Research Article

Therapeutic Efficacy of a Novel Focal Adhesion Kinase Inhibitor TAE226 in Ovarian Carcinoma

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Abstract

Focal adhesion kinase (FAK) overexpression is frequently found in ovarian and other cancers and is predictive of poor clinical outcome. In the current study, we characterized the biological and therapeutic effects of a novel FAK inhibitor, TAE226. Taxane-sensitive (SKOV3ip1 and HeyA8) and taxane-resistant (HeyA8-MDR) cell lines were used for in vitro and in vivo therapy experiments using TAE226 alone and in combination with docetaxel. Assessment of cytotoxicity, cell proliferation [proliferating cell nuclear antigen (PCNA)], angiogenesis (CD31), and apoptosis (terminal nucleotidyl transferase–mediated nick end labeling) were done by immunohistochemistry and immunofluorescence. In vitro, TAE226 inhibited the phosphorylation of FAK at both Y397 and Y861 sites, inhibited cell growth in a time- and dose-dependent manner, and enhanced docetaxel-mediated growth inhibition by 10- and 20-fold in the taxane-sensitive and taxane-resistant cell lines, respectively. In vivo, FAK inhibition by TAE226 significantly reduced tumor burden in the HeyA8, SKOV3ip1, and HeyA8-MDR models (46–64%) compared with vehicle-treated controls. However, the greatest efficacy was observed with concomitant administration of TAE226 and docetaxel in all three models (85–97% reduction, all P values <0.01). In addition, TAE226 alone and in combination with chemotherapy significantly prolonged survival in tumor-bearing mice. Even in larger tumors, combination therapy with TAE226 and docetaxel resulted in tumor regression. The therapeutic efficacy was related to reduced pericyte coverage, induction of apoptosis of tumor-associated endothelial cells, and reduced microvessel density and tumor cell proliferation. The novel FAK inhibitor, TAE226, offers an attractive therapeutic approach in ovarian carcinoma. [Cancer Res 2007;67(22):10976–83]

Introduction

Ovarian cancer is the fifth most common cause of cancer-related deaths in women and the most frequent cause of death from a gynecologic malignancy (1). Despite advances in surgical and chemotherapeutic approaches, the resistance of cancer cells to traditional cytotoxic agents is a major obstacle in clinical cancer therapy, and most patients eventually succumb to their disease (2). Therefore, novel therapeutic approaches for ovarian cancer are needed.

Focal adhesion kinase (FAK) is a 125-kDa non-receptor protein tyrosine kinase that was first identified in Src-transformed fibroblasts (3). FAK is overexpressed in many tumors, including those derived from the head and neck, colon, breast, prostate, liver, and thyroid (4–13). We have previously shown that FAK is overexpressed in a substantial proportion of ovarian cancers and is predictive of poor clinical outcome. The overexpression of FAK may be related to FAK gene amplification (14). FAK has been shown to play a significant role in cell survival, migration, and invasion (15–19). For example, decreased FAK phosphorylation by the dominant negative FAK-related non-kinase resulted in reduced migration and invasion of ovarian cancer cells in vitro (18, 20, 21). FAK overexpression has also been suggested to protect cells from stressors such as chemotherapy by activation of the phosphoinositide-3-kinase–AKT survival pathway, activation of nuclear factor-kB, and induction of inhibitor-of-apoptosis proteins (22). We have previously shown that FAK is cleaved after treatment with docetaxel chemotherapy in a caspase-3–dependent manner, and that FAK down-regulation promoted docetaxel cytotoxicity in ovarian cancer cells (23). Moreover, in vivo FAK silencing using small interfering RNA (siRNA) incorporated in a neutral nanoparticle resulted in antitumor and antiangiogenic effects in orthotopic models of ovarian carcinoma (24). In both studies, FAK silencing enhanced the effects of chemotherapy (23, 24). Although siRNA-based therapeutic approaches are gradually being developed for human use, small-molecule inhibitors have been developed against many targets and have been integrated into clinical trials. Based on the known role of FAK in ovarian cancer pathogenesis and the encouraging results with FAK gene silencing, we tested the efficacy of a novel FAK inhibitor, TAE226, in multiple ovarian cancer models.

Materials and Methods

Cell lines and culture conditions. The derivation, source, and propagation of the human ovarian cancer cell lines, SKOV3ip1 and HeyA8, have been described previously (25). The taxane-resistant cell line, HeyA8-MDR, was a kind gift from Dr. Isaiah Fidler (The University of Texas M. D. Anderson Cancer Center) and was maintained in RPMI 1640 supplemented with 300 ug/mL of paclitaxel (Bristol-Myers Squibb Company). Because there are no true human pericyte cell lines, we used a pericyte-like cell line, 10T1/2. These cells represent undifferentiated mesenchymal cells and have...
previously been used as presumptive mural cell precursors (26). All in vitro experiments were conducted with 70% to 80% confluent cultures.

Reagents. Leupeptin, aprotinin, and sodium orthovanadate were obtained from Sigma-Aldrich Company; EDTA was obtained from Life Technologies Invitrogen; and docetaxel was obtained from Sanofi-Aventis. The FAK inhibitor, TAE226, was obtained from Novartis Pharma AG (Fig. 1A; ref. 27). TAE226 inhibits the FAK kinase domain with an IC50 of 0.0055 μM/L in in vitro kinase assays (27). The primary antibodies used were mouse anti-FAK (Biosource International), anti–phospho-FAK [Y397] (Biosource), anti–vascular endothelial growth factor (anti-VEGF; Santa Cruz Biotechnology), anti–matrix metalloproteinase-9 (anti–MMP-9; Chemicon-Biosource), anti–proliferating cell nuclear antigen (anti-PCNA) clone PC10 (DAKO), mouse anti-CD31 (PharMingen) and mouse anti-desmin (DAKO). The following secondary antibodies were used for colorimetric immunohistochemical (IHC) analysis: horseradish peroxidase (HRP)–conjugated goat anti-mouse IgG2a (Biocare Medical), HRP-conjugated goat anti-rat IgG (Jackson ImmunoResearch Laboratories, Inc.), and fluorescent Alexa 488–conjugated goat anti-rabbit IgG (Molecular Probes Invitrogen).

Western blot analysis. Whole cell lysate for SDS-PAGE and Western blot analysis for FAK expression was prepared as previously reported (24). To prepare lysate from dissected in vivo tumors, samples were snap frozen in liquid nitrogen immediately after sacrificing the animals and stored at −80°C. The lysate was incubated on ice in radioimmunoprecipitation assay buffer for 2 h before being homogenized using a mortar and pestle. The homogenized sample was centrifuged, and the supernatant was collected and stored at −80°C. Protein quantification and Western blot for FAK were performed as described previously (23). Briefly, 2,000 cells per well were seeded onto 96-well plates and allowed to adhere overnight, after which the FAK inhibitor, TAE226, was added. Twenty-four hours after incubation, the media was exchanged for one containing increasing concentrations of docetaxel dissolved in ethanol. After 96 h of docetaxel exposure, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was done. The absorbance at 570 nm was recorded, and the IC50 was determined.

Cytochemistry and histology. Immunohistochemical (IHC) analysis was performed as previously described (24). Equal loading was confirmed with β-actin (0.1 μg/mL, Sigma Chemical). Densitometric analysis was done using the Scion Imaging software (Scion Corporation), using total FAK or actin as a control for each sample.

Figure 1. In vitro cytotoxicity and dose and time kinetics of TAE226. A, chemical structure of TAE 226. B, ovarian cancer cell lines plated on collagen I–coated and non-coated plates were exposed to increasing doses of TAE226. Cell lysates were collected and examined by Western blot analysis for pFAK Y397 and total FAK. Time-kinetic experiments were done to determine the onset and duration of action of TAE226 in down-regulating FAK phosphorylation (c, vehicle control). After treating cells with 1 μM/L of TAE226, lysates were collected at 3, 24, 48, and 72 h and then analyzed for down-regulation of pFAK at residue Y397. C, lysates from collagen I–coated and non-coated plates were also examined by Western blot analysis for pFAK relative to FAK. D, cytochemistry was ascertained for docetaxel both alone (hatched line) and in conjunction with TAE226 (solid line) in HeyA8 and HeyA8-MDR cell lines. Points, means of three independent experiments. Bars, SE.
mass, and the number of tumor nodules were recorded. In animals bearing SKOV3ip1 tumors, the volume of malignant ascites was recorded. Tumors were either fixed in formalin and embedded in paraffin or snap frozen in optimal cutting temperature (OCT) compound (Sakura Finetek USA, Inc.) in liquid nitrogen.

**Therapy for established i.p. tumors in nude mice.** Before evaluating the therapeutic effect of TAE226 combined with docetaxel in our mouse model, we first did preliminary dose-response experiments for TAE226. Seventeen days after HeyA8 cell inoculation (when i.p. tumors were palpable), a single i.p. dose (PBS or TAE226 15, 30, and 100 mg/kg) was given. Three mice from each treatment group were randomly sacrificed 8, 24, or 48 h after treatment, and tumors were collected for IHC.

To determine the antitumor effects of TAE226, SKOV3ip1, HeyA8, and HeyA8-MDR, tumors were injected i.p., and therapy with one of four regimens was initiated 7 days later. Treatment regimens were as follows: vehicle, TAE226 30 mg/kg p.o. daily, docetaxel 2 mg/kg (SKOV3ip1) or 2.5 mg/kg (HeyA8 and HeyA8-MDR) i.p. weekly, or TAE226 daily combined with docetaxel weekly.

**Immunohistochemistry.** Phosphorylated FAK (pFAK[Y397]) was assessed by IHC. Formalin-fixed, paraffin-embedded tissues were heated and then deparaffinized using xylene and declining grades of ethanol before being rehydrated in 0.1% Triton X-100 for 10 min. Endogenous peroxidases were blocked with 6% hydrogen peroxide for 30 min. Nonspecific epitopes were blocked with 5% normal horse serum and 1% normal goat serum for 30 min. All sections were then incubated with anti-pFAK[Y397] antibody (1:25) overnight at 4°C. After washing with PBS followed by Optimax buffer, the appropriate secondary antibody was applied, and visualization was done using the Vectastain ABC detection kit (Vector Labs) according to the manufacturer’s instructions. The chromogenic reaction was done with 3,3’-diaminobenzidine (DAB, Phoenix Biotechnologies).

VEGF, MMP-9, and PCNA IHC analyses were done as previously described (28). Briefly, after deparaffinization and rehydration, antigen retrieval was done using either heated citrate buffer (0.1 mol/L; pH 6.0) in a microwave (MMP-9, PCNA) or pepsin in a 37°C humidified incubator (VEGF). Endogenous peroxidases and nonspecific epitopes were blocked with 3% H2O2/methanol and 5% normal horse serum with 1% normal goat serum, respectively. Slides were then incubated with the appropriate primary antibody at the following dilutions: VEGF, 1:100; MMP-9, 1:400; anti-PCNA, 1:50, in blocking solution overnight at 4°C. After incubating with the appropriate secondary antibody conjugated to HRP, detection was achieved with DAB substrate and counterstained with Gill’s No. 3 hematoxylin (Sigma). The proliferative index was calculated as the percentage of PCNA-positive cells in 10 randomly selected high-power field (HPF) per slide.

CD31 IHC was done using freshly cut frozen tissue as previously described (23). Briefly, slides were fixed in cold acetone and then incubated with anti-CD31 [platelet/endothelial cell adhesion molecule 1 (PECAM-1)]. Microvessel density (MVD) was quantified by counting the number of microvessels per 100× HPF over 10 randomly selected 0.159-mm2 fields. A microvessel was defined as a discrete CD31+ cluster or single cell adjacent to a lumen.

**Terminal nucleotide transferase–mediated nick end labeling assay.** Immunofluorescence staining for terminal nucleotide transferase–mediated nick end labeling (TUNEL) was done on frozen sections as previously described (29). To quantify TUNEL-positive cells, the number of green fluorescence–positive cells was counted in 10 random 0.011-mm2 fields at 400× magnification.

**Immunofluorescence imaging.** The luciferase-transfected HeyA8 cell line (HeyA8-Luc) was established using a Lentivirus system, as previously described (29). Treatment with TAE226 and docetaxel was initiated 17 days after i.p. injection with HeyA8-Luc cells (2.5 × 106 cells per mouse). Immunofluorescence imaging and data acquisition were done twice weekly as previously reported (29) using the IVIS 100 imaging system coupled to the Living Image software ( Xenogen).

**Immunofluorescence staining for CD31 and desmin.** Freshly cut, snap-frozen sections were double stained for CD31 and desmin as described previously (28). Briefly, after fixation in cold acetone and blocking of nonspecific epitopes, sections were incubated with CD31 antibody followed by the fluorescence-conjugated antibody. After washing with PBS, samples were incubated with desmin antibody (1:400), followed by incubation with fluorescence-conjugated antibody. Samples were counterstained with Hoechst for 5 min and mounted. Pericyle coverage was determined by the percent of vessels with 50% or more coverage by the green fluorescence of associated desmin–positive cells in five random fields at 200 × magnification for each tumor.

**Microscopic analysis.** IHC-stained slides were examined with a 10× objective on a Microphot-FX microscope (Nikon) equipped with a three-chip charge-coupled device color video camera (model DXC990, Sony Corp.). Immunofluorescence microscopy was done using a 20× objective on a Microphot-FX microscope (Nikon) equipped with an HBO 100 mercury lamp and narrow band-pass filters to individually select for green, red, and blue fluorescence (Chroma Technology). Images were captured using a cooled charge-coupled device camera (model 5810, Hamamatsu) and Optimas Image Analysis software (Media Cybernetics).

**Quantitative real-time reverse transcription-PCR.** RNA was extracted, and reverse transcription was done using an oligo(dT) primer and Moloney murine leukemia virus (M-MLV) reverse transcriptase (Life Technologies). After PCR amplification, quantitative values were obtained, as previously described (30). The primers were obtained from Applied Biosystems.

**Statistical analyses.** Differences in continuous variables were analyzed using Student’s t test or ANOVA as appropriate. A P value ≤0.05 was considered statistically significant. For values that were not normally distributed, the Mann-Whitney rank sum test was used. Survival analyses were done using the Kaplan-Meier method. The Statistical Package for the Social Sciences (SPSS, SPSS Inc.) was used for all statistical analyses.

**Results**

**Effects of TAE226 on FAK phosphorylation and ovarian cancer growth.** Before in vivo experiments, we first tested the in vitro effects of TAE226 on FAK phosphorylation and growth of ovarian cancer cell lines. Given that FAK is known to bind to the j1 integrins, which consequently leads to FAK activation and autophosphorylation, we tested the effects of TAE226 on ovarian cancer cell plates plated on collagen I–coated plates (16). The expression of pFAK[Y397] was significantly inhibited by TAE226 in a dose-dependent manner on collagen I–coated plates and completely suppressed on noncoated plates following treatment with TAE226 for 1 h (Fig. 1B). Although decreases in FAK phosphorylation were noted at the 0.5-μmol/L dose, the maximal suppression occurred at 1 μmol/L. TAE226 inhibited pFAK[Y397] expression up to 24 h, with gradual return of expression by 72 h (Fig. 1B). Similar results were obtained with the HeyA8 cell line (data not shown). Because FAK phosphorylation at Y861 is also known to potentially influence tumor vasculature and, hence, tumorigenicity (31), we tested the efficacy of TAE226 in inhibiting pFAK[Y861]. TAE226 decreased pFAK[Y861] in a dose-dependent manner; however, doses up to 5 μmol/L were required to decrease phosphorylation at this site (Fig. 1C).

Based on our prior data with sensitization of ovarian cancer cells to taxanes following FAK silencing, we tested the effect of TAE226 on ovarian cancer cell growth. The IC50 with TAE226 alone was 10-fold greater in combination with docetaxel compared to docetaxel alone in HeyA8 cells (Fig. 1D) and 20-fold greater in the taxane-resistant HeyA8-MDR cells (Fig. 1D). Similar results were observed with the SKOV3ip1 and SKOV3-TR cells (data not shown).

**Effect of TAE226 on ovarian tumor growth in vivo.** In light of our in vitro data on the effect of TAE226 in sensitizing ovarian cancer cells to docetaxel, we examined the in vivo therapeutic
potential of TAE226. Before therapy experiments, dose-finding studies were done. Mice bearing HeyA8 tumors were given a single p.o. dose of TAE226 at 15, 30, or 100 mg/kg and then sacrificed 8, 24, or 48 h after a single TAE226 administration (15, 30, or 100 mg/kg). IHC staining for pFAKY397 shows down-regulation of pFAK by 24 h with return of expression by 48 h from TAE226 administration (30 mg/kg). Pictures are taken at 100x. Graph shows band intensity of pFAK relative to FAK.

To determine the in vivo therapeutic potential of TAE226, a series of in vivo experiments were carried out. Female nude mice bearing HeyA8 tumors in peritoneal cavity were randomly allocated to one of four treatment groups (n = 10 per group): (a) daily PBS p.o. and weekly PBS i.p. (control), (b) daily p.o. TAE226, (c) weekly i.p. docetaxel, and (d) both TAE226 and docetaxel. Therapy experiments were terminated when the control animals developed signs of significant tumor burden and seemed moribund (3 to 4 weeks of therapy depending on the cell line). There was a 54% to 79% reduction in tumor burden either by TAE226 or docetaxel monotherapy (Fig. 2B). Combination therapy resulted in the greatest tumor reduction, with 89% reduction in the HeyA8 model (P < 0.002 when compared with controls). The same therapy schema was carried out in the SKOV3ip1 model, and a 97% tumor
reduction was observed in the combination group compared with controls ($P < 0.001$). These were no differences in body weight, feeding habits, or mobility between the groups.

Because chemotherapy resistance is a common problem in ovarian carcinoma, we also did experiments with the HeyA8-MDR model, which is resistant to several chemotherapy agents including taxanes. Interestingly, TAE226 alone showed 46% reduction in tumor weight even in this model ($P < 0.04$; Fig. 2B), but docetaxel alone was not effective. The combination of TAE226 and docetaxel yielded an 85% reduction in tumor growth in this model ($P = 0.01$) compared with controls.

To further evaluate the effects of TAE226 therapy on metastatic tumor growth, tumor incidence and number of nodules were examined from the therapy experiments described above (Table 1). Monotherapy with either TAE226 or docetaxel resulted in a modest reduction in the number of tumor nodules in the HeyA8 and SKOV3ip1 models, but had no effect in the HeyA8 MDR model. However, combination therapy consistently produced fewer tumor nodules in all groups (HeyA8: 78% reduction, $P = 0.006$; SKOV3ip1: 92%, $P = 0.001$; HeyA8-MDR: 14% reduction, $P = NS$). In addition, all treatments were effective in blocking the development of ascites in the SKOV3ip1 model: mean volume, 1.93 ± 0.58 mL in controls compared with the TAE226 (mean volume, 0.26 ± 0.11 mL), docetaxel (mean volume, 0.31 ± 0.13 mL), or combination groups (mean volume 0; $P < 0.001$). There were no significant differences in body weight, feeding habits, or mobility between the groups.

Based on the encouraging results with TAE226-based therapy, we next examined its effects on survival using a moribund state as the end point. TAE226 and docetaxel each prolonged survival compared with controls, but the combination resulted in the greatest prolongation of survival (median 96 days; $P < 0.001$; Fig. 2C).

**Effects of TAE226 on established ovarian tumors.** Although the experiments described above used a model of small volume disease, patients with recurrent cancer will frequently have larger tumor burden. To test the effects of TAE226-based therapy on larger tumors, we used the HeyA8-Luc cell line. Therapy was started 2 weeks after tumor cell injection when the palpable tumor size ranged between 0.5 and 1 cm. In the TAE226 or docetaxel monotherapy groups, the rate of tumor growth was slower, but no apparent tumor regression was noted. Remarkably, in the combination group, there was 60% tumor regression noted by 15 days after initiating therapy (Fig. 2D).

**Effect of pFAK<sup>Y397</sup> targeting on apoptosis, angiogenesis, and cell proliferation.** To examine potential mechanisms responsible for the therapeutic effects of TAE226 and docetaxel, a series of experiments were done. First, we examined tumor samples at the conclusion of therapy for sustained FAK suppression. Indeed, TAE226 treatment resulted in decreased pFAK<sup>Y397</sup> compared with controls at the conclusion of therapy (Fig. 3A). Because of recent studies (4, 32) related to FAK and tumor angiogenesis, we next examined the effects on vessel density using CD31 immunostaining (Fig. 3B). TAE226 and docetaxel each reduced MVD compared with controls ($P = 0.007$ and 0.008, respectively). The combination of these agents had an even greater effect on vessel density compared with either treatment alone ($P < 0.002$). Based on the known FAK-mediated regulation of VEGF and MMP-9 (32–34), we examined the expression of these angiogenic factors in tumors harvested from the various therapy groups (Fig. 3B). The expression of both VEGF and MMP-9 was significantly lower in the TAE226-based therapy groups, suggesting that the antitumor effect may be mediated, in part, by suppression of angiogenesis. To further characterize the anti-vascular mechanism, we also evaluated the effects of therapy on tumor cell and tumor-associated endothelial cell apoptosis (Fig. 3C and D). Tumors from vehicle-treated animals had largely absent apoptosis in the endothelium. Apoptotic endothelial cells were apparent in the TAE226-treated group and the docetaxel-treated group. In the combination therapy group, both endothelial cells and tumor cells had significantly greater apoptosis compared with all other groups (Fig. 3C).

To assess the effects of TAE226 therapy on tumor proliferation, we did IHC analysis for PCNA (Fig. 3D). Compared with tumors from mice receiving vehicle alone, the percentage of PCNA-positive cells was reduced to 22 ± 1.8% in tumors from mice that received both TAE226 and docetaxel ($P < 0.005$). The number of PCNA-positive cells was minimally reduced by treatment with TAE226 alone or docetaxel alone compared with vehicle. Finally, we examined tumor cell apoptosis by using the TUNEL method.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment group</th>
<th>Tumor incidence (%)</th>
<th>Mean tumor weight ± SD (g)</th>
<th>$P$ (tumor weight)*</th>
<th>Number of nodules (mean ± SD)</th>
<th>$P$ (number of nodules)*</th>
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<tbody>
<tr>
<td>HeyA8</td>
<td>Vehicle</td>
<td>100</td>
<td>1.1 ± 0.51</td>
<td>NS</td>
<td>4.8 ± 1.5</td>
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<td>TAE 226</td>
<td>90</td>
<td>0.50 ± 0.25</td>
<td>0.01</td>
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<td></td>
<td>Docetaxel</td>
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<td>3.1 ± 1.2</td>
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<td></td>
<td>TAE 226 + docetaxel</td>
<td>60</td>
<td>0.11 ± 0.04</td>
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<td>1.1 ± 0.4</td>
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<td>SKOV3ip1</td>
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<td>1.2 ± 0.6</td>
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<td>2.26 ± 0.5</td>
<td>NS</td>
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* $P$ values reflect comparison to vehicle controls.
Minimal apoptosis occurred in the single-agent treatment groups. Combination therapy resulted in substantially more apoptotic cells than vehicle, TAE226 alone, or docetaxel alone (Fig. 3D).

**Effect of anti-FAK therapy on vessel maturation.** Based on the efficacy of TAE226 in causing tumor regression, we next asked whether the mature tumor vasculature was also affected. FAK is known to play a role in the migration of vascular smooth muscle cells (35). However, the effects of FAK inhibition on perivascular cells are not known. Therefore, we examined the extent of pericyte coverage in the tumors harvested from the therapy experiments described above. Docetaxel alone had no effect on pericyte coverage, but the extent of pericyte coverage was significantly lower in the TAE226 groups (Fig. 4A). It is known that pericytes may reduce the effectiveness of antiangiogenic therapy by providing local survival signals for endothelial cells. One of the mechanisms by which pericytes may protect tumor-associated endothelial cells is by producing VEGF locally in response to...
Discussion

The main findings from our study are that FAK inhibition by TAE226 has significant therapeutic efficacy in both chemotherapy-sensitive and chemotherapy-resistant ovarian cancer models. The therapeutic efficacy was the greatest when FAK inhibition was combined with docetaxel and occurred by both direct effects on tumor cells and indirect effects on the tumor vasculature.

Ovarian cancer remains the most deadly gynecologic malignancy despite advances in chemotherapy and surgery. Although alterations in dosing and routes of administration for chemotherapy have provided incremental improvements in the survival of patients (36), the addition of more chemotherapy agents does not seem to provide any further benefit. Therefore, attention has turned toward targeting key biological pathways in both tumor cells and the microenvironment. Targeting the VEGF pathway has provided promising proof that antiangiogenic therapies can indeed improve therapeutic outcomes among cancer patients (37). Initial clinical trials with bevacizumab in ovarian cancer have shown encouraging results (38, 39). However, it is likely that additional targets may be required to achieve further gains in patient survival. FAK is an attractive therapeutic target because it is a key convergence point for many growth factor pathways required for survival and metastatic functions of cancer cells. FAK is overexpressed in a substantial proportion of ovarian cancers and is predictive of poor clinical outcome (11, 14). FAK is also overexpressed in tumor-associated endothelial cells (40) and represents a unique opportunity for concomitant targeting of tumor cells and the microenvironment.

FAK is an important regulator of signaling processes between the extracellular matrix and tumor cells (41, 42). FAK phosphorylation at Y397 follows integrin stimulation or ligand binding by growth factor receptors (43, 44). Subsequently, phosphorylation at Y397 promotes the Src homology domain 2 (SH2)–dependent binding of Src-family tyrosine kinases and the formation of an activated FAK–Src complex. FAK activation at focal adhesion sites enhances cytoskeletal reorganization, cellular adhesion, and cell survival (15, 45). FAK also functions in tumor cell migration and invasion (11). However, there are a limited number of approaches currently available for targeting FAK (31). TAE226 is a novel potent inhibitor of FAK activity that has been shown to have therapeutic efficacy in a glioma model (27). Remarkably, our study shows that TAE226-based therapy was indeed effective even in chemotherapy-resistant ovarian cancer models.

FAK is a particularly appealing target because it is overexpressed in both tumor cells and tumor-associated endothelial cells (14, 40). The effects of tumor cell FAK on the tumor microenvironment can be mediated by the regulation of angiogenic cytokines. Sheta et al. (46) showed that transcriptional induction of VEGF occurs via FAK. Subsequently, Mitra et al. (33) showed that 4T1 breast cancer cells have reduced levels of VEGF due to the inhibition of FAK activity. We have shown that either FAK silencing or suppression of FAK activity reduced VEGF and MMP-9 expression in vivo. This reduction contributed to apoptosis of tumor-associated endothelial cells. However, FAK is likely to have direct functional significance for the tumor vasculature as well. FAK is expressed in angiogenic blood vessels of malignant astrocytomas where it facilitates angiogenesis by enabling haptotactic migration toward ECM proteins (47). Recently, we isolated tumor-associated endothelial cells from human ovarian cancers and showed that FAK was overexpressed in these cells (40). Moreover, FAK inhibition in endothelial cells inhibited cell motility and tube formation assays in vitro. Therefore, the therapeutic efficacy of FAK inhibition likely results from both direct antitumor and anti-vascular effects. We extend our previous findings by demonstrating an additional mechanism whereby pericytes were also affected by the anti-FAK therapy. Pericytes provide survival signals for endothelial cells (26, 48). FAK is known to play an important role in PDGF-stimulated pericyte migration (35). Our results indicate that FAK inhibition results in reduced pericyte coverage, thereby providing an additional explanation for the observed anti-vascular effects.

A potential confounding factor with the use of small-molecule inhibitors is that several targets may be affected. For example, TAE226 can also inhibit the insulin-like growth factor-1 receptor (IGF-IR) and the proline-rich tyrosine kinase 2 (Pyk2), which may play a role in the growth of ovarian and other cancers (27, 49). However, we have previously shown that FAK silencing in vivo using siRNA incorporated in neutral liposomes results in substantial antitumor activity, suggesting that FAK is indeed an important therapeutic target (24).
In summary, we have shown that TAE226 is an effective inhibitor of FAK activation. Moreover, inhibition of FAK phosphorylation in combination with doxetaxel effectively inhibited ovarian cancer growth by both direct and indirect anti-vascular mechanisms. Based on these findings, FAK represents an attractive therapeutic target in ovarian cancer and supports further consideration of anti-FAK therapies for clinical development.

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