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Knockdown of focal adhesion kinase reverses colon carcinoma multicellular resistance

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Chemotherapy resistance in solid tumors is broad and encompasses diverse unrelated drugs. Three-dimensional multicellular spheroids (MCSs) are a good model for studying *in vitro* drug resistance. In the current study, we investigated the role of focal adhesion kinase (FAK) in 5-fluorouracil (5-FU) chemoresistance in colon carcinoma MCS culture cells. The expression of FAK was inhibited significantly by specific small hairpin RNA targeting FAK. The suppression of FAK expression did not affect the growth of spheroid cells. However, silencing of FAK combined with 5-FU treatment significantly decreased the 50% inhibitory concentration (IC_{50}) of 5-FU and markedly increased the population of apoptosis cells, which was associated with the reduction of the levels of Akt and nuclear factor–kappa B (NF-κB). Moreover, knockdown of FAK could inhibit tumor growth and increase the sensitivity of the tumor to 5-FU in the nude mouse xenograft. These results indicate that while not affecting cellular proliferation in the absence of 5-FU, RNA interference targeting FAK potentiated 5-FU-induced cytotoxicity *in vitro* and *in vivo*, and partially reversed multicellular resistance, which may contribute to its chemosensitizing effect through efficiently suppressing Akt/NF-κB activity. (*Cancer Sci* 2009; 100: 1708–1713)

Chemotherapy resistance in solid tumors including colon carcinoma is broad and encompasses diverse unrelated drugs, suggesting more than one mechanism of resistance. Three-dimensional (3D) MCSs are a good model for studying *in vitro* drug resistance.[1] Due to their 3D architecture, multicellular tumor spheroids mimic avascular tumor areas comprising the establishment of diffusion gradients, reduced proliferation rates, and increased drug resistance.[2] Compared with monolayer culture, tumor cells cultured as multicellular aggregates (spheroids) exhibit much higher levels of resistance to chemotherapeutic agents, a phenomenon known as MCR. MCR is produced because multicellular aggregation (spheroid) is formed with a cell-cell connection when cultured in 3D.[3] However, the molecular mechanisms of the MCR of colon cancer cells are not fully known.

Focal adhesion kinase (FAK), a non-receptor tyrosine kinase, is involved in the control of cell–extracellular interactions such as spreading, migration, motility, and survival.[4–6] Moreover, FAK is an important regulator of cellular signaling, migration, apoptosis, and cell cycle progression.[4] FAK has been shown to become overexpressed in tumor cells,[7–12] providing survival signals that suppress apoptosis in human pancreatic adenocarcinoma cells.[13] Overexpression of FAK has been reported to protect cells from stressors including ionizing radiation,[14] hydrogen peroxide exposure,[15] and chemotherapeutic treatment.[16] Moreover, a potential role for FAK in chemoresistance has been demonstrated;[17] however, the mechanisms are not fully understood.

Here we used a MCS culture model to study the role of FAK in the MCR of colon carcinoma to chemotherapeutic agents. We sought to determine whether suppressing expression of FAK would increase the susceptibility of colon carcinoma cells to 5-FU. Here we show, for the first time, that suppression of FAK expression by RNAi increases the susceptibility of colon carcinoma MCS culture cells to 5-FU *in vitro*. We also demonstrate that FAK RNAi can suppress FAK expression *in vivo* and that this treatment enhances the therapeutic efficacy of 5-FU in a nude mouse xenograft model of colon carcinoma. These findings identify FAK as not only a determinant of colon carcinoma chemoresistance, but also a promising therapeutic target.

Materials and Methods

Monolayer cell culture. HT29 human adenocarcinoma cells were preserved by the Department of Oncology, Southwest Hospital of the Third Military Medical University (Chongqing, China). Cells were maintained in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated FCS (Hangzhou Sijiqing Biological Engineering Materials, Hangzhou, China), 100 IU/ml penicillin, and 100 μg/mL streptomycin. Cells were kept at 37°C in a humidified atmosphere of 5% CO_2/95% air.

Multicellular spheroid (MCS) cell culture. MCS cells were initiated by seeding 5×10^4 single HT29 cells into 75 cm^2 flasks, previously coated with 2% L-agarose. The flasks were placed on magnetic plates (Integra Biosciences, Chur, Switzerland) at 60 r.p.m. in 5% CO_2 and 37°C humidified atmosphere. When spheroids reached 100–150 mm in diameter after 3–4 days' culture, they were used for experiments.

Antibodies and reagents. Monoclonal anti-FAK (4.47) antibody was obtained from Upstate Biotechnology (Waltham, MA, USA), anti-phospho-FAK (Y397) antibody was from BD Biosciences (San Diego, CA, USA), Akt antibody was from Cell Signaling (Beverly, MA, USA), and NF-κB antibody and monoclonal β-actin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Recombinant plasmids construction. One specific pair of oligonucleotides[18] with a short hairpin and its negative control sequence were designed and synthesized based on FAK cDNA sequences (GenBank accession no. L13616), then inserted into pGenesil-l vector (a gift from Dr You-Zhao Jiang, Department of Endocrine, Southwest Hospital of the Third Military Medical University, Chongqing, China) to generate the recombinant plasmids. Oligonucleotides were designed with the following primers: FAK shRNA, forward: 5′-GATCCCCCCACCTGGGCCAGTATTATT TCAAGAGAATAATACTGGCCCAGGTGGTTTTTGGAAA-3′, reverse: 3′-GGGGGTGGACCCGGTCATAATAAAGTTCTCTT ATTATGACCGGGTCCACCAAAAACCTTTTCGA-5′; and control shRNA: forward: 5′-GATCCCCGACGTGGGACTGAAG GGGTTTCAAGAGAACCCCTTCAGTCCCACGTCTTTTTGG AAA-3′, reverse: 3′-GGGCTGCACCCTGACTTCCCCAAAGTT CTCTTGGGGAAGTCAGGGTGCAGAAAAACCTTTTCGA-5′,

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bearing no homology with any relevant human genes. All constructs were verified by DNA sequencing.

Recombinant plasmids transfection. The 1×10^5 HT29 monolayer culture cells were plated into 24-well plates and allowed to adhere for 24 h. The recombinant plasmid transfection was performed with Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The stably transfected cells were selected in a medium containing 1000 μg/mL geneticin G418 for 3 weeks. Then the cells used post-experiment were divided into three groups: untreated group, control shRNA group, and FAK shRNA group.

Total RNA preparation and reverse transcriptase reaction. Total RNA of three group cells was extracted with Tripure reagent (Roche, Basel, Switzerland) according to the manufacturer's instructions. cDNA was synthesized from 1 μg of total RNA using RT reagents (TaKaRa) as per the manufacturer's instructions. cDNA was stored at −20°C.

Quantitative real-time PCR analysis. Real-time PCR was performed with SYBR *Premix Ex Taq* Perfect Real Time supermix (TaKaRa Dalian) according to the manufacturer's instructions. Amplification was performed using the Opticon TM 2 Real-Time PCR Detection System (MJ Research, Waltham, MA, USA). The PCR program was followed by: (i) an initial denaturing step at 95°C for 30 s; (ii) 45 cycles, with one cycle consisting of denaturation at 95°C for 15 s, annealing at 59°C for 10 s, and extension at 72°C for 10 s; and (iii) a final extension step at 72°C for 5 min. All assays were identical in primer concentration and annealing temperature. Primers were synthesized by SBS Bio Technologies (Beijing, China). FAK, forward: 5′-ACATTATTGGCCACTGT GGATGAG-3′, reverse: 5′-GGCCAGTTTCATCTTGTTGATGAG-3′, producing a 125-bp fragment. β-Actin, forward: 5′-CTTAGTTGC GTTACACCCTTTCTTG-3′, reverse: 5′-CTGCTGTCACCTT CACCGTTCC-3′, producing a 159-bp amplicon. As an internal control and for normalization purposes, the level of mRNA expression for β-actin was measured simultaneous to FAK expression for each sample. The results are presented as relative amounts of FAK mRNA *versus* the β-actin mRNA value for the same sample.

Western blotting analysis. The 2×10^6 cells of three groups were harvested and the total protein was extracted respectively with radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitors PMSF. 50 ng total protein was subjected to 8% SDS-PAGE and the resolved proteins were transferred electrophoretically to PVDF membranes (Millipore, Bedford, MA, USA). Membranes were blocked overnight with 5% skimmed milk in TBS buffer containing 0.05% Tween-20 at 4°C. Membranes were then incubated with antibodies to FAK (1:2500), FAKp (1:1000), Akt (1:1500), NF-κB (1:500), and β-actin (1:1500) for 3 h at 25°C followed by incubation with their respective secondary antibody for 1 h. Membranes incubated with ECL (Pierce, Rockford, IL, USA) for 1 min were exposed to the film for 1–5 min.

Cell growth. Three groups of cells (1×10^5 cells per well) seeded in 24-well plates coated with 2% agarose were cultured as MCSs for 12 days at 37°C. Three wells of spheroid cells per group were trypsinized for cell counting using a hemocytometer. Cells were counted every other day.

Determination of cytotoxicity. Tetrazolium salt, MTT, was used to assess 5-FU cytotoxicity. 5×10^3 cells per well were seeded into a 96-well plate, MCSs were cultured and maintained in drug-free medium in for 72 h, and 5-FU was added at final concentrations from 0 to 500 μg/mL. After 72 h of incubation, cell viability was determined by dissociation of spheroids, 3-h incubation with MTT 2.5 mg/mL (Sigma, St. Louis, MO, USA), and measurement of optical densities at 490 nm conducted using a microplate spectrophotometer (Bio-Rad, Hercules, CA, USA). The surviving fraction of cells was determined by dividing the mean absorbance values of treated samples by the mean absorbance of untreated control samples. Each independent experiment was performed three times. The IC_{50} of 5-FU was calculated from these data.

Table 1. Five-day *in vivo* treatment regimen ($n = 5$ per group)

Group	Vaccinated cells	Treatment
1	Untransfected cells	0.9% isotonic Na chloride
2	Transfected control shRNA cells	0.9% isotonic Na chloride
3	Transfected FAK shRNA cells	0.9% isotonic Na chloride
4	Untransfected cells	5-FU (20 mg/kg)
5	Transfected control shRNA cells	5-FU (20 mg/kg)
6	Transfected FAK shRNA cells	5-FU (20 mg/kg)

5-FU, 5-fluorouracil; FAK, focal adhesion kinase.

Apoptosis detection. 5-FU-induced apoptosis of MCSs was detected by FCM. Following exposure to 200 μg/mL 5-FU for 72 h, three groups of MCS cultured cells were washed and resuspended in 0.5 mL PBS, fixed in 70% alcohol solution for 24 h, and 50 μg/mL propidium iodide was added. Cells were incubated for 30 min on ice and then analyzed by FCM (FACScan; Becton-Dickinson, San Jose, CA, USA). All observations were reproduced three times in independent experiments.

Nude mouse xenograft model. Male 5-week-old BALB/c nu/nμ mice, weighing 17–21 g, and being specific pathogen-free were obtained from the Animal Laboratory Center of the Third Military Medical University (Chongqing, China). Mice were housed in micro-isolator cages with autoclaved bedding in a specific pathogen-free environment. They received water and food ad libitum.

To determine the effect of FAK RNAi in combination with 5-FU on nude mouse xenograft growth, mice were vaccinated subcutaneously 1×10^7 three group cells respectively. Seven days following vaccination, mice were allocated randomly to six treatment groups (Table 1). Each nude mouse in groups 1–3 received 200 μL 0.9% isotonic Na chloride by intraperitoneal injection once a day, for 5 consecutive days. Each nude mouse in groups 4–6 received 200 μL 5-FU (20 mg/kg) by intraperitoneal injection once a day, for 5 consecutive days. After 5 days of treatment, animals were observed for signs of tumor growth, activity, feeding, and pain for 2 weeks. Then necropsy was performed, primary tumors were excised and immediately weighed. Tumor growth inhibition was calculated.

Statistical analysis. Data are presented as mean ± SD and analyzed using SPSS13.0 software. Differences between groups were analyzed for statistical significance using one-way ANOVA, and considered significant when $P < 0.05$.

Results

Knockdown of FAK by specific shRNA. To knockdown the expression of FAK, a DNA construct was generated to express shRNA targeted against FAK (FAK shRNA) and cell clones stably expressing shRNA-FAK were created. First, western blotting was used to investigate the effect of FAK shRNA on FAK levels in monolayer culture cells. As shown in Figure 1a, the expression of FAK was evidently inhibited by FAK shRNA compared with the non-functional control shRNA. Further, shRNA FAK also specifically silenced the expression of FAK in MCS culture cells (Fig. 1b). Quantification analysis showed that more than 75% of FAK was knockdown in both monolayer and MCS culture cells (Fig. 1c). In addition, the level of FAK that was phosphorylated at tyrosine 397 in both monolayer and MCS culture cells was also decreased by FAK shRNA (Fig. 1a,b). Moreover, the FAK mRNA level in MCS culture cells was detected to be significantly reduced by real-time RT-PCR (Fig. 1d), which demonstrated that FAK shRNA efficiently and specifically inhibited the expression of FAK at a post-transcription level.

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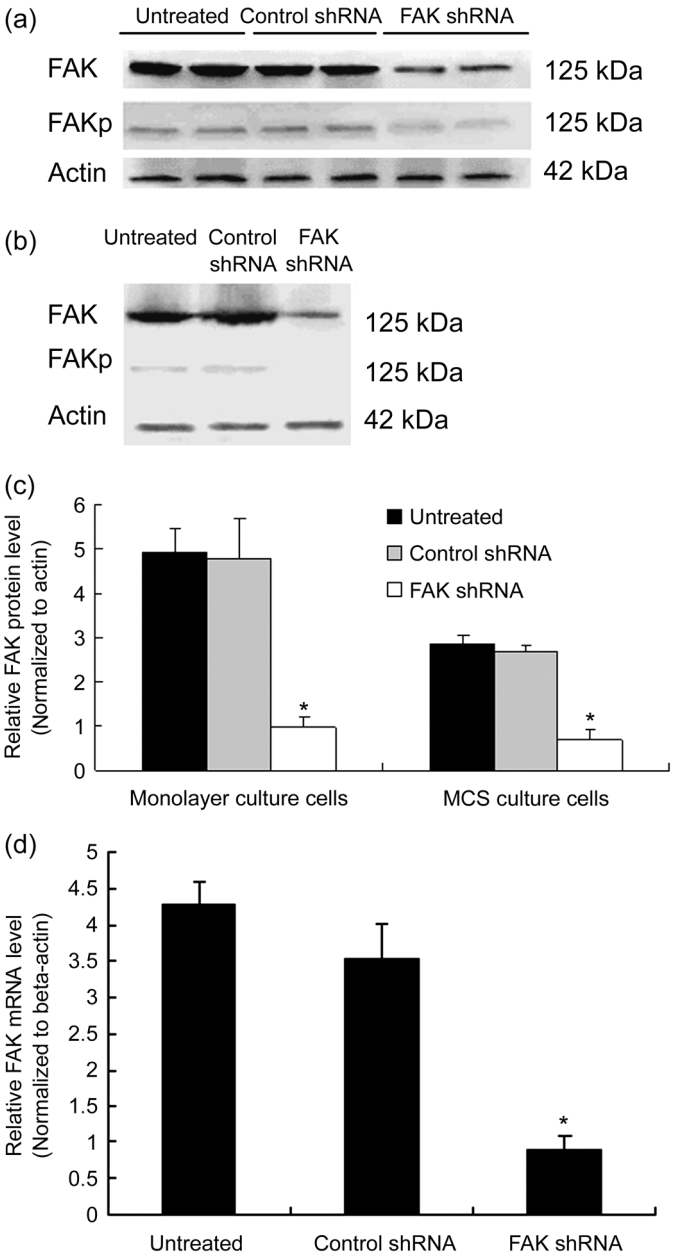

Fig. 1. Knockdown of focal adhesion kinase (FAK) by FAK shRNA in monolayer and multicellular spheroid MCS culture cells. (a and b) Representative western blotting for FAK and FAKp following treatment with control shRNA or FAK shRNA in monolayer (a) and MCS (b) culture cells. Control shRNA did not significantly affect FAK expression compared to untreated cells, whereas FAK-specific shRNA induced marked suppression of FAK expression. Actin expression was unaffected by either shRNA treatment. (c) Relative FAK protein level in monolayer and MCS culture cells by densitometry. Values are means (± SD) of three independent determinations. *$P < 0.05$ *versus* control shRNA. (d) Relative FAK mRNA level in MCS culture cells by quantitative real-time PCR. Values are means (± SD) of three independent determinations. *$P < 0.05$ *versus* control shRNA.

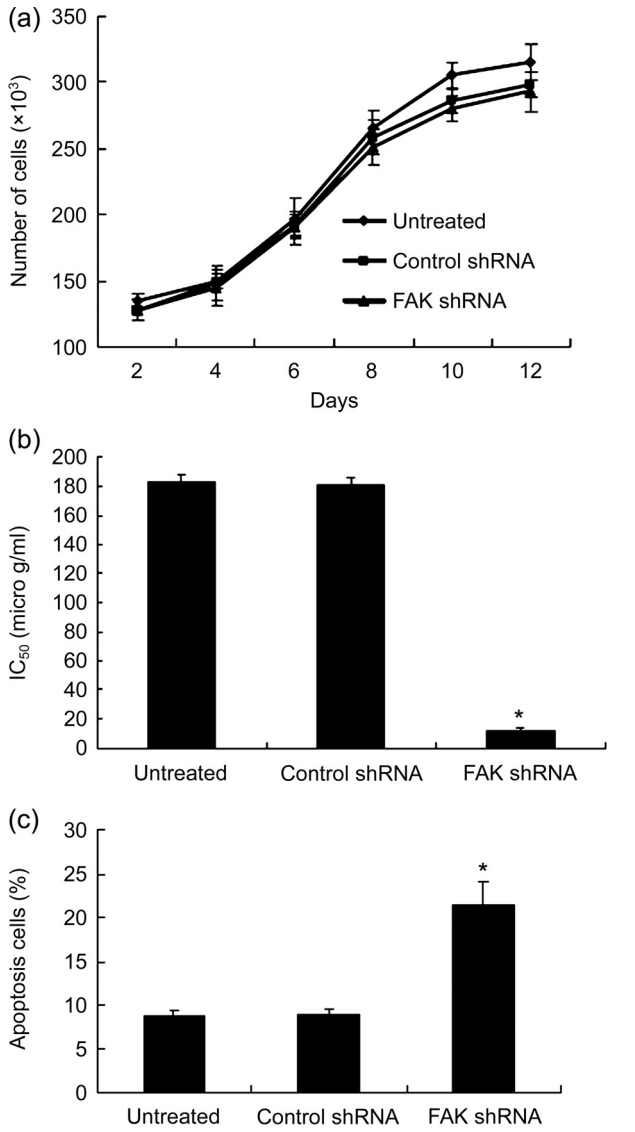

Fig. 2. Focal adhesion kinase (FAK) shRNA promoted 5-fluorouracil (5-FU)-induced cytotoxicity *in vitro*. (a) Cell viability was not affected by FAK shRNA. (b) 5-FU IC_{50} was decreased by FAK shRNA. (c) The apoptosis of MCS culture cells following 5-FU exposure was increased by FAK shRNA. *$P < 0.05$ *versus* control siRNA.

Focal adhesion kinase (FAK) shRNA does not affect cellular proliferation *in vitro*. We tested the effect of suppressing FAK expression on the proliferation of HT29 cells in MCS culture. As shown in Figure 2a, neither control nor FAK shRNA induced any change in cell proliferation compared with untreated cells.

Focal adhesion kinase (FAK) gene silencing potentiates 5-FU-induced apoptosis in MCS culture cells. Next, we sought to determine the effect of FAK shRNA administration on the 5-FU IC_{50} of colon carcinoma MCS culture cells *in vitro*. Cells were exposed to 0–500 μg/mL 5-FU for 72 h of incubation. The IC_{50} was determined at this time by MTT assay. Treatment with control shRNA had no effect on the IC_{50} of the colon carcinoma cells. In contrast, FAK shRNA induced a marked decrease in the 5-FU IC_{50}, which was 11.85 ± 2.29 μg/mL compared with 181.20 ± 5.21 μg/mL in control shRNA-treated cells (Fig. 2b). In addition, FAK shRNA treatment induced a large increase apoptosis of MCS culture cells following 5-FU treatment detected by flow cytometry (Fig. 2c).

Focal adhesion kinase (FAK) gene silencing suppresses Akt activity in HT29 MCS cells. Increased PI3-K/Akt pathway activity has been reported following FAK overexpression,[19] and simultaneous inhibition of FAK and Src has been reported to decrease Akt activity in colon cancer cell lines with high inherent Src kinase activity.[20] For these reasons we determined the effect of FAK shRNA on the Akt level. Following FAK shRNA treatment, the Akt level was significantly suppressed (Fig. 3). In addition, the level of NF-κB was also decreased by the knockdown of FAK (Fig. 3).

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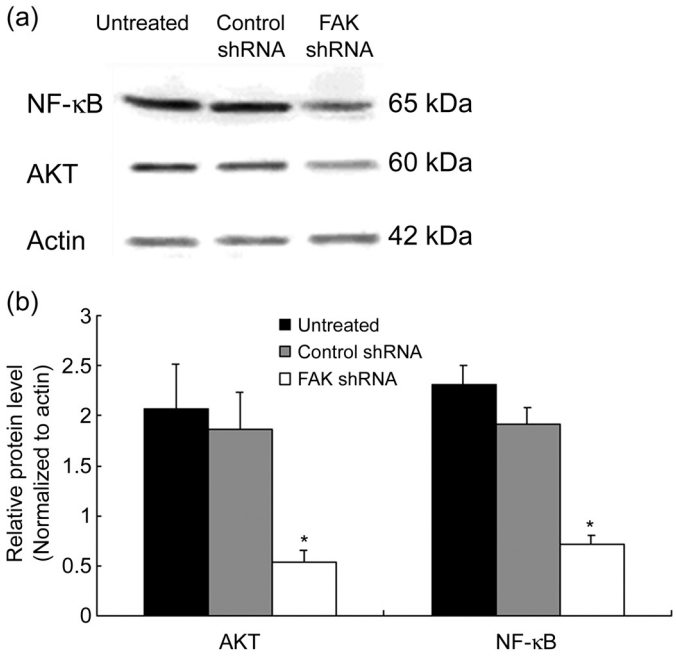

Fig. 3. Effect of silence of focal adhesion kinase (FAK) on the expression of nuclear factor–kappa B (NF-κB) and Akt in multicellular spheroid (MCS) culture cells. The protein levels of NF-κB and Akt in MCS culture cells treated with control shRNA or FAK shRNA was detected by western blotting with specific antibodies (a). Actin expression was unaffected by either shRNA treatment. Relative protein level in MCS culture cells was quantified by densitometry (b). Values are means (± SD) of three independent determinations. *$P < 0.05$ *versus* control shRNA.

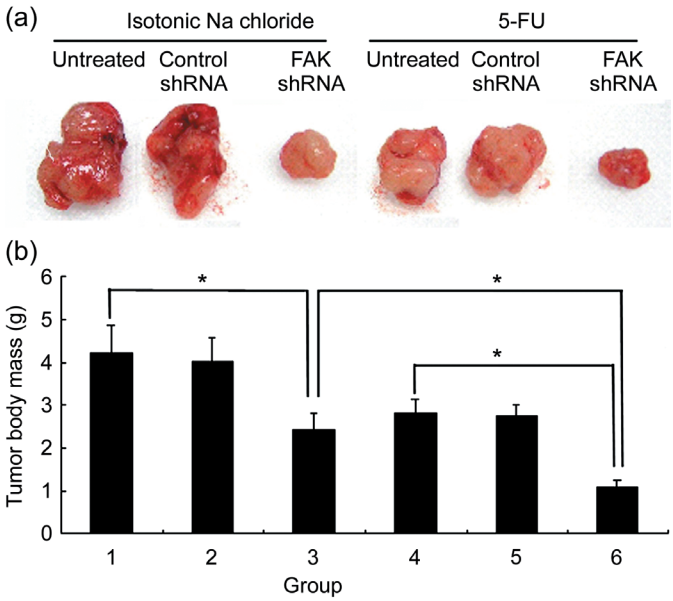

Fig. 4. Focal adhesion kinase (FAK) shRNA promotes 5-fluorouracil (5-FU)-induced cytotoxicity in the nude mouse xenograft model. (a) Representative figures of the tumor body. (b) Quantification analysis of tumor body mass. $n = 5$, *$P < 0.05$.

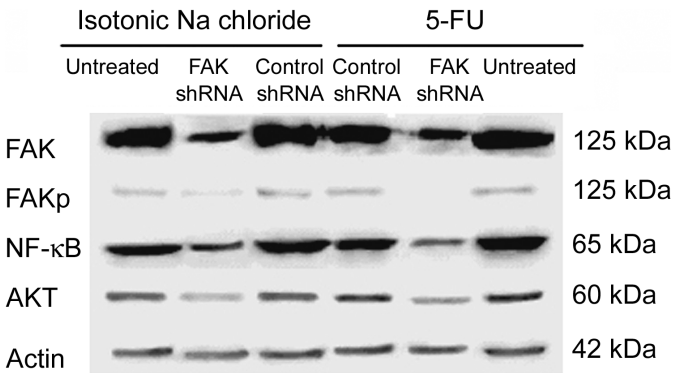

Fig. 5. Effect of focal adhesion kinase (FAK) shRNA on the expression of FAK, FAKp, nuclear factor–kappa B (NF-κB), and Akt in the nude mouse xenograft. The protein levels of FAK, FAKp, NF-κB, and Akt treated with control shRNA or FAK shRNA in the presence of 5-FU or not was detected by western blotting with specific antibodies. The expression of FAK and FAKp was knocked down by FAK shRNA, which evidently decreased the expression of NF-κB and Akt compared with control shRNA.

Short hairpin RNA (shRNA) targeting FAK promotes 5-FU cytotoxicity in the nude mouse xenograft model. In view of these findings *in vitro*, we tested the efficacy of shRNA targeting FAK as an *in vivo* chemosensitizing strategy in a nude mouse xenograft model. As shown in Figure 4, control shRNA (group 2) did not affect the growth of tumor, whereas mean tumor mass in mice treated with FAK shRNA (group 3) was significantly decreased compared that in mice treated with control shRNA. The efficacy of tumor growth inhibition with 5-FU treatment was better than that of isotonic Na chloride treatment. Moreover, the mean tumor mass of group 6 which was treated with the combination of 5-FU and FAK shRNA was significantly reduced to 25% of that in group 2, with a tumor growth inhibition of 75%. It also equated to a tumor growth inhibition of 60% ($P < 0.05$) compared to mice treated with the combination of 5-FU and control shRNA (group 5), and 55% ($P < 0.05$) compared to mice treated with FAK shRNA alone (group 3). Therefore, shRNA targeting FAK attenuated FAK expression, synergistically promoted 5-FU-induced cytotoxicity, and inhibited tumor growth in the nude mouse xenograft model. In addition, compared with the control groups, no significant body weight change or other toxicity was observed in the treatment groups (data not shown).

To explore the molecule mechanisms of suppressing expression of FAK leading to tumor growth inhibition in the nude mouse xenograft, the tumor protein expression of FAK, FAK pY397, Akt, and NF-κB were detected by western blotting (Fig. 5). We found that after the expression of FAK was suppressed, the levels of FAKp, Akt, and NF-κB were simultaneously significantly decreased.

Discussion

Colon cancer cells show increased resistance to chemotherapeutic agents compared to breast cancer cells.[21] Until now, the molecular mechanisms of the drug resistance of colon cancer cells have not been fully known. In the past, studies on drug resistance have adopted the monolayer cell culture model, and their clinical effects are not usually valid, especially when used in solid tumors like colon cancer. When grown as 3D MCS structures, tumor cells can reproduce some features of solid tumors such as the presence of a subpopulation of cells with a restricted oxygen supply, responsible for a decrease in cell proliferation and development of necrotic regions, and can acquire an additional MCR to apoptosis that may mimic the chemoresistance found in solid tumors. In this study we used the MCS culture model to study the MCR of colon cancer to chemotherapy.

Focal adhesion kinase (FAK) is a protein of 125 kDa that localizes to focal adhesions and activates tyrosine phosphorylation in response to integrin clustering. Several studies have demonstrated up-regulation of FAK expression in colorectal cancer,[7,22–24] and it

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appears that colon cells up-regulate expression of FAK at early stages of tumorigenesis, even before carcinoma has been detected.[7] In addition, FAK was identified to be involved in cellular adhesion, growth, and motility, and the invasion of colon cancer cells.[25–27] FAK plays an important role in colon cancer progression, and may therefore be a good specific target for human colon cancer.

It has been reported that siRNA has advantages over oligonucleotide-based techniques both *in vitro* and *in vivo*,[28,29] and quantitative analyses of the gene-silencing effect revealed that pshRNA has more durable effects than siRNA.[30] So we chose pshRNA gene-silencing as a means to mediate FAK expression. In this study, we have demonstrated that the recombinant vectors of RNA interference targeting FAK were constructed and transfected successfully, and that the expression of the target gene *FAK* was inhibited effectively.

Focal adhesion kinase (FAK) is an important mediator of cell survival and apoptosis. We have shown that suppressing expression of FAK caused increased apoptosis in colon cancer cell lines. Some data show that the down-regulation of FAK by antisense oligonucleotides combined with 5-FU chemotherapy results in a greater loss of adhesion and greater apoptosis in melanoma cells than treatment with either agent alone.[31] It has been reported that antisense oligonucleotides targeting FAK have also been shown to increase the sensitivity of breast cancer cells to the camptothecin derivative topotecan.[32] Here, we observed that suppression of FAK expression by shRNA interference did not affect colon carcinoma cellular proliferation, but promoted apoptosis in suspended MCS culture cells in the absence of 5-FU (data not shown). These results indicate that suppression of FAK expression can promote anoikis induced by inadequate or inappropriate cell substrate adhesion when cells were cultured as suspended MCS, which is consistent with reports on human pancreatic adenocarcinoma cells.[33] Combined with 5-FU chemotherapy, suppression of FAK expression could induce a greater apoptosis in suspended MCS culture cells in this study. These results imply that FAK plays an important role in cell apoptosis. Over-expression of FAK provides protection of colon cancer cell lines from apoptosis. Moreover, our results show that suppressing FAK expression can inhibit tumor growth in the nude mouse xenograft, and synergistically increase the sensitivity of tumors to 5-FU *in vivo*, which was associated with decreased FAKpY397 activity. These data imply that FAK mediates colon carcinoma MCR.

Tyrosine 397 is an autophosphorylation site of FAK, which is a critical component in downstream signaling[34] and important for the anti-apoptotic activity of FAK and activation of Akt pathways. FAK activation leads to stimulation of the MAP kinase cascade and of PKB/Akt/NF-κB. The suppressing expression of FAK could be alterations of these signaling pathways. In addition, FAK induces PI3K/Akt dependent anti-apoptotic pathways.[19] In this study, we found that FAK expression in MCS cells were suppressed notably after RNAi targeting FAK, and FAKpY397/Akt/NF-κB expression was simultaneously significantly suppressed, which implies that the signaling pathway FAK/Akt/NF-κB plays an important role in colorectal cancer progression and may be involved in the MCR of colon cancer. In addition to the promotion of apoptosis, cell adhesion alternation may be another important factor that affects the sensitivity of MCS culture cells to 5-FU. FAK can modulate cell adhesion strengthening via integrin activation.[35] Suppressing FAK expression could weaken the cell adhesion of the spheroid cells, so that MCS culture cells may be not so easy to aggregate and form into MCSs. Indeed, we observed that the forming rate of MCSs was slowed down by FAK shRNA (data not shown), which may induce an increase in the exposure efficiency of spheroid cells to 5-FU.

Finally, we were very interested by our observation that knockdown of FAK did not affect the growth of spheroid cells *in vitro*, while it reduced the tumor body mass in the absence of 5-FU *in vivo*. The reason may partially be that suppression of FAK can promote suspended MCS culture cells' anoikis induced by inadequate or inappropriate cell substrate adhesion as seen in human pancreatic adenocarcinoma cells.[33] In addition, the MCS model imitates avascular microenvironment of the solid tumor[36] and FAK mainly plays a role in anti-apoptosis in the *in vitro* system. But the *in vivo* system FAK plays a further role: it can control vascular pathological processes including angiogenesis,[37] which promotes tumor growth. Thus, knockdown of FAK can inhibit the angiogenesis of tumors and reduce the tumor body mass *in vivo*.

In summary, suppression of FAK expression by shRNA significantly increases the sensitivity of colon cancer cells to 5-FU both *in vivo* and *in vitro*. This is the first demonstration of the suppression of FAK expression by RNAi *in vitro* to study the MCR of colon cancer to chemotherapy using a MCS culture model. The signaling pathway FAK/Akt/NF-κB is one of mechanisms of FAK mediating the MCR of colon cancer. Thus, these data demonstrate that FAK is an important determinant of malignant cellular behavior and a promising target for the therapeutic intervention of colon cancer.

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Abbreviations

5-FU	5-fluorouracil
DMEM	Dulbecco's modified Eagle's medium
ECL	Enhanced chemiluminescence
FAK	Focal adhesion kinase
FCM	Flow cytometry
FCS	Fetal calf serum
IC_{50}	Inhibitory concentration
MCR	Multicellular resistance
MCS	Multicellular spheroid
NF-κB	Nuclear factor–kappa B
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI3K	Phosphoinositide 3-kinase
pshRNA	Short hairpin RNA expressing plasmid
RNAi	RNA interference
RT-PCR	Reverse transcription–PCR
shRNA	Short hairpin RNA

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