ORIGINAL ARTICLE

Fangchinoline as a kinase inhibitor targets FAK and suppresses FAK-mediated signaling pathway in A549

Bingyu Guo, Jingyuan Su, Tingting Zhang, Kaiwen Wang, and Xiaoming Li

Institute of Neurology, General Hospital of Shenyang Military Command, Shenyang, Liaoning, China

Abstract

Background: Fangchinoline as a novel anti-tumor agent has been paid attention in several types of cancers cells except lung cancer. Here we have investigated the effect of fangchinoline on A549 cells and its underlying mechanism.

Purpose: The purpose of this work was to study the effect of fangchinoline on A549 cells.

Methods: Four lung cancer cell lines (A549, NCI-H292, NCI-H446, and NCI-H460) were exposed to varying concentrations (10–40 μmol/l) of fangchinoline to observe the effect of fangchinoline on the four lung cancer cell lines and to observe the changes of the lung cancer cell on proliferation, apoptosis, and invasion.

Results: Fangchinoline effectively suppressed proliferation and invasion of A549 cell line but not NCI-H292, NCI-H446, and NCI-H460 cell lines by inhibiting the phosphorylation of FAK (Tyr397) and its downstream pathways, due to the significant differences of Fak expression between A549 and the other three cell lines. And all FAK-paxillin/MMP2/MMP9 pathway, FAK-Akt pathway, and FAK-MEK-ERK1/2 pathway could be inhibited by fangchinoline.

Discussion: Fangchinoline effectively suppressed proliferation and invasion of A549 cell line by inhibiting the phosphorylation of FAK (Tyr397) and its downstream pathways.

Conclusion: Fangchinoline could inhibit the phosphorylation of FAKp-Tyr397, at least partially. Fangchinoline as a kinase inhibitor targets FAK and suppresses FAK-mediated signaling pathway and inhibits the growth and the invasion in tumor cells which highly expressed FAK such as A549 cell line.

Keywords

FAK, fangchinoline, lung cancer cell, phosphorylation, signaling pathway

Introduction

Focal adhesion kinase (FAK) is involved in the regulation of many cellular processes, including cell survival and death, proliferation, and migration [1]. FAK as a cytoplasm non-receptor tyrosine kinase, belonging to the tyrosine kinase super family, also called as PTK II. FAK can integrate signals from integrin, growth factors, and mechanical stress, activate the PI3K-Akt and MEK-ERK1/2 pathway, and regulate cell growth. In addition, FAK is also involved in extracellular matrix (ECM)/integrin-mediated signaling pathways which are tightly linked to the migration [2,3]. In cancer cells, combined with paxillin [4] and the matrix metalloproteinases (MMP) [5], FAK has been shown to regulate cell migration and invasion through distinct pathways by promoting the dynamic regulation of focal adhesion and peripheral actin structures [6–8]. After integrin activation, FAK exhibits increased kinase activity through phosphorylation at its Tyr397 site [9]. Indeed, FAK activity was found to be up-regulated in cancer cell lines and tissue lysates obtained from patients with metastatic disease [10]. Phase I clinical trials showed that FAK kinase inhibitors which inhibit FAK phosphorylation on Tyr (397) significantly suppressed a variety of tumors [11,12]. Consistent with these views, FAK is an important target in cancer therapy.

Fangchinoline is a bis-benzylisoquinoline alkaloid with a complex structure and has been identified as a new compound, sharing structural features with tetrandrine, another compound with a wide spectrum of anti-tumor activity in various cancer cells [13]. The potent anti-tumor activity of tetrandrine has been extensively reported with its proposed mechanism of inducing G1/S and G2/M arrest and stimulating apoptotic cell death [14–16]. However, the report of the anti-tumor activity of fangchinoline and its underlying mechanism is little. There are experiments showing that fangchinoline inhibits cell proliferation via Akt/GSK-3beta/CyclinD1 signaling and induces apoptosis in breast cancer cell lines and induces autophagic cell death via p53/sestrin2/AMPK signaling in human hepatocellular carcinoma cells [13,17–19].

Here we report that fangchinoline could effectively suppress the proliferation and the invasion of lung cancer cells A549, promoting its early apoptosis. More importantly, we provide a novel mechanism that fangchinoline as a kinase inhibitor targets FAK, the important tyrosine kinase which promotes tumor cell survival and invasion, by suppressing the
Materials and methods

Cell culture

Human lung cancer cell lines NCI-H292, NCI-H446, NCI-H460, A549, and HEK293 cells (as the control cells) were cultured in DMEM (Invitrogen, Waltham, MA) supplemented with 10% fetal calf serum (Invitrogen, Waltham, MA) at 37°C in incubator with humidified atmosphere of 5% CO2 and 95% air.

MTT assays

Human cancer cells (1 × 10⁴/well) were plated in 0.1 ml of the medium containing 10% FBS in 96-well plates; 24 h later, the medium was removed and replaced with 0.1 ml medium containing the indicated concentrations of fangchinoline and incubated for 12, 24, 36, 48, and 60 h. At the end of the incubation, the capability of cellular proliferation was measured by the modified tetrazolium salt-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. For this, 0.01 ml of MTT solution (5 mg/ml in PBS) was added to each well. After 4 h incubation at 37°C, the medium was replaced with 0.15 ml DMSO. After 15 min incubation at 37°C, the optical densities at 490 nm were measured using a Microplate Reader (BIO-RAD, Hercules, CA).

Cell-cycle analysis by flow cytometry

A549 cells were incubated with the indicated concentrations of fangchinoline for 24 h. After incubation, the cells were collected, washed with PBS, and then suspended in a staining buffer (10 μg/ml propidium iodide, 0.5% Tween 20, 0.1% RNase in PBS). The cells were analyzed using a FACS Vantage flow cytometer with the CellQuest acquisition and analysis software program (Becton Dickinson and Co., San Jose, CA). Gating was set to exclude cell debris, doublets, and clumps.

Cell migration and invasion assay

Migration and invasion assays were performed using modified Boyden chambers with polycarbonate nucleopore membrane. Precoated filters (6.5 mm in diameter, 8-μm pore size, Matrigel 100 μg/cm²) were rehydrated with 100 μl medium. Then, 1 × 10⁵ cells in 100 μl serum-free DMEM supplemented with 0.1% bovine serum albumin were placed in the upper part of each chamber, whereas the lower compartments were filled with 600 μl DMEM containing 10% serum. After incubating for 18 h at 37°C, non-invaded cells were removed from the upper surface of the filter with a cotton swab, and the invaded cells on the lower surface of the filter were fixed, stained, photographed, and counted under high-power magnification.

Cell apoptosis

Cell apoptosis was determined by ANNEXIN-V-FITC apoptosis detection kit instructions; the specific steps are as follows: cells were washed twice with cold PBS, then resuspended with binding buffer cells at a concentration of 1 × 10⁶ cells/ml. About 5 μl of ANNEXIN-V-FITC and 10 μl of PI were added. The cells were incubated in dark at room temperature for 15 min. Finally, 400 μl binding buffer was added to each tube and the apoptosis rate was measured by flow cytometry within 1 h.

Hoechst 33258 staining

A549 cells were incubated with the indicated concentrations of fangchinoline for 24 h. After incubation, cells were fixed with 4% polyoxymethylene, then washed twice with PBS, incubated with 10 μg/ml hoechst 33258 for 5 min at room temperature, and then washed with PBS for three times. Cells were observed with fluorescence microscope.

Mitochondrial membrane potential

About 1 × 10⁵ cells were cultured in 6-well plates for the assay. Then cells were collected, centrifuged, and re-suspended in 0.5 ml DMEM medium. The cells were washed twice in staining buffer and then incubated in 0.5 ml JC-1 staining buffer at room temperature in dark. Flow cytometry was used to determine the fluorescence intensity of the red/green ratio semi-quantitatively.

Reverse transcription and quantitative real-time PCR

Total cellular RNA from DMSO and fangchinoline-treated A549 cells were extracted after 24 h using TRIzol (Invitrogen, Waltham, MA) according to the protocol of the manufacturer. One microgram of total RNA was reverse transcribed to cDNA in a total volume of 20 μl system using a RT reaction kit (Promega, Madison, WI). Real-time PCR was performed using an Mx 3000P real-time PCR system (Applied Biosystems, Waltham, MA) according to the instruction of the manufacturer and SYBR Premix Ex Taq (TaKaRa, Berkeley, CA) was used as a DNA-specific fluorescent dye. PCR was carried out for 50 cycles of 95°C for 10 s and 60°C for 30 s. Primer sequences for detection of mRNA expression were synthesized as in Table 1.

All the reactions were repeated at least three times. Gene expression levels were calculated relative to the housekeeping β-actin by using Stratagene Mx 3000P software (Agilent Technologies Inc., Santa Clara, CA).

Transfection of sh-RNA

To stably silence FAK, cells were transfected with a set of shRNA constructs against human FAK, pRS-shFAK (Shanghai GeneChem Company, Shanghai, China), and then selected with puromycin (1.5 μg/ml). The pRS vector was used as a control.

Western blot analyses

To determine the expression of protein, whole cell extracts (lysate) were prepared from 1 × 10⁶ cells in lysis buffer (20 mM Tris pH7.4, 250 mM sodium chloride, 0.1% TritonX-100, 2 mM EDTA, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, 4 mM sodium orthovanadate, and 1 mM DTT), and 60 μg of the protein was
resolved on 10% SDS-polyacrylamide gels. After electrophoresis, the proteins were electrotransferred to nitrocellulose filters, the membrane (Amersham, Pittsburgh, PA) was blocked with 5% nonfat dry milk in TBS-T (20 mM Tris, pH 7.6, 137 mM NaCl, 0.05% Tween-20) for 1 h at room temperature, and the proteins were probed with specific antibodies – CyclinD1, CDK4, CDK6, MMP2, MMP9 (Bioworld, Dublin, OH), paxillin, phospho-paxillin (Tyr118), phospho-MEK-1 (Ser298), MEK-1, ERK1/2, phosphor-ERK1/2 (Thr202/Tyr204), paxillin, phospho-CDK4, CDK6, MMP2, MMP9 (Bioworld, Dublin, OH), and then cell-cycle analysis was performed. Fangchinoline at 40 μmol/l markedly inhibited the level of FAK p-Tyr397 (Figure 1F). While, after fangchinoline treatment, there was little difference in the degree of phosphorylation of FAKp-Tyr397 between A549/wild-type cell line and A549/FAK knockdown cell line (Figure 1G), indicating that fangchinoline could inhibit the phosphorylation of FAKp-Tyr397, at least partially.

Fangchinoline inhibits the proliferation of A549 by inhibiting FAK-MEK-ERK1/2 pathway

To further investigate the mechanisms of fangchinoline inhibiting the growth of lung cancer cells, A549 cells were exposed to various concentrations of fangchinoline for 24 h, and then cell-cycle analysis was performed. Fangchinoline prominently induced a dose-dependent increase in the percentage of cells in the G1 phase and a decrease in the

### Table 1. Primer sequences.

| Name     | Forward primer (5’ → 3’) | Reverse primer (5’ → 3’) |
|----------|--------------------------|--------------------------|
| Cyclin D1| CCGAGGAGCTGCTGCAAATGGAGCT| TGAATCTGGCCGGTCTATTGCGG |
| CDK4     | CAGAGCTTTAGCAGGACGCT     | GGCACGACACAAATTTCAG     |
| CDK6     | AGTCTGATTAACCACGCGCCG   | CCTCGAAGCGGAAGTCCTCAA   |
| Caspase9 | GTTAGGCCACCAATTGCCAGCC   | TCAGACATCTGCTGTTGAC     |
| Caspase3 | TGTTAGGGCGGTGTGAGAGTT   | GGTCCACATACGATCTCAC     |
| Bcl-2    | GGTGACCTGCTGGGAGGATGG   | GACCCAGGGTTAATGGAAGA    |
| Bax      | ACGTGAGCCGATGTTCTCAA    | GTTCAATTGGTGACCTAAC     |
| MMP2     | GCGATCTGGGGGTCTTTAAACAT | TGACGACAACAGGTTCGAG     |
| MMP9     | CGAGGCTCTTCTCAGTTACCAG  | TTGTATCCGCGAACAAGTGGCT |
| β-Actin  | TCGTGCAGTGACATTAAGAGG  | ATGCCAGGTTACATGGTGT     |

### Results

**Fangchinoline inhibits the phosphorylation of FAK (Tyr397)**

NCI-H292, NCI-H446, NCI-H460, A549, and HEK293 cells were used to detect the inhibitory effect of fangchinoline on growth of these cells. As shown in MTT assay, fangchinoline significantly inhibited the proliferation of A549 cells in a concentration-dependent manner than the other cell lines (Figure 1B and C). Since FAK is known as a kind of cytoplasmic non-receptor protein-tyrosine kinases, it could promote cell survival, inhibit cell adhesion, and promote anchorage-independent growth [1]. The expression level of FAK in lung cancer cell lines was examined. Interestingly, the level of FAK was dramatically higher in A549 cells than that in the other lung cancer cells and HEK-293 cells (Figure 1D), indicating that FAK might be targeted by fangchinoline and be involved in fangchinoline-induced growth inhibition of lung cancer cells. Then the inhibitory effect of fangchinoline on FAK kinase was examined by Kinase Glo Luminescent assay. Data showed that fangchinoline inhibited FAK activity with an IC50 value of 33 μmol/l (Figure 1E). Furthermore, we examined whether fangchinoline inhibited the FAK kinase activity in A549 cells. Fangchinoline at 40 μmol/l markedly inhibited the level of FAKp-Tyr397 (Figure 1F). While, after fangchinoline treatment, there was little difference in the degree of phosphorylation of FAKp-Tyr397 between A549/wild-type cell line and A549/FAK knockdown cell line (Figure 1G), indicating that fangchinoline could inhibit the phosphorylation of FAKp-Tyr397, at least partially.

**Fangchinoline inhibits the proliferation of A549 by inhibiting FAK-MEK-ERK1/2 pathway**

To further investigate the mechanisms of fangchinoline inhibiting the growth of lung cancer cells, A549 cells were exposed to various concentrations of fangchinoline for 24 h, and then cell-cycle analysis was performed. Fangchinoline prominently induced a dose-dependent increase in the percentage of cells in the G1 phase and a decrease in the
Figure 1. Fangchinoline inhibits the phosphorylation of FAK (Tyr397). (A) The structure of fangchinoline. (B) MTT assay tested the effect of indicated concentrations of fangchinoline on different cells. Results represent the mean ± SD of three experiments done in triplicate. (C) The inhibition rate of 80 μmol/l of fangchinoline at 24 h on different cell lines. Data are shown as mean ± SEM. Statistical significance was analyzed by one-way ANOVA. Scale bars: 50 μm. **p < 0.01 versus HEK293; ***p < 0.001 versus HEK293; #p < 0.05 versus A549; ##p < 0.01 versus A549. (D) Total proteins of the different cell lines were extracted to detect FAK level. (E) FAK kinases were pre-incubated with the indicated concentrations of fangchinoline for 1 h, then Kinase Glo assay were performed and inhibition rate was calculated, linear fit curve was drawn with an equation of y = -0.032x + 0.673. (F) A549 cells were treated with DMSO alone or indicated concentration of fangchinoline for 24 h, proteins were extracted and subsequently analyzed by immunoblotting with anti-FAKp-Tyr397 and anti-FAK. (G) A549/wild type, A549/NC (negative control) and A549/sh-FAK cell line were treated with 20 μmol/l of fangchinoline for 24 h, proteins were extracted and subjected to western blot analyzing with anti-FAK and anti-FAKp-Tyr397.
S phase compared with the control (Figure 2A), indicating that fangchinoline arrests A549 cells at the G1 phase of the cell cycle. Since CyclinD1 and CDK4/6 are key regulators in the G1 phase of the cell cycle, here we examined the indicated regulators expression level in fangchinoline-treated cells. Western blot analysis showed that exposure of A549 to 10/20/40 μmol/l fangchinoline for 24 h dramatically decreased protein expression of CyclinD1 and CDK2/4 (Figure 2B), indicating that fangchinoline arrests cells at the G1 phase and then suppresses cells growth via down-regulated CyclinD1
and CDK4/6. Furthermore, real-time RT-PCR showed that expression of Cyclin D1 and CDK4/6 in A549 was down-regulated at the mRNA level after exposure to fangchinoline (Figure 2C). Western blot analysis showed that ERK1/2p-Thr202/Tyr204 and MEK-1p-Ser298 and FAKp-Tyr397 were down-regulated in a dose-dependent manner, but without affecting the total expression of them (Figure 2D).

**Fangchinoline induce the apoptosis of A549 by inhibiting FAK-Akt pathway**

To inquire whether fangchinoline induces apoptosis of A549 cells, we detected the apoptosis rate by Hochest 33258 staining. To observe the fangchinoline-induced apoptotic nucleus of A549 cells, we detected the apoptosis rate by Hochest 33258 staining and AV-PI. Hochest33258 staining was performed to observe the change of the mitochondrial membrane potential, JC-1 was used to stain the cells and then analyzed them through flow cytometry. Results showed that the number of cells with lost ΔΨm increased after treatment with fangchinoline (Figure 3C). Then real-time RT-PCR showed that the expression of Caspase3, Caspase9, and Bax in A549 was upregulated at mRNA level and Bcl-2 was downregulated at mRNA level after exposure to fangchinoline (Figure 3D). Furthermore, the expression of apoptosis regulators was examined by western blot. The expression of Bcl-2 was obviously decreased and the levels of Caspase3, Caspase9, and Bax were increased in fangchinoline-treated A549 cells, and Aktp-Ser308 was dramatically downregulated without changing the expression of Akt (Figure 3E).

**Fangchinoline represses the migratory and invasive potential of A549 by inhibiting FAK-paxillin/MMP2/ MMP9 pathway**

Inhibitory effect of fangchinoline on migration and invasion of NCI-H292, NCI-H446, NCI-H460, and A549 cells were analyzed by transwell assay (with or without matrigel). Results showed that fangchinoline significantly decreased invasion and migration potential of lung cancer cells A549 (Figure 4A–D) in a dose-dependent manner, but weakly decreased invasion and migration potential of NCI-H292, NCI-H446, and NCI-H460 cells (Supplementary figure SA–F). Real-time RT-PCR showed that expression of MMP2 and MMP9 in A549 was down-regulated at mRNA level after exposure to fangchinoline (Figure 4E). Western blot analysis showed that exposure of A549 to fangchinoline (10/20/40 μmol/l) for 24 h dramatically decreased levels of MMP2, MMP9, and paxillinp-Tyr118 but have little effect on paxillin (Figure 4F). The anti-tumorigenic effect of fangchinoline on A549 cells was further illustrated in vivo in a nude mouse xenograft. On the day of sacrifice (day 16), fangchinoline treatments at the given doses resulted in about 55.89% tumor suppression (Figure 4G). These results indicated that fangchinoline effectively suppressed proliferation and invasion of A549 by inhibiting FAK-paxillin/MMP2/MMP9 pathway (Figure 4H).

**Discussion**

Fangchinoline is a novel anti-tumor agent with little known of its mechanisms on tumor cells. Fangchinoline inhibits cell proliferation and induces apoptosis in several cancer cell lines, such as MDA-MB-231 and HepG2 cells [13,17–19]. But the effects of fangchinoline on lung cancer cells have not been reported. Our data showed that fangchinoline treatment inhibited the proliferation, migration, and invasion of A549 cells in a concentration-dependent manner but have little effect on NCI-H292, NCI-H446, and NCI-H460 cell lines, and the control cell line HEK293. In elucidating the mechanism, we found that it exhibited high-expression of FAK in A549 cell lines but only little expression in NCI-H292, NCI-H446, and NCI-H460 cell lines, and the control cell HEK293. Interestingly, we found that fangchinoline could inhibit FAK activity in vitro by Kinase Glo Luminescent assay and suppress the phosphorylation of FAK in A549 cells, which implying fangchinoline-targeted FAK in tumor cells highly expressing FAK and inhibit the proliferation, migration, and invasion of them. We also had confirmed that fangchinoline had anti-tumorigenic effects of A549 cells in nude mouse xenograft.

FAK is considered as a key regulator in cancer cell signaling. It was reported that FAK kinase inhibitor could suppress the proliferation of prostate cancer cells [12] and promote the H125 cells apoptosis [11]. In our study, activated FAK level was markedly decreased at 40 μmol/l concentration of fangchinoline in A549. And in vitro, kinase activity of FAK was dramatically decreased in a dose-dependent manner by fangchinoline and IC50 values got by Kinase Glo assay. All these results indicated fangchinoline acted as a novel, potent kinase inhibitor of FAK and suppressed A549 cell line proliferation via FAK.

It was reported that FAK receives signals from integrins or fibronectin and activates PI3K-Akt pathway to regulate cell growth [21]. FAK controls cell proliferation by Ras–Raf–MAPK pathway and regulates cell cycle by activating ERK1/2 [22,23]. Our data showed that fangchinoline arrested A549 cells at the G1 phase by decreasing the protein levels of CyclinD1, CDK4/6, which act as key regulators of the G1-S check point. We also found that fangchinoline promotes A549 apoptosis by decreasing Bcl-2 level and increasing Caspase3 and Caspase9 expressions. At the same time, CyclinD1 and Caspase3 are the downstream proteins of FAK-MEK-ERK1/2 pathway and FAK-Akt pathway [21,23]. All these indicated that fangchinoline adjusts A549 growth by FAK-MEK-ERK1/2 and FAK-Akt pathways in a FAK-dependent manner.

In addition to the effect on cell proliferation, we also demonstrated the cell migratory and invasive inhibition effect of fangchinoline on A549. It was reported that overexpression of FAK in cells enhances cell migration [24], combined with paxillin [25] and the matrix metalloproteinases (MMP) [5]. Our results showed that fangchinoline significantly suppressed the migratory and invasive ability of A549 in parallel with down-regulation of paxillinp-Tyr31, MMP9 and MMP2.
Figure 3. Fangchinoline induces the apoptosis of A549 through inhibiting FAK-Akt pathway. (A) A549 cells were pre-incubated with fangchinoline for 24 h, then cells were stained with Hoechst33258, and observed with fluorescence microscope. (B) A549 cells were pre-incubated with fangchinoline for 24 h then cells were treated with ANNEXIN-V-FITC apoptosis detection kit and analyzed with FCAS. The experiment was repeated for three independent times. (C) A549 cells were pre-incubated with fangchinoline for 24 h, then stained with JC-1 and analyzed by flow cytometry. Cell percentage of the Q4 phase indicating mitochondrial membrane potential lost of three experiments was analyzed. (D) A549 cells were treated with DMSO alone or indicated concentration of fangchinoline for 24 h, and the mRNA expression was detected by real-time RT-PCR, results represent the mean ± SD of three experiments done triplicate. (E) A549 cells were treated with DMSO alone or indicated concentration of fangchinoline for 24 h, the protein expression was detected by western blot.
Figure 4. Fangchinoline represses the migratory and invasive potential of A549 by inhibiting FAK-paxillin/MMP2/MMP9 pathway. (A) A549 cells were pre-incubated with fangchinoline for 24 h, transwell assay without matrigel was performed. (B) Cells were counted and results represent the mean ± SD of three experiments. (C) A549 cells were pre-incubated with fangchinoline for 24 h, and transwell assay with matrigel was performed. (D) Cells were counted and results represent the mean ± SD of three experiments. (E) A549 cells were treated with DMSO alone or indicated concentrations of fangchinoline for 24 h, and the mRNA expression of MMP2 and MMP9 was detected by real-time RT-PCR, results represent the mean ± SD of three experiments done in triplicate. (F) A549 cells were treated with DMSO alone or indicated concentration of fangchinoline for 24 h, the protein expression was detected by western blot. (G) Photographic of tumors from control solvent and fangchinoline-treated nude mice on the day of sacrifice (day 16) and statistics tumor weights in the graph. (H) The ideograph showed that fangchinoline effectively suppressed proliferation and invasion of A549 by inhibiting FAKp-Tyr397, then inhibiting its downstream pathways.
So it was reasonable to speculate that fangchinoline inhibited cell invasion and metastasis by FAK-paxillin/MMP2/MMP9 pathway.

Nowadays, there are several compounds which could inhibit FAK activity, such as PF-573228, PF-562271, and some synthetic glycosphingolipids [11,12]. But they have not been used in clinical trials. However, fangchinoline which we identified as the novel kinase inhibitor of FAK was derived from the dry roots of Stephaniae tetrandrine S that has been used in cardiovascular disease clinical therapy. So it is expected that fangchinoline might offer a novel therapeutic strategy for advanced metastatic lung cancer.

In summary, fangchinoline was identified to be capable of inhibiting FAK kinase activity and its downstream signaling pathways and suppressing FAK-mediated A549 behaviors including growth, migration, and invasion. Although these results are warranted further testing in experimental models in vivo, results presented in our current study have broadened the scope of the exploitation and application about FAK inhibitors and may offer a novel therapeutic strategy for advanced metastatic lung cancer.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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