Int J Clin Exp Pathol 2015;8(8):8937-8947
www.ijcep.com /ISSN:1936-2625/IJCEP0012100

Original Article

Caveolin-1 regulates cell apoptosis and invasion ability in paclitaxel-induced multidrug-resistant A549 lung cancer cells

Fei Han[1,2], Long Zhang[1], Yongxin Zhou[3], Xianghua Yi[1]

[1]Department of Pathology, Shanghai Tongji Hospital Affiliated to Shanghai Tongji University, Shanghai 200065, China; [2]Department of Pathology, Shanghai Chest Hospital Affiliated to Shanghai Jiao Tong University, Shanghai 200030, China; [3]Department of Thoracic-Cardiovascular Surgery, Tongji Hospital of Tongji University, 389 Xincun Rd, Shanghai, China

Received June 29, 2015; Accepted July 29, 2015; Epub August 1, 2015; Published August 15, 2015

Abstract: The aim of the study was to investigate the effect and potential mechanism of caveolin-1 (Cav1) knockdown in paclitaxel-resistant lung cancer A549/Taxol cells. The human paclitaxel-resistant lung cancer cell line A549/Taxol was transfected with a Cav1 shRNA lentiviral vector. Interference efficiency for Cav1 was detected by real-time PCR and Western blotting. A MTT assay was used to determine cell proliferation, and flow cytometry was used to detect the cell cycle stage and apoptosis. Cell migration and invasion capability were detected by a transwell assay. Protein levels of related signaling molecules were detected by Western blotting. We successfully constructed a stable A549/Taxol cell line expressing low levels of Cav1. Cav1 knockdown significantly inhibited cell proliferation and induced G0/G1 arrest and cell apoptosis *in vitro* and *in vivo*. In addition, these effects correlated significantly with a reduction in cyclin D1 expression and activation of the Bcl-2/Bax-mediated mitochondrial apoptosis pathway. Furthermore, knockdown of Cav1 inhibited cell migration and invasion, and this may be related to the inhibition of AKT and the subsequent decreased protein expression of MMP2, MMP7 and MMP9.

Keywords: Caveolin-1, multidrug-resistant, A549/Taxol, apoptosis, invasion

Introduction

Caveolin-1 (Cav1) is a 22-24 kDa structural protein component of caveolae in the plasma membrane that regulates multiple cancer-associated processes including tumor growth, cell death and survival, and migration and invasion [1, 2]. In non-small cell lung cancers (NSCLC), Cav1 expression is associated with poor prognosis in squamous cell carcinomas, large cell carcinomas, and pleomorphic carcinomas of the lung [3-5]. Recently, several studies have shown that Cav1 plays an essential role in cancer cell radio- and chemoresistance. Cav1 is usually up-regulated in multidrug resistant tumor cells including those in colon, ovarian and lung cancers [6-9]. Increased Cav1 expression was also significantly correlated with drug resistance and poor prognosis in advanced NSCLC patients after gemcitabine-based chemotherapy [10].

Paclitaxel (Taxol) is a cytotoxic microtubule-stabilizing agent often used in first-line or second-line treatment of middle to late stage NSCLC, but the development of paclitaxel resistance is common. Yang et al. found that Cav1 levels were elevated in taxol-resistant A549 lung carcinoma cells [11]. However, the effects and molecular mechanism of Cav1 up-regulation in paclitaxel-induced multidrug resistant A549 lung cancer cell are currently poorly understood.

In this study, we successfully constructed a stable A/T-Cav1 KD cell line expressing low levels of Cav1, and found that Cav1 knockdown inhibited cell proliferation, induced G0/G1 arrest, and stimulated cell apoptosis. These roles were associated with decreased expression of Cyclin D1 and activation of an intrinsic apoptosis pathway. Cav1 knockdown also reduced the migration and invasion abilities of paclitaxel-resistant A549 lung cancer cells. This effect may be correlated with inhibition of the PI3K/Akt signaling pathway and subsequent decreased protein expression of MMP2, MMP7 and MMP9.

Materials and methods

Cell culture and chemical reagents

Human lung adenocarcinoma cell lines A549 and A549/Taxol (paclitaxel-sensitive and -resistant cell lines, respectively) were obtained from Shanghai (Chest Hospital, Shanghai Jiaotong University, China). The A549/Taxol sub-line, derived from A549, was generated using a previously described protocol [12]. Briefly, A549/Taxol cell lines were selected by continuous culture in medium containing stepwise increases in the paclitaxel (Bristol-Myers Squibb, Princeton, NJ, USA) concentration over 8 months, up to 1 mg/ml. Cells were cultured in DMEM (Gibco BRL, Karlsruhe, Germany) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. Paclitaxel was added to the culture medium at a final concentration of 5 μM to maintain resistance. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulphoxide (DMSO), Hoechst 33258 and β-actin antibody were purchased from Sigma (St. Louis, MO, USA). Primary antibodies against Cav1, Bcl-2, Bax and all secondary antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Primary antibodies against poly (ADP-ribose) polymerase (PARP), caspase-3 and -9, cyclin D1, p-Akt (Ser473) and Akt, MMP2, MMP7, and MMP9 as well as the PI3K inhibitor LY294002 were purchasedfrom Cell Signaling Technology, Inc (Danvers,MA). Lipofectamine 2000 was obtained from Invitrogen (Carlsbad, CA, USA).

Lentivirus-mediated Cav1 RNA interference

We established a stable Cav1-silenced paclitaxel-resistant A549cell line by infecting the cells with a lentivirus encoding Cav1 shRNA (GenePharma, Shanghai, China). The lentivirus packaging shRNA expression vector was established as reported previously [13]. Briefly, the sequences for targeting the Cav1 gene (GenBank accession no. NM_001753) were selected using theBIOCK-iT RNAi Designer (Invitrogen, Carlsbad, CA, USA). Then, three short hairpin RNA oligonucleotides were designed (RNAi#1: 5'-ACCTTCACTGTGACGAA AT-3'; RNAi#2: 5'-TTTGTGATTCAATCTGTAA-3'; RNAi#3: 5'-CCCACTCTTTGAAGCTGTT-3') and cloned into the pGLV-U6-GFP lentiviral vector (GenePharmaShanghai, China). The negative vector contained a nonsense shRNA (5'-TTCT-CCGAACGTGTCACGT-3') to control for any non-RNAi-mediated effects. A549/Taxol cells were exposed to lentivirus-containing supernatant for 24 h in the presence of polybrene (Sigma, 8 μg/ml). Stable transfectants were selected with puromycin (2 μg/ml) for 8 days and verified by real-time PCR and Western blotting.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted using the RNeasy mini kit (Qiagen, Hilden, Germany), and genomic DNA was isolated using the RNase-free DNase kit (Promega, Madison, WI, USA). First-strand complementary DNA (cDNA) was generated using the Reverse Transcription System Kit (Promega, Madison, WI, USA). qRT-PCR was then performed in a 20-μl reaction including 2 μg cDNA, 1 mM of each primer, and 2× SYBR Green mix (Takara, Shuzo, Kyoto, Japan). Changes in mRNA expression levels were calculated following normalization with glyceraldehyde-3-phosphatedehydrogenase (GAPDH). Relative gene expression was determined by the fluorescence intensity ratio of the target gene to GAPDH. The primers for Cav1 and GAPDH were designed using Primer 5.0, as follows: forward, 5'-ACA TCT CTA CAC CGT TCC CAT-3' and reverse, 5'-TGT GTG TCC CTT CTGGTTCTG-3' for Cav1; forward, 5'-AACGG-ATTTGGTCGTATTG-3' and reverse, 5'-GGAAGAT-GGTGATGGGATT-3' for GAPDH.

Cell proliferation assay

Cells were plated in 96-well plates at a density of 5×10^3/well, and viable cells were counted from days 1 to 7. On each day, cells were stained with 20 μl sterile MTT dye (5 mg/ml) at 37°C for 4 h followed by removal of the culture medium and thorough mixing with 150 μl DMSO for 10 min. Spectrometric absorbance at 490 nm was measured using a microplate reader. Each cell line was established in quadruplicate wells, and the results were repeated three times.

Hoechst 33258 staining

Cells were seeded and incubated overnight with 5 μM paclitaxel on sterile cover glasses,

then fixed with methanol for 5 min at room temperature. The fixed cells were washed twice with phosphate buffered saline (PBS), incubated with Hoechst 33258 for 5 min at room temperature, and then observed under a fluorescence microscope. The presence of fragmented or condensed nuclei was used to define apoptotic cells.

Serum starvation and flow cytometry

Cells at 80-90% confluence were incubated for 36 h in DMEM containing 0.5% FBS, then in DMEM with 10% FBS for 12 h. Cells were harvested and fixed in citric acid buffer for 30 min. After centrifugation, the cell pellets were resuspended and treated with 100 mg/L RNase and 10 mg/L propidium iodide in PBS for 20 min. The apoptotic cell fraction and cell cycle distribution were analyzed by FACS can cytometry (Becton-Dickinson, San Jose, CA, USA).

Migration and invasion assay

A 24-well plate containing an 8-mm-pore size chamber insert (BD Biosciences, Franklin Lakes, NJ, USA) was used to evaluate the migration and invasion of tumor cells. For the migration assay, 5×10^4 cells were seeded in the upper chamber. For the invasion assay, the membrane was coated with Matrigel (BD Biosciences) to form a matrix barrier, and then 1×10^5 cells were placed in the upper chamber. In each lower chamber, 500 μL DMEM with 10% FBS were added. After 36 h of incubation at 37°C, the cells that had migrated through the pore were fixed by methanol, stained with 4',6-diamidino-2-phenylindole (DAPI) (Sigma, 0.1 μg/ml), and counted. Each assay was carried out in triplicate.

Xenograft studies

Fifteen male BALB/c-nu/nu mice (weight, ~20 g; age, 4 weeks) were purchased from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). Mice were randomly divided into three groups consisting of five mice each. Cells (1×10^7 cells in 200 μL) were suspended in DMEM medium and injected subcutaneously into the flank of each mouse. Tumor size was measured every 3 days using a vernier caliper and was calculated as 0.52× length× width× height. All mice were sacrificed on day 30, and the xenografts were immediately removed, weighed and then fixed, embedded and cut into 3-μm-thick sections for further studies. All experiments were performed according to the guidelines of the local Animal Use and Care Committee.

In situ apoptosis detection by TUNEL staining

A terminal deoxynucleotidyl transferase-mediated dUTP biotin nick end labeling (TUNEL) assay was performed using the In Situ Cell Death Detection kit, POD (Roche Diagnostics) according to the protocol supplied by the manufacturer. The average apoptotic index (AI) of each tumor sample was calculated as follows: AI = (apoptotic cells/total cells from 10 different random fields) ×100%.

Western blot analysis

Cells were washed twice with ice-cold PBS and then lysed in RIPA buffer with protease and phosphatase inhibitors (Abcam Inc., Cambridge, MA). Protein concentrations were determined using a BCA protein assay kit (KeyGen Biotech). Equivalent amounts of total proteins (40 μg) were loaded onto a 10% SDS-polyacrylamide gel and then transferred onto polyvinylidine difluoride membranes. The transferred membranes were blocked for 1 h in 5% nonfat milk and then incubated with the appropriate primary antibodies at 4°C overnight. Membranes were washed three times with TBST for 10 min and incubated with horseradish peroxidase-coupled secondary antibodies for 1 h at room temperature. Enhanced chemiluminescence reagents were used for detection. The autoradiographed films were scanned using an Odyssey Infrared Imaging system. The densitometric value of each protein band was normalized to the β-actin level.

Statistical analysis

Data are expressed as the means ± standard deviations (SD). The SPSS 16.0 software package (SPSS, Inc. Chicago, IL, USA) was used for statistical analysis. Statistical comparisons were performed using the Student's t-test and one-way ANOVA. $P<0.05$ was considered to indicate statistical significance.

Results

Cav1 knockdown inhibited paclitaxel-resistant A549/Taxol lung cancer cell proliferation

To evaluate the effect of Cav1 knockdown on the biological behavior of A549/Taxol, three

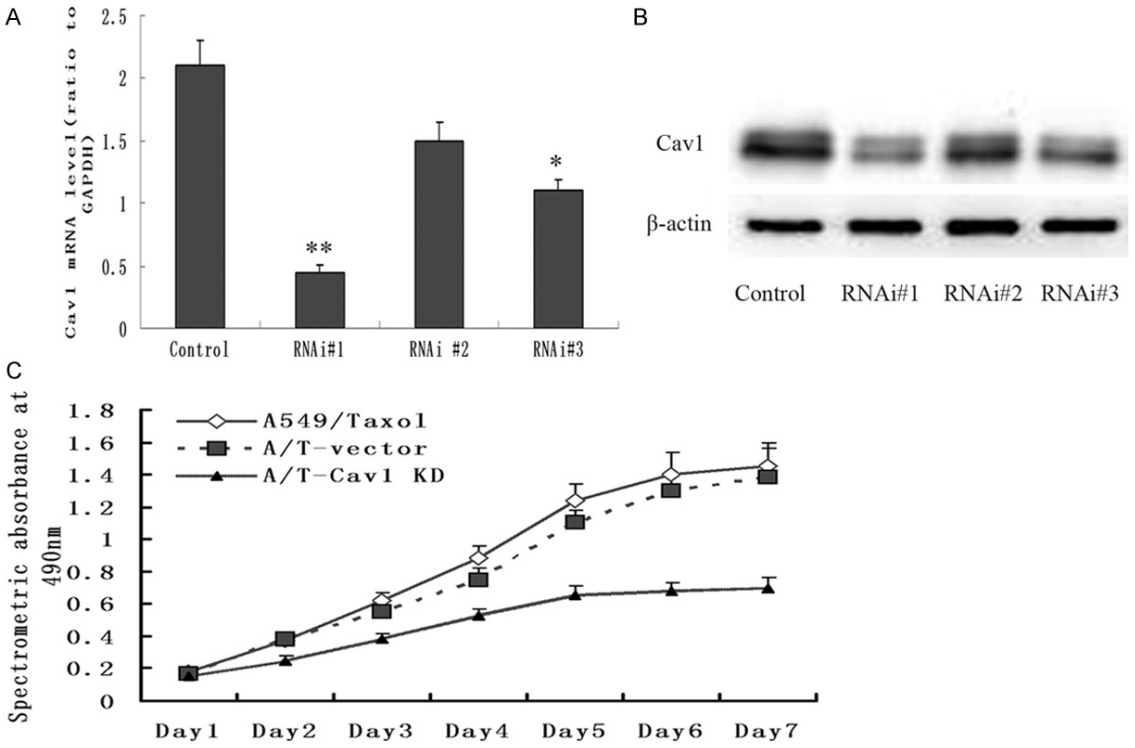

Figure 1. Identification of the most effective Cav1 shRNA vector and cell proliferation assay. A. The mRNA expression levels of Cav1 was detected by quantitative real-time PCR. The cell transfected with control shRNA was used as a negative control. *P<0.05, **P<0.01, compared with control. B. Protein level of Cav1 by western blot analysis. β-actin level was used as an internal control. RNAi#1 was the most effective shRNA vector that was used for infecting A549/Taxol cell, named A/T-Cav1 KD cell for further studies. C. MTT assay was carried out in A549/Taxol, A/T-vector and A/T-Cav1 KD cells. Each point indicates the mean of spectrometric absorbance ± SD of three independent experiments.

pairs of interfering vectors were designed for specific interference of the endogenous Cav1 gene. The expression of Cav1 was detected by real-time PCR and Western blot analysis. As shown in **Figure 1**, RNAi#1 was the most effective shRNA vector for Cav1 knockdown, and was thus used to establish an A549/Taxol stable cell line expressing the lowest Cav1 levels, named A/T-Cav1 KD. The control shRNA vector-transfected cell line was denoted A/T-vector. MTT assay showed that Cav1 knockdown significantly inhibited growth of A549/Taxol cells (**Figure 1C**).

Cav1 knockdown induced G0/G1 arrest and cell apoptosis in A549/Taxol cells.

To further confirm that the effect of Cav1 on cell growth is due to proliferation and/or apoptosis pathways, we measured cell cycle and apoptosis by flow cytometry and Hoechst staining. As shown in **Figure 2A**, fluorescence microscopy revealed that, compared with controls, condensed and fragmented apoptotic nuclei were more readily observed in Cav1 down-regulated A/T-Cav1 KD cells. Flow cytometric cell cycle analysis demonstrated that Cav1 knockdown resulted in cell cycle arrest in the G0/G1 phase, and a reduction of cells in the S and G2/M phases. Furthermore, the proportion of apoptotic cells was increased by 3.7-fold in the A/T-Cav1 KD group compared with the A/T-vector group (**Figure 2B-D**, P<0.01).

Cav1 knockdown inhibited the migration and invasion of A549/Taxol cells

To investigate the effects of Cav1 on the migration and invasion ability of A549/Taxol cells, a transwell chamber assay (both with and without Matrigel) was performed. The migrated and invasive cells underneath the membrane were fixed and stained after 36 h. Filters were viewed under a 10× objective, and counting was per-

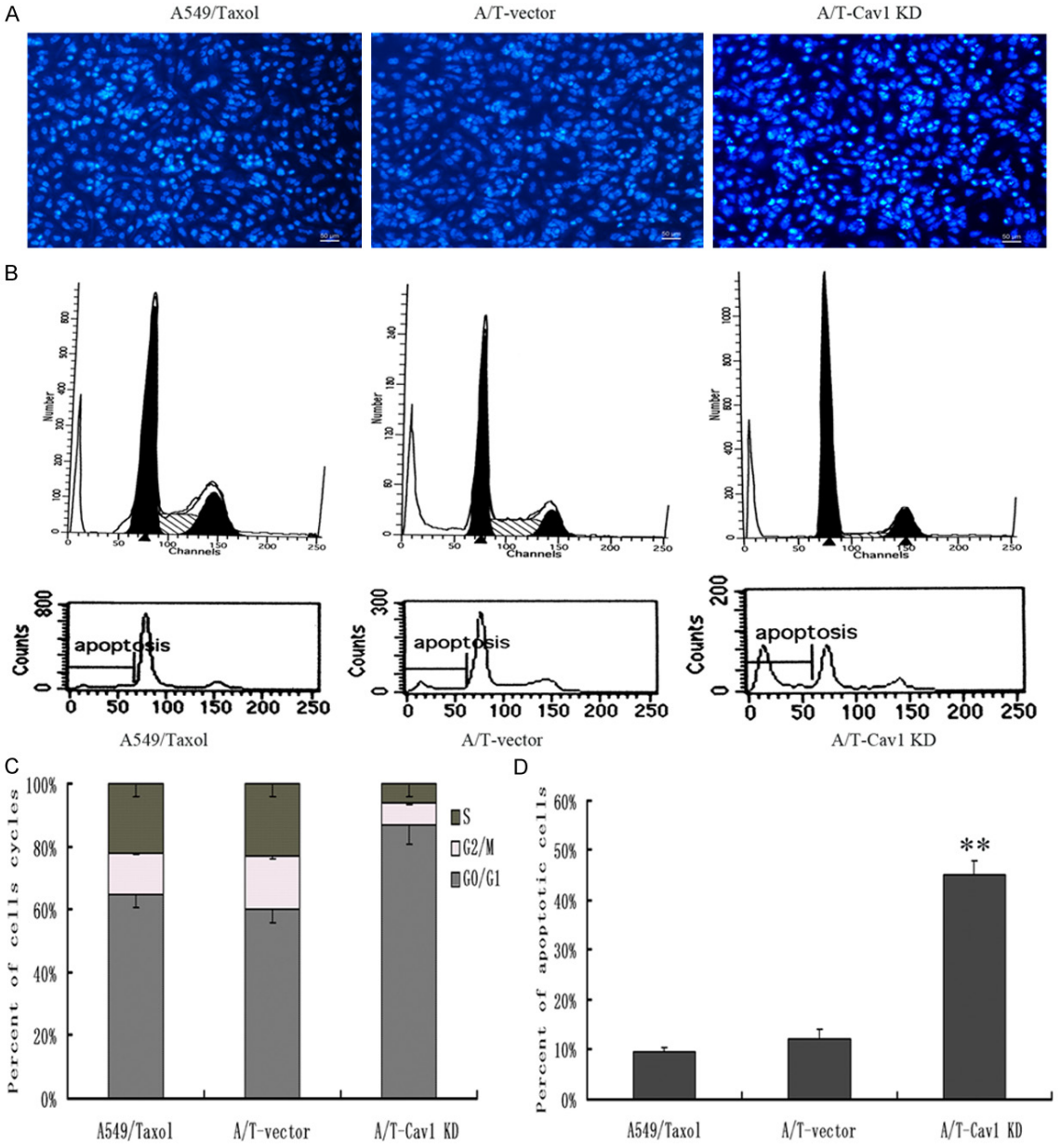

Figure 2. Effects of Cav1 knockdown on cell cycle and apoptosis. A. Cell morphological change detected by Hoechst 33258 staining. All fields are representative of multiple fields observed in three experiments. B-D. Cell cycle and apoptosis analyzed by flow cytometry. ***P*<0.01 indicates A/T-Cav1 KD versus A/T-vector cells.

formed for six random fields in each group. As shown in **Figure 3**, the capacity of the A/T-Cav1 KD cells to migrate through the polycarbonate membrane of the transwell chambers was decreased 3-fold compared with the A/T-vector group, whereas no significant difference between A549/Taxol and A/T-vector cells was found. A markedly decreased invasive ability was also observed in the A/T-Cav1 KD cells compared with the A/T-vector cells, but there was no difference between A549/Taxol and A/T-vector cells. These results indicate that Cav1 promotes the metastastic potential of A549/Taxol cells.

Cav1 knockdown inhibited the tumorigenicity of A549/Taxol cells in vivo

To determine the effect of Cav1 knockdown on cellular growth *in vivo*, we xenografted A549/

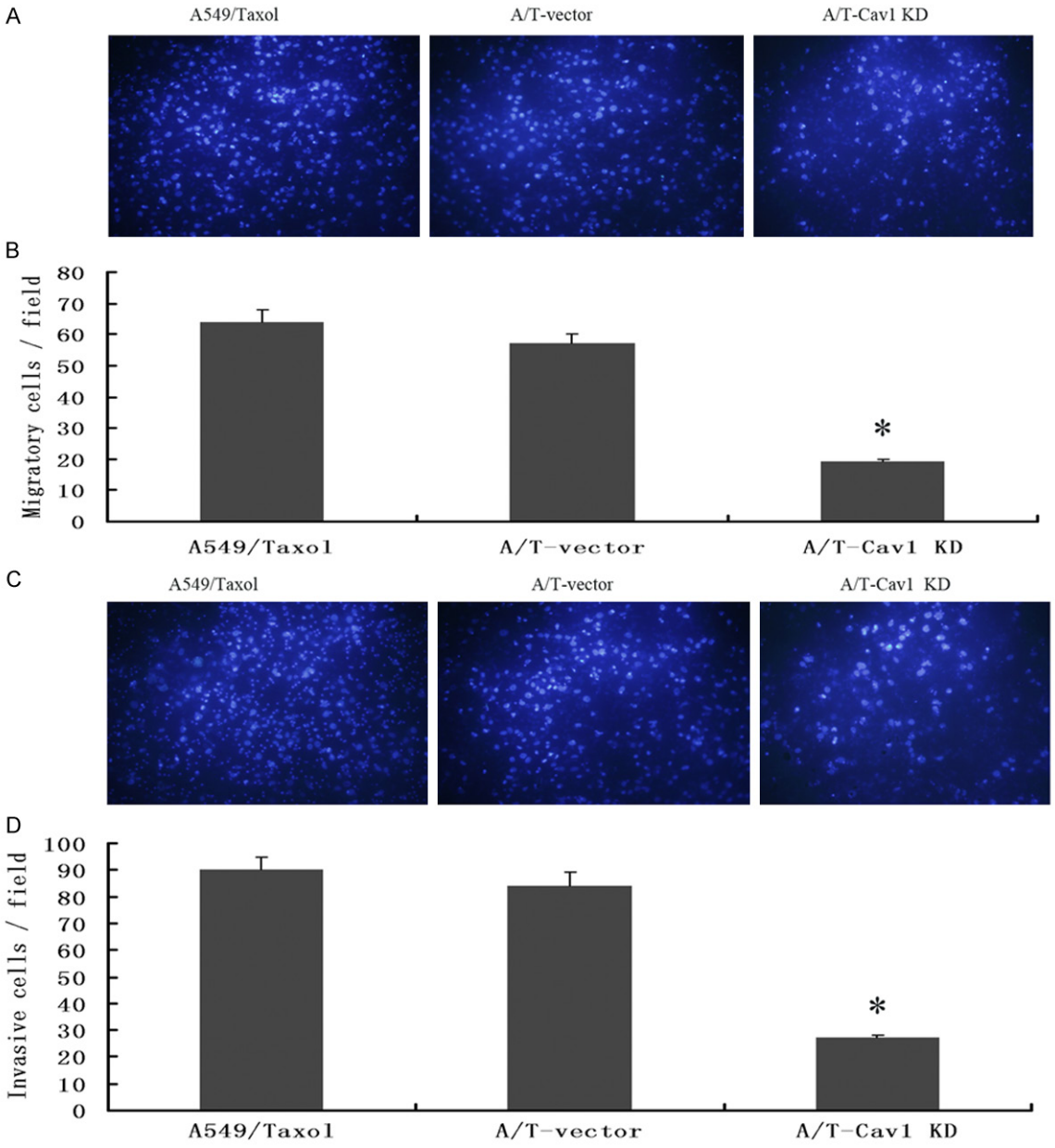

Figure 3. Cav1 knockdown inhibited cell migration and invasion. A and B. Transwell migration (DAPI staining, original magnification ×100). A/T-Cav1 KD cells had much less cells migrated through the basement membrane than those of A/T-vector and A549/Taxol cells (*P<0.05). No significant difference was observed between A/T-vector and A549/Taxol cells (P>0.05). C and D. Transwell invasion (DAPI staining, original magnification ×100). A/T-Cav1 KD cells had much less cells invaded through the basement membrane than those of A/T-vector and A549/Taxol cells (*P<0.05). There was no distinction between A/T-vector and A549/Taxol cells (P>0.05).

Taxol, A/T-vector and A/T-Cav1 KD cells into nude mice. At a postmortem examination conducted after 30 days, we found tumor volumes and weights derived from A/T-Cav1 KD cells were much smaller than those derived from A/T-vector and A549/Taxol cells (**Figure 4A-C**). To further evaluate cell vitality, we conducted a TUNEL assay to detect cell apoptosis *in situ*. We found that the proportion of apoptotic cells in the A/T-Cav1 KD group was increased 2.3-fold compared with the A/T-vector group (**Figure 4D** and **4E**). These data suggest that Cav1 knockdown inhibits cell growth and induces apoptosis in A549/Taxol cells *in vivo*.

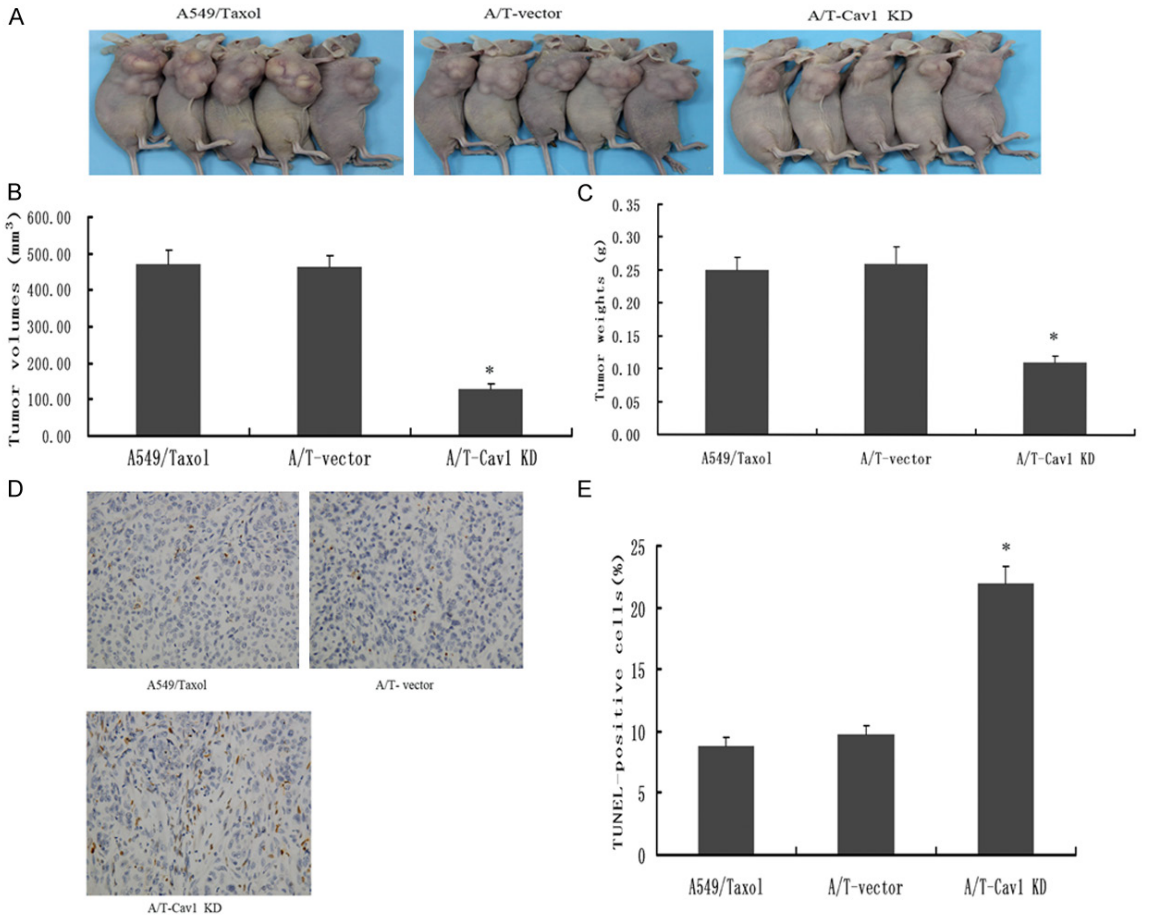

Figure 4. Cav1 knockdown inhibited tumor growth *in vivo*. A-C. A549/Taxol, A/T-vector and A/T-Cav1 KD (loss expression of Cav1) cells were xenografted in a nude mice model. The volumes and weights of tumors were evaluated in the xenograft model. D and E. TUNEL assay showed A/T-Cav1 KD group has more apoptotic cells than A/T-vector and A549/Taxol groups (*P<0.05).

Knockdown of Cav1 alters signaling pathways in A549/Taxol cells

To investigate the molecular mechanism of Cav1 down-regulation in cell proliferation, apoptosis, and invasion, we performed Western blot analysis of A549/Taxol, A/T-vector and A/T-Cav1 KD cells. As shown in **Figure 5**, Cav1 knockdown inhibited the phosphorylation of Akt (Ser473) and the expression of cell cycle-dependent protein cyclin D1. In addition, Cav1 knockdown activated the mitochondrial apoptotic pathway by affecting the Bcl-2/Bax ratio. Up-regulation of Bax and down-regulation of Bcl-2 protein expression were detected in A/T-Cav1 KD cells compared with controls. Meanwhile, increased levels of cleaved caspase-9, caspase-3 and PARP (a known substrate of caspase-3) were observed in A/T-Cav1 KD cells. To identify metastasis-related molecules in A/T-Cav1 KD cells, Western blot analysis was used to detect the protein expression of MMP2, MMP7 and MMP9. Decreased protein expression of MMP2, MMP7 and MMP9 were observed in A/T-Cav1 KD cells compared with controls. Interestingly, protein levels of MMP2, MMP7 and MMP9 were decreased in A/T-vector cells treated with 25 μM LY294002 (an inhibitor of PI3K/Akt signaling pathway) for 24 h, compared with the negative control. Protein levels of MMP2, MMP7 and MMP9 were also lower in A/T-Cav1 KD cells compared with A/T-vector cells with both LY294002 treatments. Cav1 knockdown had a synergistic inhibitory effect with LY294002 treatment on the protein expression of MMP2, MMP7 and MMP9. These results suggest Cav1 might regulate the invasion and metastasis capacities of A549/Taxol cells by activating the PI3K/Akt signaling pathway.

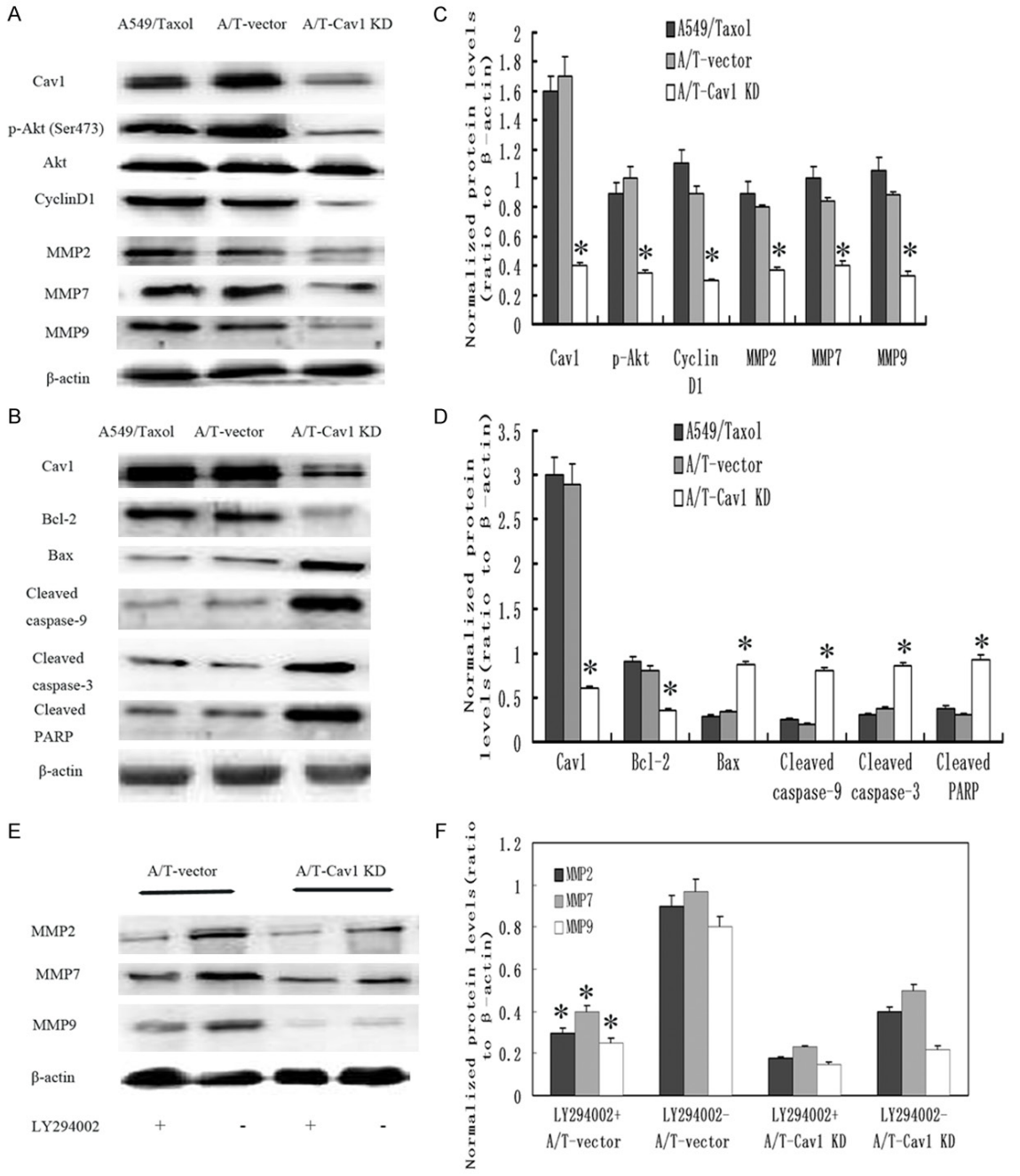

Figure 5. Involving changes of signal pathways in Cav1 knockdown A549/Taxol cells. A and C. Cell lysates were prepared from A549/Taxol, A/T-vector and A/T-Cav1 KD cells. Protein expressions of Cav1, p-Akt (Ser473), Akt, Cyclin D1, MMP2, MMP7 and MMP9 were detected by Western blot assay.β-actin level was used as a loading control. B and D. Protein expressions of Bcl-2, Bax, Cleaved caspase-9 and caspase-3, cleaved PARP by western blot analysis. β-actin level was used as a loading control. *P<0.05 indicates A/T-Cav1 KD versus A/T-vector cells. E and F. A/T-vector and A/T-Cav1 KD cells were treated with DMSO or LY294002 (25μM for 24 h), protein expression of MMP2, MMP7 and MMP9 by western blot assay. Inhibition of Akt activity significantly inhibited protein expressions of MMP2, MMP7 and MMP9 (*P<0.05).

Discussion

Lung cancer is the leading cause of cancer death worldwide, and nearly 80% of NSCLC patients die of tumor recurrence and metastasis. Multidrug resistant cancer cells are apt to develop apoptosis resistance and form metastases following chemotherapy [14]. Recent

studies have implicated Cav1 in acquired drug resistance. For example, Park et al [15] reported that Cav1 knockdown sensitized human renal carcinoma cells to doxorubicin-induced apoptosis and reduced lung metastasis in a mouse model. Cav1 knockdown radiosensitized pancreatic cancer cell lines to ionizing radiation, and enhanced radiosensitivity of Cav1-deficient mice was associated with increased apoptosis *in vivo* [16, 17]. Despite these findings, the precise role of Cav1 in paclitaxel-induced multidrug resistant A549 lung cancer cell has not been extensively investigated.

In current study, we used paclitaxel-resistant lung cancer A549/Taxol cells, which were derived from the sensitive A549 cell line, constructed in a previous study and expressing high levels of Cav1 [12]. We chose lentivirus-mediated Cav1 RNA interference to infect A549/Taxol cells and established a stable cell line expressing low levels of Cav1 for the following studies. A cell proliferation assay showed that Cav1 knockdown significantly inhibited cell growth. Flow cytometry showed that Cav1 knockdown induced G0/G1 arrest and cell apoptosis in A549/Taxol cells. We further established a subcutaneous xenotransplantation lung cancer mouse model. Consistent with our *in vitro* results, a significant reduction in tumor growth and an increase in cell apoptosis were observed following Cav1 knockdown. To investigate the underlying molecular mechanism, the protein expression of several signaling molecules was detected by Western blotting. We found that Cav1 knockdown reduced the phosphorylation level of Akt (Ser473) in A549/Taxol cells. Cyclin D1, a G1 cyclin, controls the transition from G1 to S of the cell cycle. Our study showed that knockdown of Cav1 decreased the protein expression of Cyclin D1. Moreover, Cav1 knockdown altered the Bcl-2/Bax ratio and activated the mitochondrial apoptotic pathway, inducing the caspase-9 and caspase-3 cascade effect and the expression of cleaved PARP. These results suggest that Cav1 might promote cell survival by affecting both apoptosis and proliferation pathways mediated through Akt activation.

Although previous studies have shown that Cav1 up-regulation correlates with metastatic potential and predicts poor prognosis in various cancers including prostate cancer, breast cancer, lung cancer and renal cell carcinoma [18-22], the role of Cav1 in invasive ability of paclitaxel-induced multidrug resistant A549 lung cancer cell remains largely unknown. Our studies demonstrate that Cav1 down-regulation remarkably inhibited cell migration and invasion abilities in A549/Taxol cells. The matrix metalloproteinases (MMPs) are a family of zinc-containing proteolytic enzymes that break down extracellular matrix proteins and play an important role in tumor invasion and metastasis. To better understand the mechanisms that inhibit invasion of A549/Taxol cell by Cav1 down-regulation, the protein levels of various MMPs were analyzed using immunoblotting. Our data showed that knockdown of Cav1 significantly decreased the protein expression of MMP2, MM7 and MMP9, which was also inhibited by a PI3K inhibitor, LY294002 (25 μM). Cav1 knockdown mimicked and enhanced the inhibitive effect of LY294002 in A/T-Cav1 KD cells. These findings suggest that Cav1-induced MMP expression may be mediated by the PI3K/Akt signaling pathway in paclitaxel-resistant lung cancer cells.

In conclusion, Cav1 knockdown inhibited proliferation and invasion capabilities and induced cell apoptosis in paclitaxel-induced multidrug resistant A549/Taxol cell; moreover, these effects may be related to the activation of an intrinsic apoptosis pathway and the reduction of MMP2, MMP7 and MMP9 protein expression via the PI3K/Akt signaling pathway.

Acknowledgements

This study was supported by a grant from the National Natural Science Foundation of China (No. 81201838) for Dr Fei Han. It was also partly supported by National Natural Science foundation (81570053). We also sincerely thank the members of the Department of Pathology at Tongji Hospital for their assistance in this work.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xianghua Yi, Department of Pathology, Shanghai Tongji Hospital

Affiliated to Shanghai Tongji University, 389 Xincun Rd, Shanghai 200065, China. Tel: +0086 21-66111162; E-mail: yixhxff@163.com; Dr. Yongxin Zhou, Department of Thoracic-Cardiovascular Surgery, Tongji Hospital of Tongji University, 389 Xincun Rd, Shanghai 200065, China. E-mail: zhou6302@tongji.edu.cn

References

[1] Burgermeister E, Liscovitch M, Rochen C, Schmid RM, Ebert MP. Caveats of caveolin-1 in cancer progression. Cancer Lett 2008; 268: 187-201.

[2] Parton RG, Simons K. The multiple faces of caveolae. Nat Rev Mol Cell Biol 2007; 8: 185-194.

[3] Yoo SH, Park YS, Kim HR, Sung SW, Kim JH, Shim YS, Lee SD, Choi YL, Kim MK, Chung DH. Expression of caveolin-1 is associated with poor prognosis of patients with squamous cell carcinoma of the lung. Lung Cancer 2003; 42: 195-202.

[4] Han F, Zhang J, Shao JC, Yi XH. Caveolin-1 promotes an invasive phenotype and predicts poor prognosis in large cell lung carcinoma. Pathol Res Pract 2014; 210: 514-520.

[5] Moon KC, Lee GK, Yoo SH, Jeon YK, Chung JH, Han J, Chung DH. Expression of caveolin-1 in pleomorphic carcinoma of the lung is correlated with a poor prognosis. Anticancer Res 2005; 25: 4631-4637.

[6] Hehlgans S, Eke L, Storch K, Haase M, Baretton GB, Cordes N. Caveolin-1 mediated radioresistance of 3D grown pancreatic cancer cells. Radiother Oncol 2009; 92: 362-370.

[7] Belanger MM, Roussel E, Couet J. Up-regulation of caveolin-1 expression by cytotoxic agents in drug-sensitive cancer cells. Anticancer Drugs 2003; 14: 281-287.

[8] Lavie Y, Fiucci G, Liscovitch M. Up-regulation of caveolae and caveolar constituents in multidrug-resistant cancer cells. J Biol Chem 1998; 273: 32380-32383.

[9] Lavie Y, Liscovitch M. Changes in lipid and protein constituents of rafts and caveolae in multidrug resistant cancer cells and their functional consequences. Glycoconjugate J 2000; 17: 253-259.

[10] Ho CC, Kuo SH, Huang PH, Huang HY, Yang CH, Yang PC. Caveolin-1 expression is significantly associated with drug resistance and poor prognosis in advanced non-small cell lung cancer patients treated with gemcitabine-based chemotherapy. Lung Cancer 2008; 59: 105-110.

[11] Yang CP, Galbiati F, Volonte D, Horwitz SB, Lisanti MP. Upregulation of caveolin-1 and caveolae organelles in Taxol-resistant A549 cells. FEBS Lett 1998; 439: 368-372.

[12] Sun QL, Sha HF, Yang XH, Bao GL, Lu J, Xie YY. Comparative proteomic analysis of paclitaxel sensitive A549 lung adenocarcinoma cell line and its resistant counterpart A549-Taxol. J Cancer Res Clin Oncol 2011; 137: 521-532.

[13] Shen YH, Song GX, Liu YQ, Sun W, Zhou LJ, Liu HL. Silencing of FABP3 promotes apoptosis and induced mitochondrion impairment in embryonic carcinoma cells. J Bioenerg Biomembr 2012; 44: 317-323.

[14] Rooney C, Sethi T. The epithelial cell and lung cancer: the link between chronic obstructive pulmonary disease and lung cancer. Respiration 2011; 81: 89-104.

[15] Park J, Bae E, Lee C, Yoon SS, Chae YS, Ahn KS, Won NH. RNA interference-directed caveolin-1 knockdown sensitizes SN12CPM6 cells to doxorubicin-induced apoptosis and reduces lung metastasis. Tumour Biol 2010; 31: 643-650.

[16] Li J, Hassan GS, Williams TM, Minetti C, Pestell RG, Tanowitz HB, Frank PG, Sotgia F, Lisanti MP. Loss of caveolin-1 causes the hyperproliferation of intestinal crypt stem cells with increased sensitivity to whole body gamma-radiation. Cell Cycle 2005; 4: 1817-1825.

[17] Cordes N, Frick S, Brunner TB, Pilarsky C, Grutzmann R, Sipos B, Kloppel G, Mckenna WG, Bernhard EJ. Human pancreatic tumor cells are sensitized to ionizing radiation by knockdown of caveolin-1. Oncogene 2007; 26: 6851-6862.

[18] Zhou H, Jia L, Wang S, Wang H, Chu H, Hu Y, Cao J, Zhang J. Divergent expression and roles for caveolin-1 in mouse hepatocarcinoma cell lines with varying invasive ability. Biochem Biophys Res Commun 2006; 345: 486-494.

[19] Li L, Yang G, Ebara S, Satoh T, Nasu Y, Timme TL, Ren C, Wang J, Tahir SA, Thompson TC. Caveolin-1 mediates testosterone-stimulated survival/clonal growth and promotes metastatic activities in prostate cancer cells. Cancer Research 2001; 61: 4386-4392.

[20] Savage K, Lambros MB, Robertson D, Jones RL, Jones C, Mackay A, James M, Hornick JL, Pereira EM, Milanezi F. Caveolin-1 is overexpressed and amplified in s subset of basal-like and metaplastic breast carcinomas: A morphologic, ultrastructural, immunohistochemical, and in situ hybridization analysis. Clin Cancer Res 2007; 13: 90-101.

[21] Ho CC, Huang PH, Huang HY, Chen YH, Yang PC, Hsu SM. Up-regulation of caveolin-1 accen-

tuates the metastasis capability of lung adenocarcinoma by inducing filopodia formation. Am J Pathol 2002; 161: 1647-1656.

[22] Joo HJ, Oh DK, Kim YS, Lee KB, Kim SJ. Increased expression of caveolin-1 and microvessel density correlated with metastasis and poor prognosis in clear cell renal cell carcinoma. BJU Int 2004; 93: 291-296.