lung tumors. *Clin Cancer Res* 2008;14:2458–64.

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