Targeting Focal Adhesion Kinase with Dominant-Negative FRNK or Hsp90 Inhibitor 17-DMAG Suppresses Tumor Growth and Metastasis of SiHa Cervical Xenografts

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Abstract

Focal adhesion kinase (FAK), a nonreceptor protein tyrosine kinase and key modulator of integrin signaling, is widely expressed in different tissues and cell types. Recent evidence indicates a central function of FAK in neoplasia where the kinase contributes to cell proliferation, resistance to apoptosis and anoikis, invasiveness, and metastasis. FAK, like other signaling kinases, is dependent on the chaperone heat shock protein 90 (Hsp90) for its stability and proper function. Thus, inhibition of Hsp90 might be a way of disrupting FAK signaling and, consequently, tumor progression. FAK is expressed in high-grade squamous intraepithelial lesions and metastatic cervical carcinomas but not in nonneoplastic cervical mucosa. In SiHa, a cervical cancer cell line with characteristics of epithelial-to-mesenchymal transition, the stable expression of dominant-negative FAK-related nonkinase decreases anchorage independence and delays xenograft growth. FAK-related nonkinase as well as the Hsp90 inhibitor 17-dimethylaminoethylamino-17-demethoxygeldanamycin both negatively interfere with FAK signaling and focal adhesion turnover. Short-term 17-dimethylaminoethylamino-17-demethoxygeldanamycin treatment prolongs survival in a SiHa lung metastasis model and chronic administration suppresses tumor growth as well as metastatic spread. [Cancer Res 2009;69(11):4750–9]

Introduction

Focal adhesion kinase (FAK), a nonreceptor protein tyrosine kinase involved in integrin signaling, fulfills complex biological tasks that require its kinase activity as well as its scaffolding function. (1). Recent reports highlight the significance of FAK in neoplasia where it has been implicated in cell proliferation, protection from apoptosis and anoikis, migration, invasion, cell spreading, adhesion, and angiogenesis, thereby influencing each step of the metastatic cascade (2). This functional diversity and the finding that FAK is overexpressed in different tumors has led to an increasing interest in the integrin-FAK-Src signaling complex as potential target for therapeutic intervention (3–5). The expression of the COOH-terminal, noncatalytic domain of FAK, termed FAK-related nonkinase (FRNK) (ref. 6), has been instrumental for the exploration of the cellular functions of the kinase as well as the therapeutic potential of FAK inhibition.

Notably, not only Src but also FAK has been described as “client” of heat shock protein 90 (Hsp90), which is responsible for maintaining the functional conformation of a range of signaling kinases (7). Hsp90 is abundant and active intracellularly as a flexible dimer. Each monomer consists of an ATPase containing N-domain, which is connected to the M-domain by a flexible, charged linker and followed by the COOH-terminal dimerization domain. Substrate specificity and chaperone function are regulated by an array of cofactors, and an increased activity of the entire complex in cancer cells has been held responsible for the relative tumor specificity of Hsp90 inhibitors (8). The uniqueness of the NH2-terminal ATP-binding pocket is the structural cause for the binding of benzoquinone ansamycins, which mimic the kinked conformation of ATP inside this pocket (9). Geldanamycin was the first compound of this group and initially recognized for its cytotoxicity and ability to revert the phenotype of v-Src-transformed cells (10). Because of instability and hepatotoxicity, semisynthetic derivatives were developed. 17-Allylamino-17-demethoxygeldanamycin and the water-soluble 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) have entered clinical trials. 17-Allylamino-17-demethoxygeldanamycin is most advanced in clinical testing and encouraging results have been reported (11–13). Efforts are currently directed toward the development of fully synthetic Hsp90 inhibitors with improved solubility and side effect profile (14) and toward resolving problems associated with resistance mechanisms such as the heat shock response (15).

Recent studies have shown that Hsp90 contributes to cell migration and, in malignancies, to tumor progression (16, 17). These studies focused mainly on the secreted Hsp90α isoform. However, Hsp90 also maintains the stability of intracellular kinases in charge of cell motility. Thus, inhibitors of Hsp90 should suppress not only tumor growth but also invasion and metastasis. Although cancer of the uterine cervix can be detected at early stages through screening, therapeutic options for metastatic disease are currently limited to cisplatin and radiation (18). In this study, we provide a rationale for FAK targeting in cervical cancer. Using stable FRNK expression as a model, we show that interference with FAK signaling decreases tumor growth and metastatic colonization. Importantly, we provide evidence that pharmacologic inhibition of Hsp90 leads to decreased FAK signaling and,
consequently, displays growth-inhibitory and antimetastatic effects similar to FAK inhibition alone.

**Materials and Methods**

**Patient tissue and histology.** Formalin-fixed, paraffin-embedded tissues of 20 cases each of high-grade squamous intraepithelial lesion (HGSIL) and metastatic squamous cell carcinoma (SCC) of the uterine cervix with one paired lymph node metastasis were selected from files of the Institute of Pathology Leipzig (1997-2007). Ethics approval was obtained from institutional committees at the University of Leipzig (protocol 037-2007) and the University Health Network (protocol 06-0804-T). Histology was examined by a senior pathologist (L.-C.H.) and a pathologist-in-training (J.S.). Eighteen HGSIL, 20 SCC, and 18 metastasis contained sufficient tissue for scoring. Nonneoplastic epithelium was present in 5 HGSIL and 11 SCC. Immunohistochemistry was scored for intensity (0-3; Supplementary Fig. S1) and percent area was estimated for each intensity level. Both values were multiplied to give a histology score (H-score: 0-300).

**Reagents, cells, and viruses.** Geldanamycin (Biomol) was dissolved in DMSO at 10 mmol/L and stored at -20°C. 17-DMAG (NSC707545) was supplied by the National Cancer Institute, stored at -20°C, and dissolved in water or saline before use. SiHa and ME180 cells were obtained from the American Type Culture Collection and cultured as recommended. DsRed2 fluorescently labeled SiHa cells were obtained from Dr. R.P. Hill (University of Toronto). Absence of Mycoplasma was confirmed using Hoechst 33342 staining (Sigma-Aldrich) and MycoAlert Detection Kit (Lonza). FRNK cDNA, a gift from Dr. M.D. Schaller (University of North Carolina), was subcloned into a retroviral vector (pBMN-I-GFP; Addgene plasmid 1736; http://www.stanford.edu/group/nolan/plasmid_maps/pmaps.html) and stable cell lines were generated using an amphotrophic Phoenix-MMULV system provided by Dr. G.P. Nolan (Stanford University). Vector-control cells were generated using the empty construct. GFP-expressing cells were subsequently isolated by fluorescence-activated cell sorting resulting in a 98% enriched population. FRNK expression was monitored by reverse transcription-PCR and immunoblotting. Total RNA was isolated using RNeasy Mini Kit (Qiagen) and reverse transcribed with SuperScript II (Invitrogen). An equivalent of 10 ng cDNA, SYBR Green Master Mix (Applied Biosystems), and the Mx3000P PCR system (Stratagene) were used to amplify FRNK and RPL15 housekeeping control. For primer sequences, see Supplementary Information.

**In vitro assays.** Migration and invasion were assessed as described (19). Human fibronectin (5 µg/mL; BD Biosciences) or epidermal growth factor (50 ng/mL; Sigma-Aldrich) served as chemoattractants. Cells were used after 4 h serum withdrawal. 17-DMAG was added into the bottom chamber for up to 24 h. Cells on the opposite filter surface were stained with Diff-Quik (Dade Behring) and counted at ×250 magnification in one random field/quadrant (migration) or the entire filter (invasion). Colony formation was tested with 100 and 250 cells per 10 cm dish (triplicates). Colony number and size were determined after 3 weeks. Anchorage-independent growth was tested in soft agar, and 2% α-MEM was mixed with select agar (Invitrogen) to form a 1% (v/v) base and a 0.4% top layer in 6-well plates. Colonies were grown for 24 to 48 h on type I collagen (100 µg/mL; BD Biosciences)-coated coverslips before drug exposure for 24 h. After fixation with 4% formalin and permeabilization with 0.1% (v/v) Triton X-100, primary antibodies in 1% (v/v) bovine serum albumin/PBS were incubated for 1 h. For antibodies and dilutions, see Supplementary Information. Clone 77/FAK used for immunofluorescence was generated with an immunogen not overlapping with FRNK (amino acids 354-533). Secondary antibody goat anti-mouse IgG Alexa Fluor 555 was incubated together with Alexa Fluor 488-phallolidin for 30 min and coverslips were mounted with ProLong Gold containing 4,6-diamidino-2-phenylindole (Invitrogen). Confocal images were acquired using an Olympus IX81 microscope and Fluoview FV1000 software.

**Animal xenografts.** Female SCID mice aged 6 to 12 weeks were bred and housed at the Ontario Cancer Institute animal facility. Experiments were done according to regulations of the Canadian Council on Animal Care. Subcutaneous and orthotopic cervical xenografts were done as described (20, 22). To determine a limited dose-response relationship, orthotopic xenografts were established over 4 weeks and treated with four doses of 10 or 30 mg/kg 17-DMAG at 12 h intervals. Mice were killed and tumors were sampled 4 h after the last treatment. For chronic administration, 17-DMAG was given by intraperitoneal injection three times a week at 25 mg/kg as described (23) starting 4 h after implantation. Controls received saline. Mice were killed when control tumors approached ~1 cm diameter. Tumors were removed and remaining organs were examined for DsRed2-positive metastases using a Leica MZFLIII fluorescence stereomicroscope. Subsequently, the retroperitoneum was dissected. Interval levels (200 µm) were cut to confirm lymph node and organ metastasis by histology. Experimental lung metastases were initiated by tail vein injection. Cell viability in suspension was determined by ViaCount Assay (Guava Technologies) until all injections were completed. Four doses of 30 mg/kg 17-DMAG intraperitoneally were given at 12 h intervals, the first dose at ~30 min before tail vein inoculation. Mice were monitored for distress and isolated if sickness occurred. Sick mice were euthanized by CO2 asphyxia if there was no clinical improvement.

**Animal computer tomography (microCT).** Development of lung metastases was monitored by serial microCT. Images were acquired on a GE Locus Ultra microCT (80 kVp, 50 mA, voxel size: 150 µm) and analysis was conducted on GE Microview (GE Healthcare Bio-Sciences). Mice were maintained under isoflurane anesthesia during image acquisition.

**High-performance liquid chromatography.** 17-DMAG drug tissue concentrations were measured in xenograft tumors using a high-performance liquid chromatography method as described previously (20).

**Statistical analysis.** Statistical analysis was done using Mann-Whitney test and ANOVA. Two-tailed P values < 0.05 were considered significant. Kaplan-Meier survival data were compared using the log-rank test. GraphPad Prism version 5.01 software was used.

**Results**

**FAK is overexpressed in HGSIL and cervical SCC.** Previous research indicated that FAK overexpression is an early event in cancer development (24). We aimed to reproduce these results in our own cohort. Because transition from a preinvasive state toward a more aggressive lesion is often associated with loss of cell-cell adhesions, we also examined E-cadherin. Adjacent squamous epithelium without intraepithelial neoplasia or signs of human papillomavirus infection was scored for comparison. We observed an increase of FAK expression from essentially unstained nonneoplastic epithelium to HGSIL and metastatic cancer. HGSIL and cancer displayed mostly weak to moderate staining with some...
exceptions, for example, strong focal FAK expression at the invasion front or in a diffusely infiltrating carcinoma with partial E-cadherin loss (Fig. 1). E-cadherin expression was strongest in nonneoplastic epithelium and reduced or lost in HGSIL and metastatic cancer. Differences in H-scores were only significant between nonneoplastic epithelium and all other categories, suggesting that both FAK expression and E-cadherin loss are indeed early events during tumorigenesis.

FRNK decreases anchorage independence, migration, and focal adhesion signaling. To test if FAK is active in cervical cancer cells, we generated FRNK-SiHa, a cell line with stable expression of FRNK. The SiHa cell line was selected because of low E-cadherin expression and nuclear positivity for Snail1, suggesting a less differentiated phenotype compared with other cervical lines such as ME180 (data not shown). Both SiHa and ME180 cells, however, retain human cytokeratin expression (AE1/AE3). Cell growth in monolayer culture over 5 days and colony formation were not significantly affected in FRNK-SiHa (data not shown), but anchorage-independent growth assays showed significantly smaller FRNK-SiHa colonies (Fig. 2A).

Migration and invasion of FRNK-SiHa were tested with fibronectin as chemoattractant. Migration was decreased by 40% and consistent with a diminished fibronectin response (Fig. 2B). Invasion was decreased by 40% to 60% (data not shown) but failed to reach statistical significance likely due to the weak stimulatory effect of fibronectin. Basal phosphorylation levels of FAK Y397 (autophosphorylation) and Y861 and Y925 (Src-dependent) were reduced in FRNK-SiHa (Fig. 2C). Paxillin Y118 was 50% decreased similar to p130Cas Y165, Y249, and Y410, indicating an inhibited downstream signaling due to FRNK. Paxillin Y31 remained

Figure 1. FAK expression and E-cadherin loss are early events in cervical cancer. A, FAK increase and E-cadherin loss in HGSIL, cancer, and metastases compared with nonneoplastic (Normal) epithelium. B, focus of SCC invasion with adjacent FAK-negative epithelium (asterisk) and loss (arrow) of E-cadherin membrane staining. C, poorly differentiated SCC with FAK-positive (inset 1) infiltrating cells and lack of E-cadherin (inset 2) in this poorly differentiated component. D, HGSIL (asterisk) and cancer invasion front (inset) with pronounced FAK positivity.
unaffected. No difference was seen for Akt and extracellular signal-regulated kinase 1/2 phosphorylation (data not shown). Treatment with 10 μmol/L nocodazole for 4 h with subsequent washout was used to examine focal adhesion dynamics (25). A ~2-fold increase of FAK Y397 and Y925 in vector-control but not in FRNK-SiHa cells (Fig. 2D) suggested a decreased focal adhesion turnover.

**FRNK and 17-DMAG similarly interfere with focal adhesion turnover.** To determine the effect of Hsp90 inhibition on basal phosphorylation and total protein levels of FAK, monolayer cultures were treated with increasing doses of geldanamycin and 17-DMAG, and 100 nmol/L of either compound caused a profound decrease in FAK Y397 and a somewhat less noticeable decrease in FAK protein after 24 h (Fig. 3A). Paxillin and the established client Akt responded with a profound decrease of either protein level. Hsp70 was already induced at 10 nmol/L concentration, indicating that the heat shock response precedes client protein destabilization. Next, we addressed the question if 17-DMAG was able to cause a blunting of stimulated FAK signaling. Figure 3B shows a dose-dependent suppression of the response to epidermal growth factor. FAK activity was suppressed below baseline if the treatment was extended >20 h. Consequently, 17-DMAG also decreased SiHa cell migration against epidermal growth factor (Fig. 3C). A similar suppression of fibronectin stimulation was observed, albeit at lower overall phosphorylation levels (data not shown).

To examine if altered FAK signaling was reflected by changes in cell and focal adhesion morphology, SiHa cells were treated with 100 nmol/L geldanamycin or 17-DMAG and double-labeled for actin and either FAK, paxillin, or vinculin. Drug-treated cells were compared with FRNK-SiHa, wild-type, and vector-control cells for cell area and focal adhesion length. FRNK-SiHa and Hsp90

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**Figure 2.** FRNK decreases anchorage independence, cell migration, and FAK signaling. *A,* FRNK-SiHa colonies in soft agar are significantly smaller than wild-type (WT) and vector-control (CTRL; n = 3; columns, mean; bars, SE). *B,* migration of FRNK-SiHa is decreased by 40% (n = 6; columns, mean; bars, SE) due to a decreased fibronectin (FN) response. *C,* quantitative PCR shows specific amplification of FRNK in FRNK-SiHa but not in wild-type and vector-control compared with RPL15. FRNK leads to decreased phosphorylation of FAK, paxillin, and p130Cas. *D,* FRNK-SiHa (underlined) fails to stimulate FAK phosphorylation after nocodazole washout compared with vector-control.
inhibitor-treated cells displayed a spread-out morphology (Fig. 4A). Double labeling revealed pronounced stress fibers connected to distinct, elongated focal adhesions in FRNK-SiHa as well as drug-treated cells (Fig. 4B; Supplementary Figs. S2-S4). Vinculin confirmed our results (data not shown). Immunofluorescence for FAK using clone77 antibody produced a diminished labeling of focal adhesions in FRNK-SiHa (Fig. 4C and D), consistent with a displacement of endogenous FAK. Together, these results suggest that Hsp90 inhibition suppresses focal adhesion turnover in a manner similar to FRNK.

FRNK delays tumor growth and lung metastasis formation in SiHa xenograft models. Next, we determined if the suppressed FAK activity in FRNK-SiHa would translate into changes in tumor formation and metastatic potential. To screen for a potential difference, FRNK-SiHa and vector-control cells were initially grown in opposite flanks of single mice. This was repeated six times starting from separate cultures for each injection. All mice, except for one outlier, showed delayed tumor development for FRNK-SiHa. To confirm this result, wild-type, FRNK-SiHa, and vector-control cells were inoculated into flanks of individual mice showing a significantly delayed tumor formation with FRNK (Fig. 5A). To further examine metastatic potential and growth of these xenografts, we implanted a fragment of each dissected xenograft into the cervix of a recipient mouse. Due to extensive necrosis in large wild-type and vector-control tumors and the small size of FRNK-SiHa donors on the contrary, a high variability was seen with the resulting orthotopic xenografts and tumor weight differences were not significant. However, there was a trend toward smaller tumors with FRNK-SiHa (Supplementary Fig. S5A). Three distant retroperitoneal tumor deposits were found in each of the wild-type and vector-control groups by histology (250 μm levels) but none in FRNK-SiHa (Supplementary Fig. S5B). Finally, to address the effect of FRNK on metastatic colonization, each cell line was tail vein injected into 5 mice per group. Development of lung disease was monitored over 3 months with four microCT imaging sessions. There were three early unscheduled deaths, one in each group. The remaining mice showed a trend toward lower disease burden in FRNK-SiHa, with 1 of 4 FRNK-SiHa versus 3 of 4 wild-type and 4 of 4 vector-control mice that developed extensive lung disease (Supplementary Fig. S5C).

17-DMAG suppresses in vivo FAK signaling, tumor growth, and metastasis. Proceeding from earlier results (20), we wished to examine if 17-DMAG treatment can cause a detectable suppression of FAK signaling in vivo. Mice harboring orthotopic xenografts were treated with two different dose levels of 17-DMAG by either oral gavage or intraperitoneal injection four times in 12 h intervals. Because decreased phosphorylation often seems to precede client degradation (23), we focused on two FAK phosphorylation sites (Y397/Y861) as well as Akt S473 (Fig. 5B). Immunoblotting revealed a statistically significant FAK Y397 down-regulation and Hsp70 up-regulation at 30 mg/kg 17-DMAG. Drug tissue concentrations for oral versus intraperitoneal application were consistent with a reported oral bioavailability of ~50% (Supplementary Fig. S6A). No difference was noted between ME180 and SiHa xenografts at the same dose level. Because the FAK Y861 antibody distinctly labels cell membranes, we stained control and 30 mg/kg treated SiHa xenografts by immunohistochemistry to confirm our immunoblot results. One control specimen was excluded due to tissue disaggregation, which interfered with membrane staining and image analysis (Supplementary Fig. S6B). The remaining specimens revealed, as by immunoblotting, a 40% reduction in FAK phosphorylation (Fig. 5C).

To examine the consequences of short-term Hsp90 inhibition, we initiated a lung metastasis model (Fig. 6A). Progression of lung disease was monitored by microCT. We observed a significant
survival advantage in the 17-DMAG-treated group versus control. Most mice were euthanized because of terminal lung disease. Figure 6B and Supplementary Fig. S7A and B show representative microCT images. By autopsy, we also detected four tumor-embolic kidney infarcts (three in controls and one in 17-DMAG) and two cases of bowel ischemia (one in control and one in 17-DMAG; Supplementary Fig. S7C). Two controls developed hind-leg paresis presumably due to peripheral emboli because no brain abnormality was found. Eight treated mice lived to the end of the experiment without signs of disease.

Finally, we addressed whether chronic administration of 17-DMAG could suppress tumor growth and metastasis. DsRed2 fluorescent SiHa cells were used for easier detection of metastases from orthotopic xenografts. 17-DMAG was given as a total of 15 treatments and all mice were killed on day 33. There was a 64% decrease in mean tumor weight in the 17-DMAG group (Fig. 6C) but no change in body weight, suggesting that the treatment was well tolerated (Fig. 6D). Seven of 10 controls but none of the treated mice had detectable metastases. Histology confirmed a total of 10 metastases [4 retroperitoneal deposits partially with adjacent lymph node, 3 metastases to the diaphragm, and 1 each in liver, stomach, and kidney (Supplementary Fig. S8)].

**Discussion**

FAK has emerged as an attractive therapeutic target, particularly in the context of tumor invasion and metastasis (2, 5). Its overexpression occurs as an early event during tumorigenesis.
and has been described in different cancer entities including SCC of mucosal surfaces (24, 26–30). Increased FAK expression in precursor lesions and frankly invasive cervical cancer is an intriguing finding because an inverse relationship between p53 and FAK has been reported, which is consistent with p53 instability due to human papillomavirus E6 viral protein as essential etiologic factor (31, 32).

Ochel and colleagues first described FAK as a Hsp90 client (7), and recent research has highlighted the dependence of the kinase on intact Hsp90 chaperone action in vitro (23). However, few data on FAK inhibition in cervical cancer and the potential of Hsp90 inhibitors to target FAK function in vivo are available to date. Thus, we examined the consequences of compromised FAK signaling using FRNK and 17-DMAG in different models of cervical cancer.

FRNK has been used extensively to elucidate FAK functions (33, 34), and based on its conditional expression, van Nimwegen and colleagues (35) showed that FAK was required for the early phase of lung metastasis formation. Thus, transient inhibition of FAK at specific stages of the metastatic cascade might interrupt a process that is the most frequent cause of death in the cancer host. To pursue this path, we first determined the FAK expression in our own patient cohort, which showed a significant increase of FAK in HGSIL and cancer compared with nonneoplastic epithelium. Stable expression of FRNK in SiHa, a cell line with epithelial-to-mesenchymal transition characteristics, did not affect proliferation in monolayers but suppressed anchorage-independent growth. This is in line with previous reports that found no change of in vitro or even in vivo growth with FRNK depending on cell context (33, 35, 36). Decreased anchorage-independence in SiHa-FRNK is likely a consequence of altered FAK signaling as shown previously in classic experiments with FAK⁻/⁻ cells (37). Enlarged focal adhesions and stress fiber formation are potentially due to a decreased Rac activity downstream of p130Cas, which may shift the balance toward Rho/ROCK (38).

Changes consistent with these observations were also found after pharmacologic Hsp90 inhibition.

![Image](image.png)

Figure 5. FAK inhibition decreases xenograft growth and can be achieved with 17-DMAG in vivo. A, FRNK inhibits subcutaneous xenograft growth (2 × 10⁶ cells inoculated; n = 5 per group; **, P < 0.01; ***, P < 0.001) due to interference with FAK signaling.

B, immunoblots of orthotopic xenografts (n = 5 per group) treated with different 17-DMAG doses orally or intraperitoneally (i.p.) show a significant FAK Y397 suppression and Hsp70 induction in SiHa at 30 mg/kg intraperitoneal compared with control (values normalized to SiHa control [dotted lines]). C, FAK Y861 immunohistochemistry confirms FAK dephosphorylation by 40%. Representative images for control and treatment.

High-performance liquid chromatography results and sections of all specimens are shown in Supplementary Fig. S6.
with 17-DMAG. Although essentially correlative, our data support the notion of FAK as an important client within the focal adhesion complex. Because Hsp90 function is inextricably intertwined with the stability of a host of clients, destabilization of other promigratory molecules may contribute to the observed effect. However, it has been argued that the pleiotropism of Hsp90 inhibitors may result in a more sustained drug response; indeed, a compromised FAK function might less likely be bypassed by an increased Src activity in that context (39). Preclinical and clinical testing of small-molecule FAK inhibitors will provide further mechanistic insights into this matter.

17-DMAG significantly decreased but did not abolish FAK phosphorylation in our xenografts. Yet, we hypothesized that a short-term treatment during a critical phase of tumor progression, that is, escape from anoikis and extravasation, could be effective in preventing the metastatic colonisation of a target organ. Indeed, 17-DMAG treatment resulted in a survival advantage and lower disease burden consistent with the proposed role of FAK during the early phase of the metastatic process (35). This is encouraging considering the short plasma half-life of 17-DMAG, which might limit the exposure of circulating cells to effective drug concentrations (40). Although Hsp90 and extracellular Hsp90 undoubtedly contribute to cell migration and metastasis (16, 41), there has recently been some controversy due to prometastatic effects of Hsp90 inhibition observed in other models (42, 43). For 17-DMAG, there are only few reported studies that address antimetastatic activity based on different models reflecting parts of the metastatic cascade (44, 45). Thus, we tested 17-DMAG in a spontaneously
metastasising SiHa orthotopic xenograft model and were able to show suppressed primary tumor growth and abolished metastasis. Although our results show efficacy against xenograft growth and metastasis in SiHa, they do not strictly distinguish between these two processes. However, during this work, we also generated a FRNK-expressing variant of the keratinizing cell line ME180 using the same expression system. Although equivalent levels of FAK and FRNK were determined in monolayer cultures of SiHa and ME180, the latter cell line did not show a delayed xenograft growth in response to FRNK. Also, the number of metastases from orthotopic ME180 xenografts did not lower significantly after chronic 17-DMAG treatment despite a 54% decreased mean tumor size (data not shown). A lower FAK phosphorylation (Fig. 5b, lane 1 versus lane 6), likely due to lower FAK expression in xenografts (Supplementary Fig. S9), was noted for ME180 and left us to speculate that Hsp90-independent mechanisms may play a role in these xenografts. Indeed, FAK has been linked to the acquisition of epithelial-to-mesenchymal transition features (46, 47). By contrast, amoeboid invasion is likely independent from FAK (48).

While this work was in progress, the clinical development of 17-DMAG by Kosan Biosciences (June 2008: Bristol-Myers Squibb) was terminated. Several fully synthetic Hsp90-inhibitory compounds currently under development are expected to have better side-effect profiles (14). An enhancement of cervical cancer cell radiosensitivity by Hsp90 inhibition has been shown previously (49) and should be further pursued using those compounds. Notably, Hsp90-inhibitory and heat shock-modulating effects have recently also been recognized for cisplatin (50).

In summary, we show here that FAK is a potential therapeutic target in metastatic SCC of the uterine cervix, the function of which can be compromised by pharmacologic inhibition of Hsp90 both in vitro and in xenografts. Interference with FAK function suppressed tumor growth and metastatic colonization in two different model systems of poorly differentiated SCC. Additional work is needed to elucidate the function(s) of Hsp90 during tumor progression and to examine the efficacy of its inhibitors in the context of cancer with epithelial-to-mesenchymal transition features.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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