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The Inhibition Effect of Caveolin-1 on PANC1 Human Pancreatic Tumor Growth *In vitro* and *In vivo**

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Abstract Caveolin-1 is a transmembrane protein and essential structural constituent of the caveolae membrane. Caveolin-1 has been involved in multiple cellular functions and oncogenesis. To investigate the roles of caveolin-1, stable transfectants were established in PANC1 pancreatic adenocarcinoma cells which had up-regulated caveolin-1 expression. The plasmid pCI-neo-cav-1 and its corresponding empty vector (pCI-neo) were transfected into PANC1 cell lines. The expression of caveolin-1 in these three cell lines was determined by RT-PCR and Western blot. Cell cycle phase distribution was determined by flow cytometry. The colony formation ability of tumor cells was detected by anchorage-independent growth assay. Cell migration and invasion were assayed in MilliCell chambers. Xenograft tumor models in nude mice were developed. Immunohistochemistry was used to characterize Ki-67 levels in residual tumors, and apoptosis was evaluated by TUNEL technique. Caveolin-1 overexpression inhibited PANC1 cell proliferation by arresting the cell cycle in the G0/G1 phases and also markedly reduced the capacity of the cells to form colonies in soft agar. Additionally, caveolin-1 overexpression dramatically inhibited cells to invade and migrate. Importantly, *in vivo* experiments demonstrated that overexpression of caveolin-1 resulted in significant growth inhibition of the xenograft pancreatic tumors. Immunohistochemistry analysis demonstrated both a marked decrease in the number of proliferating tumor cells and an increase in the number of apoptotic tumor cells in PANC1/cav-1 xenograft tumors. The results provide an initial demonstration that caveolin-1 can function as a tumor suppressor rather than as a tumor promoter in PANC1 cells.

Key words caveolin-1，cell cycle，migration，invasion，xenograft
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Caveolin-1 is a transmembrane protein that forms a hairpin-like structure with both the NH_2 and COOH termini facing the cytoplasmic side of caveolae structures in the plasma membrane[1]. As a result of this structure and localization, caveolin-1 is able to interact directly with numerous signaling molecules through its scaffolding domain that comprises amino acid residue 82～101, which generally results in inhibition of these molecule transduction activities [2]. Thus, caveolin-1 can act as a scaffolding molecule to sequester and organize multi-molecular signaling complexes[3], some of which have been implicated in cell proliferation or apoptosis[4–5].

The human caveolin-1 gene maps to a location in chromosome 7q31.1/D7S522 that is closely localized to a known "tumor suppressor" locus, leading to a proposition that caveolin-1 could function as a tumor suppressor[6–7]. Conflicting results have been reported in the literature concerning the role of caveolin-1 in regulating cell proliferation/apoptosis[8–9]. For example, caveolin-1 expression in small cell lung cancer significantly inhibited soft agar colony formation, whereas caveolin-1 appears to be required for the cell survival and growth of non-small cell lung cancer [10]. Additionally, accumulating evidence shows that

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caveolin-1 plays a positive regulatory role in insulin and insulin-like growth factor-1 signaling[11]. Thus, the regulation roles for caveolin-1 in cancer proliferation still remain obscure. Pancreatic cancer is one of the most aggressive human malignancies and currently the fourth leading cause of cancer related death in the industrialized world [12]. Despite developments in surgical and non-surgical therapies, significant improvements in long-term survival rates have not been realized, making development of new therapeutic strategies is critically important. Considering caveolin-1 plays an important regulatory role in many signaling pathways and cancer proliferation, the relationships between caveolin-1 and the invasion of pancreatic cancer should be an object of study. Numerous experimental results have shown that caveolin-1 up-regulated in pancreatic adenocarcinoma [13] and in invasive intraductal papillary-mucinous tumors (IPMTs) [14] suggesting overexpression of caveolin-1 may be involved at early stage of pancreatic carcinogenesis. Recent studies show that siRNA-mediated knockdown of caveolin-1 expression results in an enhanced radiosensitization of pancreatic cancer cells[15–16] and that overexpression of caveolin-1 in pancreatic carcinoma may contribute to tumor progression with a negative prognostic predictor following surgery[17]. In contrast to these findings, it has been reported that overexpression of caveolin-1 inhibits the invasion of pancreatic carcinoma cell line SW1990, whereas the knockdown of caveolin-1 in Bxpc3 cells promotes cell invasion[18]. Also, Lin *et al*.[19] have demonstrated that caveolin-1 expression in primary pancreatic adenocarcinoma cells restricts migration and invasion, while loss of caveolin-1 expression leads to RhoC-mediated migration and invasion in metastatic pancreatic cancer cells, implying a biphasic action in response to expression of caveolin-1 in pancreatic cancer progression. While it is clear that caveolin-1 may play an important role in the development of pancreatic cancer, few papers described the growth characteristics of pancreatic cells transfected with caveolin-1 *in vitro* and *in vivo*. In the study, we overexpressed caveolin-1 in pancreatic cancer cell line (PANC1) and found that PANC1/cav-1 cells exhibited a reduced migration and invasion *in vitro* as well as a decreased ability to form xenograft tumors *in vivo* following inoculation into nude mice.

1 Materials and methods

1.1 Cell lines, plasmids and reagents

Monoclonal antibody (mAb) against caveolin-1 was purchased from Zymed Laboratories Inc. Monoclonal anti-human Ki-67 antibody was obtained from DakoCytomation. In Situ Cell Death Kit (TUNEL assay) was purchased from Roche Applied Science. Dulbecco modified Eagle medium (DMEM), Fetal Bovine Serum (FBS), L-glutamine, Trypsin-EDTA, and penicillin/streptomycin were purchased from Life Technologies Inc. G418 and Lipofectamine 2000 were purchased from Invitrogen. Matrigel was obtained from BD Biosciences. SuperScript One-Step reverse transcription polymerase chain reaction (RT-PCR) with Platinum® Taq and TRIzol® Reagent were all from Life Technologies Inc. The Human pancreatic cancer cell line PANC1 was purchased from Cell Culture Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College. The pCI-neo and pCI-neo-cav-1 plasmids were generous gifts from Dr. Eric J. Smart (Kentucky Medical School, USA). Hematoxylin, Eosin and other reagents were all from regular commercial sources with the highest available degree of purity. Female BALB/C nude mice (4 ～5 weeks old) were obtained from the animal facilities of Chinese Academy of Sciences.

1.2 Cell culture and transfections

The parental human pancreatic cancer cell line PANC1 was cultured in DMEM medium supplemented with 10% FBS, 2 mmol/L glutamine, 100 U/ml penicillin and 100 mg/L streptomycin and maintained in a humidified atmosphere of 5% CO_2 at 37℃. The plasmid pCI-neo-cav-1 and its corresponding empty vector (pCI-neo) were transfected into PANC1 cell lines using Lipofectamine 2000 according to the manufacturer's instruction [20]. The selection for transfected cells was performed using a culture medium containing 400 mg/L G418 for at least 2 weeks. After confirming caveolin-1 expression by Western blot, we then selected wells that contained only a monoclonal population for propagation. Analysis of caveolin-1 expression in some monoclonal transfectants was subsequently confirmed by RT-PCR and Western blots. We then chose a pair of monoclonal cell lines, PANC1/vector and PANC1/cav-1, for future studies.

1.3 Western blot analysis

Cell extracts were prepared and analyzed by SDS-PAGE as described previously [21]. In brief, amounts of total protein extracts were quantified using the bicinchoninic acid assay (BCA; Pierce). Cell lysates were separated by SDS-PAGE with a standard reducing condition protocol. After electrophoresis, proteins were electrotransferred onto a nitrocellulose membrane. Blots were blocked with 5% nonfat dried milk and 0.05% Tween 20 in Tris-buffered saline (10 mmol/L Tris, pH 8.0, 135 mmol/L NaCl). The membranes were incubated with the designated primary antibodies at 4℃ overnight. Horseradish peroxidase-conjugated secondary antibodies (1∶5 000 dilutions, Pierce) were used to visualize bound primary antibodies with the enhanced chemiluminescence detection system (Amersham). Experiments were repeated three times.

1.4 Reverse transcription-polymerase chain reaction

RNA from each cells were isolated with TRIzol® Reagent. To determine if caveolin-1 mRNA were expressed in cells, reverse transcription (RT)-PCR was performed using One-Step RT-PCR with Platinum® by using caveolin-1 forward primer 5′ CTA CAA GCC CAA CAA CAA GGC 3′ and caveolin-1 reverse primer 5′ AGG AAG CTC TTG ATG CAC GGT 3′[22]. 8 μl of each PCR product was electrophoresed in 1× Tris/acetate/EDTA (TAE) electrophoresis buffer on a 1.2% agarose gel, stained with ethidium bromide and detected by UV illumination. To ensure that RNA load was kept constant in all lanes, expression of glyceraldehyde-3-phosphate dehydrogenase was used as a control, using following pair of primers: 5′ ACC ACA GTC CAT GCC ATC AC 3′ and 5′ TCC ACC ACC CTG TTG CTG TA 3′.

1.5 Cell cycle analysis

We used flow cytometry to investigate effects of caveolin-1 on cell cycle distribution of PANC1 cells as described previously [23]. PANC1 transfectants were cultured in complete medium for 72 h. Then the cells were harvested and washed with ice-cold PBS. The cell suspension was fixed with ice-cold 70% ethanol and stored at –20℃ overnight. Cells were pelleted and the pellet was washed with PBS before 1×10^6 cells were resuspended in propidium iodine (PI) solution (50 mg/L in PBS, pH 7.4) containing 200 mg/L RNaseA and incubated in the dark at room temperature for 30 min. The samples were examined by flow cytometry on a Becton-Dickinson FACS-420. The data were analyzed using the Cell Quest Software[23]. Experiments were repeated three times.

1.6 Colony formation in soft agar

The colony formation ability of tumor cells was detected by anchorage-independent growth assay in 6-well plates [20]. Briefly, 1×10^5 viable cells were suspended and plated in 0.32% agar containing DMEM supplemented with 17% fetal calf serum, and layered over a 0.5% agar base with complete medium containing 20% serum. The culture plates were incubated at 37℃ in a humidified atmosphere containing 5% CO_2. Colonies were photographed at 100× magnification on the 15th day after plating in the soft agar. The number of colonies formed in soft agar was counted in three random fields of each sample. Values are represented as the $\bar{x}\pm s$, which was determined from three independent experiments.

1.7 Cell migration and invasion assays

Cell migration and invasion were assayed in MilliCell chambers (12 mm diameter with 8 μm pores; Millipore)[24]. Briefly, exponentially growing cells were trypsinized using trypsin-EDTA and washed with PBS, and then 10^5 cells in 200 μl of serum-free DMEM were seeded into duplicate chambers, either coated with (for invasion assays) or without (for migration assays) 50 μl matrigel (diluted to 1 g/L in serum free DMEM). Millicell units were placed into 24-well dishes containing 500 μl fresh DMEM medium containing 10% FBS. Cells were allowed to migrate or invade for 24 h at 37℃. Chambers were fixed in methanol for 15 min after removing the unpenetrated cell by wiping with cotton wool. Cells were stained with Hematoxylin for 10 min, rinsed in PBS, and the numbers of cells in three random fields were counted using a bright-field microscope. Data were represented as the average $\bar{x}\pm s$, which was determined from three independent experiments.

1.8 Development of xenografts in nude mice

All mice were handled according to the Guide for the Care and Use of Laboratory Animals. For inoculation into nude mice, PANC1, PANC1/vector and PANC1/cav-1 cells were washed with PBS, digested with trypsin, and then resuspended in serum-free DMEM. After centrifugation, cells were resuspended in DMEM at a concentration of 8×10^6 cells in 100 μl and injected subcutaneously into left flank of 4-week-old female BABL/C athymic (nu/nu) mice. Tumor diminutions were monitored with caliper measurement of the length and width and tumor

volume was calculated using the formula of $\pi/6 \times$ length $\times$ width^2. The mice were sacrificed about 42 days after inoculation and tumor were removed and fixed in 10% neutral buffered formalin for histologic and immunohistochemical analysis. Statistical analysis results were expressed as $\bar{x} \pm s$ from each set of 12 nude mice by three independent experiments.

1.9 Tumor immunohistochemical and histology analysis

Formalin-fixed tumors were embedded in paraffin and sectioned at 4 μm. Then deparaffinized, rehydrated, and then stained with H&E, Ki-67 immunohistochemistry or terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining. In brief, the tissue sections were mounted onto slides and stained with H&E by routine methods to confirm the presence of tumors. For Ki-67 staining, after antigen retrieval in Target Retrieval Solution(DakoCytomation) at 95℃～99℃ for 20 min, endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 30 min followed incubation for 1 h at room temperature in nonspecific protein block (5% BSA in PBS). Ki-67 was detected with primary antibody and staining was carried out by EliVision™ plus kit instructions with 3, 3-diaminobenzidine and Hematoxylin. For detection of TUNEL-positive cells, the In Situ Cell Death Detection Kit was used. Sections were pretreated with proteinase K, permeabilized with 0.1% sodium citrate buffer containing 0.1% Triton X-100 and washed with PBS three times. TUNEL staining was performed using TUNEL labeling mix and counterstaining was carried out with Hoechst 33258. Fluorescence images were captured using a Bio-Rad Radiant-2100 confocal microscope.

2 Results

2.1 Stable overexpression of caveolin-1 in PANC1 cell line

PANC1 cells were transfected with pCI +neo-caveolin-1 or pCI +neo-vector single clone were selected for clonal propagation. We generated two stable single clone cells: PANC1/cav-1 as transfectant cells overexpressing caveolin-1 and PANC1/vector as vector control cells to complement wild-type PANC1 control cells. The expression levels of caveolin-1 were detected by RT-PCR and Western-blot assay (Figure 1a and c) .The densitometry results in RT-PCR and Western-blot assay confirmed that the expression level of caveolin-1 in PANC1/cav-1 cell was about 4.2 and 3.3 fold higher than that in PANC1/vector cells (Figure 1b and d).

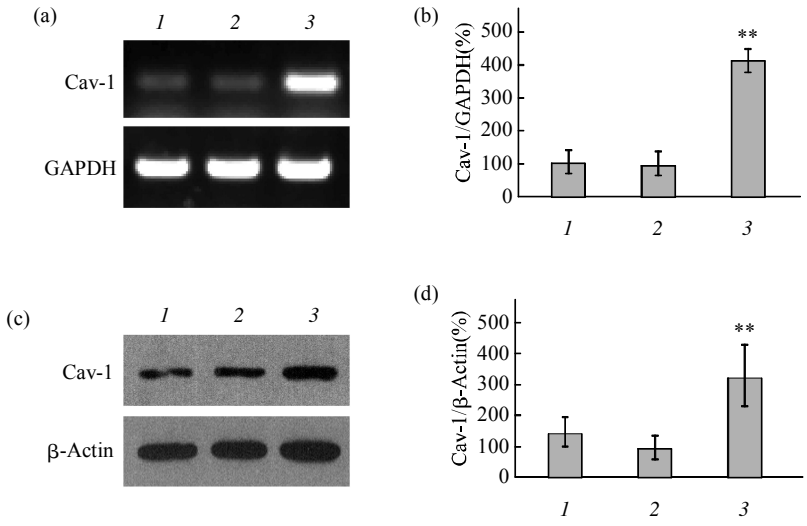
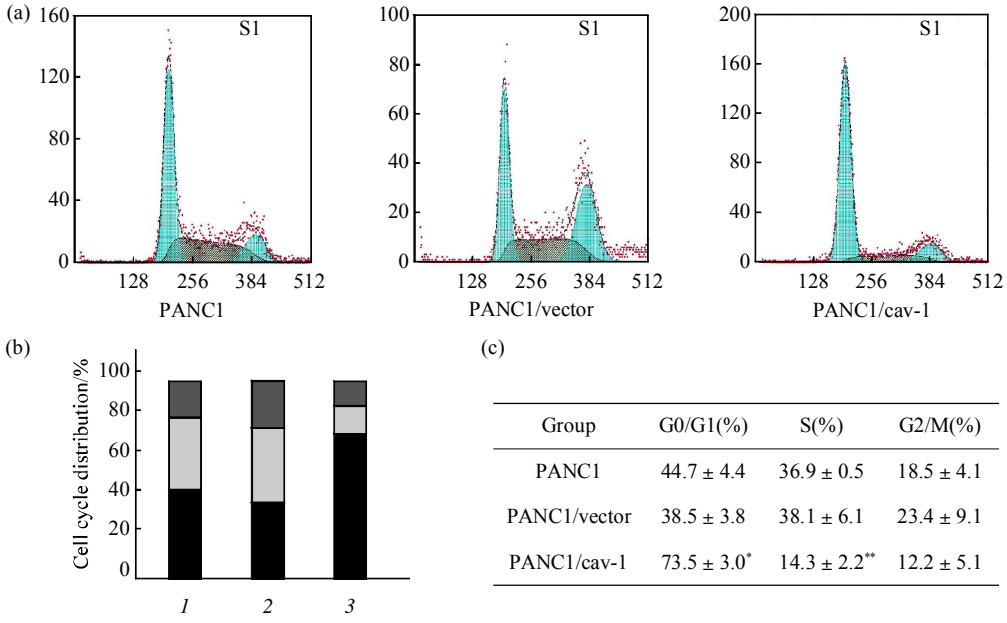


Fig. 1 Establishment of stable caveolin-1 overexpression PANC1 cells

(a) RT-PCR analysis of caveolin-1 mRNA levels in PANC1, PANC1/vector and PANC1/cav-1 cells. GAPDH mRNA levels were used as a loading control. Experiments were repeated three times. (b) Relative amounts of caveolin-1 mRNA standardized against GAPDH mRNA compared with PANC1/vector cells. $^{**}P < 0.01$. (c) Caveolin-1 expression levels were determined in PANC1 transfectants by Western blot analysis. β-Actin was used as a loading control. Experiments were repeated three times. (d) Relative amounts of caveolin-1 proteins standardized against β-actin compared with PANC1/vector cells. $^{**}P < 0.01$. *1*: PANC1; *2*: PANC1/vector; *3*: PANC1/cav-1.

2.2 Caveolin-1 overexpression arrests cell cycle at G0/G1 phase

It has been confirmed that caveolin-1 expression can inhibit cell proliferation by arresting the cell cycle at G0/G1 phase[25]. To determine whether caveolin-1 in PANC1 cells can affect cell cycle distribution, we analyzed the number of cells in G0/G1 versus S and G2/M phases of cell cycle by flow cytometry. As shown in Figure 2, PANC1/cav-1 cells undergo arrest in the G0/G1 phase of the cell cycle after differentiation (Figure 2a and b). Flow cytometry analysis in PANC1/cav-1 showed that (73.5 ± 3.0)% of cells in G0/G1 phase, whereas the percentage of cells arrested in G0/G1 phase in PANC1 and PANC1/vector cells was only (44.7 ± 4.4)% and (38.5 ± 3.8)%, respectively (Figure 2c). It is very clear that the arrest of PANC1/cav-1 cells in the G0/G1 phase of the cell cycle was significantly increased by about 91% as compared with control cells, PANC1/vector. Meanwhile, PANC1/cav-1 cells in S phase were reduced coincidentally. These results indicate that caveolin-1 expression in PANC1 cells is associated with a block of cell proliferation in the G0/G1 phase of the cell cycle.



(c)

Group	G0/G1(%)	S(%)	G2/M(%)
PANC1	44.7 ± 4.4	36.9 ± 0.5	18.5 ± 4.1
PANC1/vector	38.5 ± 3.8	38.1 ± 6.1	23.4 ± 9.1
PANC1/cav-1	73.5 ± 3.0*	14.3 ± 2.2**	12.2 ± 5.1

Fig. 2 Caveolin-1 overexpression in PANC1 cells altered cell cycle distribution

(a) Cell cycle analysis. The cells were detached and analyzed by PI staining and flow cytometry (in methods). (b) The cell cycle shown represents the averages from three independent experiments. Note that only overexpression of caveolin-1 in PANC1 cells causes G0/G1 arrest. ■: G2/M; □: S; ■: G0/G1. (c) Quantitation. Table represented the averages and SDs of three independent experiments of the cell cycle analysis shown in (a). *1*: PANC1; *2*: PANC1/vector; *3*: PANC1/cav-1.

2.3 Overexpression of caveolin-1 suppresses anchorage-independent growth of PANC1 cells

Previous studies have indicated that caveolin-1 expression negatively correlates with the cancer ability to growth in soft agar[10, 26]. Recent work confirmed that increased caveolin-1 expression in human breast Hs578T cells promotes cell growth in soft agar [20]. Here, we observed the capacity of the three types of PANC1 cells described above to form colonies in soft agar. PANC1 and PANC1/vector cells formed numerous large colonies after 15 days of growth in soft agar. PANC1 cells overexpressing caveolin-1 exhibited an obvious reduction in colony formation capacity, showing fewer colonies formed and reduced colony size (Figure 3a). These results suggest PANC1 cells colony formation is specifically inhibited by overexpression of caveolin-1.

2.4 Caveolin-1 dramatically inhibits migration and invasion in PANC1 cells

Tumor migration and invasion of basement membranes are crucial steps in the complex multistage process of metastasis[27]. Previous studies indicated that caveolin-1 expression inhibits migration and invasion in pancreatic adenocarcinoma cell, HPAF-Ⅱ. Here,

we used matrigel coating as a barrier on the top of porous filter in Boyden chambers to test the invasive behaviors of PANC1 cells. Chambers without matrigel we used to monitor the migratory characteristics of these cells. It shows that PANC1 and PANC1/vector cells have higher migration and invasion capacities than PANC1/cav-1 cells (Figure 3a). As shown in Figure 3b and 3c, expression of caveolin-1 in PANC1/cav-1 cells results in about 70% and 71% inhibition of cell migration and invasion respectively, as compared with PANC1/vector ($P < 0.01$). Our findings demonstrate that caveolin-1 is involved in two independent cellular processes, extracellular matrix dissolution and cell migration. Decreasing both the migration and invasion capacity of PANC1/cav-1 cells should significantly attenuate their metastatic capacity relative to control cell.

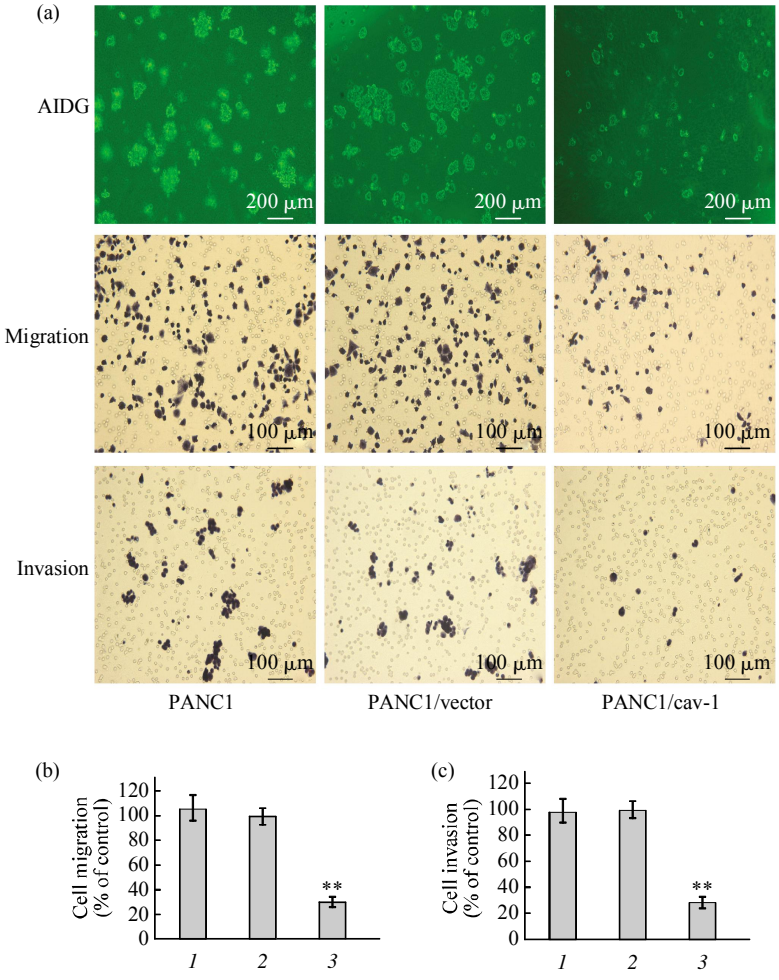


Fig. 3　Effect of caveolin-1 overexpression on anchorage-independent growth (AIDG), migration and invasion of PNAC1 cells

(a) Caveolin-1 overexpression inhibited anchorage-independent growth of PANC1. The colonies were observed after 15 days of growth in soft agar. Caveolin-1 inhibited migration and invasion of PANC1 cells. All the results were representative of three independent experiments. (b, c) The migration and invasion were quantified, respectively. The relative contents of migration and invasion were expressed as percentage of levels of PANC1/vector (control cells), respectively. Data were represented as the $\bar{x} \pm s$, which were counted in each set of nine values from three independent experiments. $^{**}P < 0.01$. *1*: PANC1; *2*: PANC1/vector; *3*: PANC1/cav-1.

2.5　Caveolin-1 expression inhibits PANC1 cell xenograft growth *in vivo*

To assess whether our *in vitro* findings would extend to an animal model, and to assess the biological significance of overexpression of caveolin-1 in PANC1 cells *in vivo*, PANC1, PANC1/vector and PANC1/cav-1

cell lines were injected into the subcutaneous space of nude mice (8×10^6 cells/each mice), respectively. Tumors were palpable one week after tumor cell injection in all animals except those implanted with PANC1/cav-1 cells, for which the earliest possible palpation of the tumors occurred at day 9 after injection. These results indicate that overexpression of caveolin-1 in PANC1 cells dramatically inhibits growth of the tumor cells *in vivo* (Figure 4c). The representative images of xenograft mice and xenograft tumors excised at day 42 after cell injection were presented in Figure 4a and 4b, respectively. Examination of excised tumors revealed that PANC1 and PANC1/vector tumors were larger and displayed grape-like clusters, while PANC1/cav-1 cells were smaller and pale form.

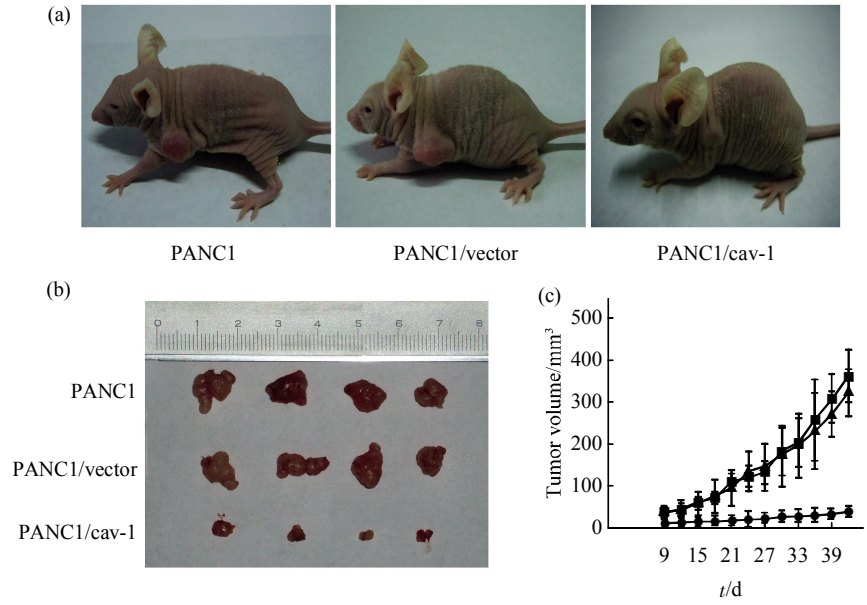


Fig. 4 Effects of caveolin-1 overexpression on PANC1 cell xenograft growth

(a) Representative xenograft tumors from three independent experiments in nude mice after inoculation for 42 days. (b) Representative tumors removed from three groups of nude mice after 42 days. (c) Tumor volume was measured from xenograft at 9 days after injection. Bars represent the $\bar{x} \pm s$, which were counted in each set of twelve nude mice from three independent experiments. ■—■: PANC1; ▲—▲: PANC1/vector; ●—●: PANC1/cav-1.

2.6 Caveolin-1 alters tumor histology and decreases proliferation but increase apoptosis of PANC1 tumor *in vivo*

To investigate the effect of caveolin-1 on the histology of PANC1 xenografts, tumor sections taken from mice were stained with H&E. As shown in Figure 5a, numerous cancer cells with pronounced nuclear fragmentation were apparent in H&E stained sections from PANC1/cav-1 tumors compared to PANC1/vector tumors. All of the tumors are clearly malignant and are morphologically carcinomas. To quantitatively compare the proliferation and apoptotic index, the tumor sections were stained for the proliferation marker Ki-67 which revealed an obvious decrease in the level of proliferating cells in PANC1/cav-1 tumor (Figure 5a). The level of proliferating cells in these tumor sections were quantified by counting of 12 randomly selected microscopic fields, showing that overexpression of caveolin-1 in PANC1 cells caused a 61% reduction cell proliferation compared to control cells (Figure 5b). TUNEL staining for DNA fragmentation detected a significant increase in the number of apoptotic cells in PANC1/cav-1 cells (Figure 5a). These tumors displayed a 4.5-fold increase of apoptotic cells compared with control tumors (Figure 5c). Our findings suggest that the dramatic inhibition of the tumor growth *in vivo* resulting from overexpression of caveolin-1 in PANC1 cells is due to the combination of enhanced apoptosis and reduced proliferation rate of PANC1/cav-1 cells.

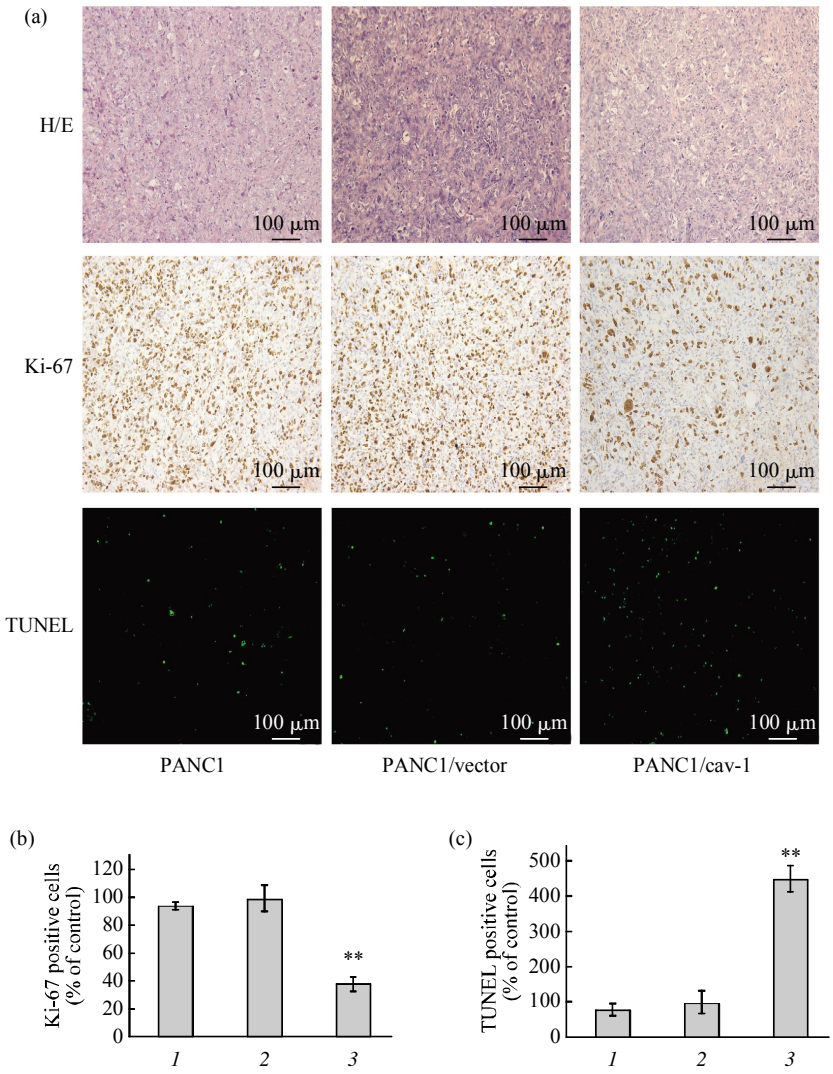


Fig. 5　Histological analysis of tumor sections from three groups of xenograft tumor models

(a) Tumor samples from representative animals were fixed, embedded in paraffin and sectioned at 4 μm onto slides, and processed for H&E, Ki-67 and TUNEL analysis. (b, c) Quantitation of the proliferation and apoptosis index. The relative contents of Ki-67 and TUNEL were expressed as percentage of level of PANC1/vector cell (control cells), respectively. Data were represented as the $\bar{x} \pm s$, which were determined in each set of twelve values from three independent experiments. $^{**}P < 0.01$. *1*: PANC1; *2*: PANC1/vector; *3*: PANC1/cav-1.

3　Discussion

In the present study, we overexpressed caveolin-1 in PANC1 cancer cells to demonstrate up-regulation of caveolin-1 in the cancer cells arrests the cell cycle at G0/G1 phase and suppresses anchorage-independent cell growth, invasion and migration *in vitro*. The findings agree with previous results with HPAF-Ⅱ pancreatic adenocarcinoma cells[19]. In order to confirm our *in vitro* observations we performed *in vivo* experiments using nude mice xenograft model, providing an important first demonstration that caveolin-1 overexpression dramatically suppresses PANC1 tumor growth with an accompanying increase in the amount of tumor cell apoptosis. It is reasonable to conclude that both decreased cell proliferation and increased cell apoptosis contribute to the antitumor activity of caveolin-1 in PANC1 cells.

Conflicting results have been reported in the literature on the role of caveolin-1 in regulating cancer growth and apoptosis. Although there is clear evidence for negative growth regulation in some cancers[10, 24],

other studies reported caveolin-1 overexpression in aggressive stages of specific malignancies, which is believed to contribute to malignant disease [28–29]. Interestingly, although both Hs578T and MCF-7 cancer cell lines represent breast adenocarcinoma cell lines, they respond quite differently when overexpressed with caveolin-1. Caveolin-1 increased tumor suppressor activity in MCF-7 cells [24] while promoting proliferation and inhibiting apoptosis in Hs578T cells[20]. Analysis of 10 pancreatic cancer cell lines revealed high levels of caveolin-1 expression in lines derived from primary tumors and low expression in those derived from metastases[19]. However, PANC1 cell line, which was derived from an invasive intraductal extension of primary tumor, displays low endogenous caveolin-1 expression levels (Figure 1). In the present study, PANC1/cav-1 transfectant cells which contain high levels of caveolin-1 may undergo a change from an invasive tumor to a primary tumor, as caveolin-1 inhibits RhoC GTPase activity and subsequent activation of the p38 MAPK pathway in primary pancreatic cancer cells to restrict migration and invasion [19]. Conversely, it was reported that caveolin-1 overexpression may contribute to the aggressiveness of pancreatic carcinoma and associate with poor prognosis for patients following surgical resection [17]. It is possible that the involvement of caveolin-1 in the regulation of cancer cell proliferation or apoptosis may be cell specific or depend on the cellular context.

Caveolin-1 regulation of tumor cell proliferation and apoptosis should involve signaling pathways. Overexpression of caveolin-1 in fibroblasts and epithelial cells has been reported to sensitize these cells to apoptotic stimuli, possibly *via* inhibition of PI (3)K and activation of caspase-3 [8]. Caveolin-1 interacts with and inhibits serine/threonine protein phosphatases PP1/PP2 leading to increased Akt activities and largely responsible for enhanced cell survival of prostate cancer cells [30]. Caveolin-1 expression appears to suppress c-myc-induced apoptosis in certain prostate cancer derived cell types [6] and caveolin-1 might play a pivotal role in regulating apoptosis as a suppressor through regulation of neutral-sphingomyelinase/ceramide signaling pathway[20]. Also, caveolin-1 plays a regulatory role in EGFR, TGF-β1 and Wnt/β-catenin signaling pathway[15, 31–32]. Torres *et al*[33] have demonstrated that both a reduction in proliferation and increase in death in HEK293T and ZR/5 cells can be observed as a consequence of increased caveolin-1 expression *via* suppressing expression of inhibitor of apoptosis protein survivin and changing cell cycle distribution. Caveolin-1 has been shown to inhibit cell proliferation by arresting cells in the G0/G1 phase of the cell cycle through a p53/p21-signaling pathway [25]. In the present paper, up-regulation of endogenous caveolin-1 in PANC1 pancreatic cancer resulted in significantly increasing the G0/G1 phase of cell cycle and decreasing the S and G2/M population of cell cycle, indicating caveolin-1 may play an important role in moderating cell cycle progression. It has been noted that the tumor suppressor protein-p53, is activated by caveolin-1 to suppress cell proliferation *via* cell cycle arrest with no increase in apoptosis[25]. However, our findings showed that overexpression of caveolin-1 in PANC1 cells induced not only reduced proliferation but also enhanced apoptosis in the tumor cells. So far, mechanisms by which caveolin-1 promotes cell apoptosis and inhibits cell proliferation through modulating cell cycle progression are not completely understood.

There is a growing body of evidence that a number of signaling molecules, such as, protein kinase C, eNOS and Src family tyrosine kinases can directly interact with a specific scaffolding domain of caveolin-1 in caveolae membranes, which consequently lead to functional inactivation of these molecules[3, 34–35]. Src kinases regulates a number of tumor cell-specific functions such as migration, adhesion, cell growth and differentiation and survival [36–37]. In pancreatic carcinoma, Src kinase overexpression and activation have been reported [38]. The inhibition of Src kinase activity by its inhibitor dasatinib in human pancreatic cancer has antitumor efficacy. Treatment with dasatinib and gemcitabine significantly reduced primary pancreatic tumor volume [36]. In the present paper, *in vivo* experiments employing xenograft tumor models demonstrated that a marked reduction in tumor volume and proliferation along with an increase in apoptotic cells in PANC1/cav-1 cells. Therefore, it is not excluded that additional caveolin-1 molecules in PANC1/cav-1 cells due to overexpression of caveolin-1 directly interact with Src kinases or other signaling molecules within caveolae to further suppress the tumor growth.

The regulations of caveolin-1 in cancer cell proliferation and apoptosis are complex multiple

cellular signaling pathways. The physiological role of caveolin-1 remains elusive and warrants further investigation. However, caveolin-1, as an important structure protein of caveolae, plays a key regulatory role in signaling transduction and cancer proliferation. Since caveolin-1 contributes to many different regulatory cascades, it may be proved be an excellent therapeutic target as we continue to examine the role of caveolin-1 in tumor genesis and cancer progression.

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Caveolin-1 抑制胰腺癌细胞 PANC1 的体内外生长和增殖 *

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摘要 窖蛋白 -1(caveolin-1)是胞膜窖(caveolae)中重要的结构和功能蛋白．Caveolin-1 参与细胞的多种生命活动并与恶性肿瘤的发生相关．为探讨 caveolin-1 对胰腺癌细胞 PANC1 的体外增殖、迁移、侵袭以及裸鼠体内成瘤能力的影响，通过基因转染技术培育 caveolin-1 过表达细胞株 PANC1/cav-1 作为实验组，转染空载体细胞株 PANC1/vector 作为对照组，采用 RT-PCR 及 Western blot 方法检测 caveolin-1 的表达量, 流式细胞术分析细胞周期，软琼脂细胞克隆实验检测细胞增殖能力, 侵袭小室实验检测癌细胞迁移和侵袭的能力，建立裸鼠皮下种植瘤模型并检测肿瘤组织的增殖与凋亡．PANC1/cav-1 中的 caveolin-1 表达稳定，表达量明显高于对照组细胞株和亲本细胞株($P < 0.01$)，细胞周期检测显示大量 PANC1/cav-1 细胞被抑制于 G0/G1 期， caveolin-1 抑制 PANC1 的增殖，迁移和侵袭能力．在裸鼠的体内实验中，caveolin-1 显著抑制 PANC1 细胞在裸鼠体内的生长，Ki-67 染色和 TUNEL 染色表明在 PANC1 细胞中过表达 caveolin-1，可以抑制肿瘤增殖并诱导肿瘤凋亡．上述结果表明，caveolin-1 可能通过对胰腺癌细胞周期的影响(抑制于 G0/G1 期)，抑制胰腺癌 PANC1 细胞在体内外的增殖、迁移和侵袭，并导致肿瘤凋亡．

关键词 caveolin-1，细胞周期，迁移，侵袭，异种移植

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