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Adenovirus-mediated Expression of Both Antisense Ornithine Decarboxylase (ODC) and S-adenosylmethionine Decarboxylase (AdoMetDC) Inhibits Lung Cancer Cell Growth And Invasion *In vitro* and *In vivo**

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Abstract Polyamine biosynthesis is controlled primarily by ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC). Antisense ODC and AdoMetDC sequences were cloned into an adenoviral vector (Ad-ODC-AdoMetDCas). To evaluated the effect of recombinant adenovirus Ad-ODC-AdoMetDCas which can simultaneously express both antisense ornithine decarboxylase (ODC) and sadenosylmethionine decarboxylase (AdoMetDC), the human lung cancer cell line A-549, was infected with Ad-ODC-AdoMetDCas as well as with control vector. Viable cell counting, determination of polyamine concentrations, cell apoptosis, and Matrigel invasion assays were performed in order to assess properties of tumor growth and invasiveness. Furthermore, Ad-ODC-AdoMetDCas's anti-tumor effect was also evaluated *in vivo* in a nude mice xenograft model. It was demonstrated that adenovirus-mediated ODC and AdoMetDC antisense expression could inhibit tumor cell growth, lead to cell apoptosis and reduce tumor cell invasiveness. Polyamine levels were significantly decreased in Ad-ODC-AdoMetDCas-treated cells compared with controls. This adenovirus also induced tumor regression in established tumors in nude mice. It was suggested that as a new anticancer reagent, the recombinant adenovirus Ad-ODC-AdoMetDCas holds promising hope for the therapy of lung cancers.

Key words ornithine decarboxylase，S-adenosylmethionine decarboxylase，polyamine，lung cancer，gene therapy

Polyamines are naturally occurring aliphatic polycations found in almost all living organisms. Polyamines include spermidine, spermine, and their diamine precursor, putrescine [1]. Polyamines have critical physiological functions in cell growth and differentiation. In mammalian cells, the intracellular polyamine biosynthetic pathway is primarily regulated by the action of two rate-limiting enzymes. Ornithine decarboxylase (ODC) is the first key enzyme required for polyamine synthesis, decarboxylating ornithine to produce putrescine[2]. The second, rate-limiting enzyme is S-adenosylmethionine decarboxylase (AdoMetDC). It generates the aminopropyl donor, decarboxylated Sadenosylmethionine (dcSAM), by decarboxylating adenosylmethionine. DcSAM donates its propylamine moiety for the formation of spermidine and spermine via catalysis by spermidine synthase and spermine synthase, respectively.

The association of increased polyamine synthesis with cell proliferation and cancer progression was first reported in the late 1960s. High polyamine levels and elevated polyamine synthesis activity were found in many tumors. Environmental and genetic risk factors for cancer, such as ultraviolet light [3] and various oncogenes[4~6], have been reported to induce high ODC activity in normal tissues. Overexpression of ODC or AdoMetDC was also reported to cause malignant transformation of NIH3T3 cells [6,7]. Therefore, inhibition of ODC and/or AdoMetDC activity might

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induce a depletion of intracellular polyamines, providing an effective anticancer treatment strategy. Previous work has primarily focused on the development of polyamine synthesis inhibitors. Difluoromethylornithine (DFMO) irreversibly inactivates ODC activity and has been used in clinical chemoprevention trials for epithelial cancers, including colon, esophageal, breast, cutaneous, and prostate malignancies [8]. AdoMetDC inhibitors, such as methylglyoxalbis (guanylhydrazone) (MGBG), have also been shown to inhibit tumor growth[9]. SAM486A is a new AdoMetDC inhibitor that has been shown to possess anti-proliferative activity in both tissue culture cells and preclinical animal studies[10].

Lung cancer is one of the most lethal cancers known to mainland in China because of the high incidence and high mortality. Metastatic lung cancer is essentially resistant to systemic cytotoxic chemotherapy, while external beam and radioisotope radiotherapy offers only symptom palliation. The development of novel therapies, such as gene therapy, is of high priority.

In the present study, we constructed a replicationdeficient recombinant adenovirus containing antisense sequences of both ODC and AdoMetDC (Ad-ODCAdoMetDCas) to downregulate their gene expression levels simultaneously. Our data show that downregulation of these two key enzymes by Ad-ODC-AdoMetDCas significantly inhibited lung cancer cell growth and tumor invasiveness *in vitro*. Polyamine levels were significantly decreased in Ad-ODC-AdoMetDCas-treated cells compared with controls. More importantly, this recombinant adenoviral vector was also shown to inhibit tumor growth in tumor xenografts in a nude mouse tumor model *in vivo*.

1 Materials and methods

1.1 Cell culture and reagents

Human lung cancer cell line A-549, obtained from The Chinese Academy of Sciences, were maintained in RPMI 1640 medium supplemented with 10% (*v*/*v*) heat-inactivated bovine serum, 100 U/ml penicillin and 100 g/ml streptomycin. HEK293 cells (transformed human embryonic kidney cells), also purchased from The Chinese Academy of Sciences, were grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen USA) containing 10% fetal bovine serum. All cells were cultured in a 5% CO_2 incubator at 37℃ . The polyamine standards (putrescine, spermidine, and spermine) and densyl chloride for high-performance liquid chromatography (HPLC) were purchased from Sigma (St. Louis, MO, USA). An anti-ODC mouse monoclonal antibody (mAb) and an anti-AdoMetDC mouse polyclonal antibody were prepared in our laboratory. An anti-p21 (sc-6246) mousemAb and an antiactin (sc-1616) goat polyclonal antibody were purchased from Santa Cruz Biotechnology. Matrigel and Transwell plates were obtained from BD Biosciences (Bedford, MA, USA) and Costar (Cambridge, MA, USA), respectively.

1.2 Construction of Ad-ODC-AdoMetDCas

The construction of the adenoviral vector, rAd-ODC/EX3as, containing antisense ODC sequence with both a cytomegalovirus (CMV) promoter and a green fluorescent protein (GFP) gene, was reported previously [11]. To construct an adenoviral vector harboring additional antisense AdoMetDC sequence, a 205 bp cDNA fragment of the 5′ end of the AdoMetDC gene was amplified by reverse-transcription polymerase chain reaction (RT-PCR) using specific primers and was subcloned downstream of the ODC gene in the pAd-ODCas vector in the reverse direction. The forward primer was 5′ GGTCTAGATTCGCTAGTCTCACGGTGAT 3′ and the reverse primer was 5′ GGCTCGAGTAAGCTT-CCTGCTTGTCAGT 3′. The sequence of the resulting clone, pAd-ODC-AdoMetDCas, was confirmed by sequencing and was then linearized by digestion with *Pme* Ⅰ and transformed into Adeasier-1 cells containing the 33 kb pAdeasy-1 vector to generate recombinant clones as previously described [12]. The recombinant adenovirus genome was digested with *Pac* Ⅰ and transfected into HEK293 cells with Lipofectamine2000 (Invitrogen USA) for the isolation of recombinant adenovirus.Recombinant viral plaques were identified and amplified by PCR in order to verify ligation success. The recombinant virus particles were purified by CsCl ultracentrifugation [13] and a standard plaque assay was performed to measure the titer of the purified viral stock.The control virus, Ad-GFP, contained no gene insertion in the multiple cloning site.

1.3 Analysis of gene transduction efficiency *in vitro*

The efficiency of adenovirus-mediated gene transfer was assessed by detection of GFP. A-549 cells (3×10^5 cells/well) seeded in 6-well plates were infected

with Ad-GFP at different multiplicities of infection (MOIs) of 5, 10, 20, 50 and 100. GFP expression was analyzed at 48 h after the infection using a flow cytometer (Beckman Coulter, Miami, FL, USA).

1.4 Western blot analysis

After the A-549 cells had been treated with phosphate-buffered saline (PBS), Ad-GFP, Ad-ODCas, and Ad-ODC-AdoMetDCas for 72 h, total cell lysates were prepared in extraction buffer containing 50 mmol/L Tris (pH 8.0), 1% NP-40, 1 mg/L aprotinin, 0.1% sodium dodecyl sulfate (SDS), 0.02% sodium azide, 150 mmol/L NaCl, and 100 mg/L phenylmethylsulfonyl fluoride. Sample protein concentrations were quantified using the bicinchoninic acid (BCA) protein assay. After electrophoresis samples were transferred onto nitrocellulose membranes (Millipore, Bedford, MA, USA). After an incubation with appropriate antibodies in PBS containing 5% nonfat dry milk and 0.02% Tween 20, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies, developed using the Western blotting luminol reagent (Santa Cruz Biotechnology, USA), and exposed to X-ray film (Kodak, Shantou, China).

1.5 Measurement of polyamine content

Polyamine content was measured as previously described[14]. After an incubation with PBS, Ad-GFP, Ad-ODCas, and Ad-ODC-AdoMetDCas for 3 days, A-549 cells were harvested by scraping and permeabilized with 5% trichloroacetic acid. The polyamines in the supernatant were separated and quantified on an ionpaired, reversed-phase HPLC system. Protein content was subsequently measured in the precipitate.

1.6 Measurement of cell growth

Viable cell counts were used to evaluate the effects of recombinant adenovirus on cell proliferation. The lung cancer cells were plated in 6-well tissue culture plates at a density of 5×10^4 cells/well. After 24 h, tumor cells were treated with Ad-GFP, Ad-ODCas, and Ad-ODCAdoMetDCas at an MOI of 50 or with PBS as a control. Cells in each treatment group were plated in triplicate and cultured for 6 days. Cells were then treated with trypsin and harvested every 24 h and subsequently stained with 0.4% trypan blue (Gibco, USA) for the identification of dead cells. Viable cells were then counted using a hemocytometer.

1.7 Cell apoptosis analyzed by terminal deoxynucleotidyl transferase-mediated biotin-dUTP nick-end labeling (TUNEL)

TUNEL assay kit was supplied by Santai Biological Company (Beijing, China) and used to detect apoptotic cells, according to the manufacturer's instructions.

1.8 Matrigel invasion assay

A-549 cells were infected with Ad-GFP, Ad-ODCas, or Ad- ODC-AdoMetDCas at an MOI of 50 for 2 days. Invasiveness was measured by counting cells that had traveled through Matrigel-coated Transwell inserts.Transwell inserts (6.5 mm) with a 8.0 μm pore size were coated with 30 μl of Matrigel and dried for 2 h at room temperature. Cells were harvested as described above. A 100 μl cell suspension containing 5×10^4 cells was added to wells in triplicate. After 24 h of incubation, nonmigrated cells were scraped from the upper side of the membrane with cotton swaps. Cells that passed through the filter into the bottom side of the membrane were fixed and stained with hematoxylin. Five representative fields in each well were quantified to determine the number of invasive cells under a light microscope at 200 × magnification.

1.9 Nude mouse tumorigenicity study

The A-549 cells were infected with Ad-ODC-AdoMetDCas at an MOI of 50 for 48 h, harvested, washed three times with PBS, and resuspended in 1640 medium. The cell suspensions (2×10^6) in a total volume of 100 μl were then injected subcutaneously into 6-week-old BALB/C nude male mice. Tumor volume was measured every week and calculated with the following formula: volume=$M1 \times M2 \times M2 \times 0.5236$ ($M1$, long axis; $M2$, short axis), according to Rockwell, *et al*.[15].

1.10 Statistical analysis

Data are reported as the $\bar{x} \pm s$. Statistical differences between control and treated cells were evaluated using Student's t-test. A value of $P < 0.05$ was considered significant.

2 Results

2.1 Ad-ODC-AdoMetDCas inhibits ODC and AdoMetDC gene expression in lung cancer cells *in vitro*

Adenovirus infects host cells through the coxsackie and adenovirus receptor (CAR)[16]. As the

CAR status in lung cancer cells is largely unknown, we first evaluated adenoviral gene transfer efficiency in tumor cells using Ad-GFP. A-549 tumor cells were infected with AdGFP at MOIs of 5, 10, 20, 50 and 100 for 48 h. We demonstrated that (75.5 ± 2.5)% of A-549 cells were positive for GFP at an MOI of 50, this MOI was used for further study. To study the inhibitory effects of adenoviral vector-gene transfer on both ODC and Ad-ODCas gene expression, A-549 cells were infected with Ad-GFP, Ad-ODCas, and Ad-ODC-AdoMetDCas at an MOI of 50 for 72 h. Protein extracted from both adenoviral vector-treated and control conditions were probed with antibodies against ODC and AdoMetDC. Figure1a , b shows that Ad-ODC-AdoMetDCas induced a greater than 60% reduction of both ODC and AdoMetDC protein in A-549 cells compared with Ad-GFP-infected or uninfected cells. Similarly, Ad-ODC-AdoMetDCas induced a greater than 75% reduction of both ODC and AdoMetDC protein in A-549 cells compared with control conditions. Not surprisingly, ODC protein levels dropped more than 60% in A-549 cells after Ad-ODCas treatment compared with Ad-GFP-infected or uninfected cells. However, there was no appreciable change in AdoMetDC protein levels in Ad-ODCas-treated cells compared with control cells.

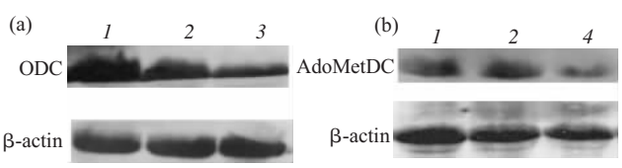

Fig. 1 Western blot analysis of ODC and AdoMetDC gene expression in A-549 cells

Total protein was extracted 3 days after infection with Ad-GFP, Ad-ODCas, or Ad-ODC-AdoMetDCas at an MOI of 50. Each lane was loaded with 50 μg protein and electro-transferred onto a nitocellulose membrane. The blot was probed with either an ODC monoclonal antibody or an AdoMetDC polyclonal antibody. *1*: PBS; *2*: Ad-GFP; *3*: Ad-ODCas; *4*: Ad-ODC-AdoMetDCas.

2.2 Ad-ODC-AdoMetDCas gene transfer decreases polyamine content in lung cancer cells

After demonstrating that Ad-ODC-AdoMetDCas depressed ODC and AdoMetDC protein expression levels in A-549 cells, we next evaluated whether the polyamine content could be decreased accordingly by adenoviral gene transfer into these tumor cells. Polyamines in adenovirus-infected or uninfected lung cancer cells were separated by ion-paired, reversed-phase HPLC. As shown in Table 1, both Ad-ODCas and Ad-ODCAdoMetDCas decreased the polyamine content of A-549 cells, correlating with the downregulation of polyamine biosynthesis. Table 1 also shows that incubation with Ad-ODCas alone caused a drop in putrescine content to undetectable levels in A-549 cells. Spermidine concentrations decreased moderately but significantly, while spermine levels remained unchanged. In cells treated with Ad-ODC-AdoMetDCas, all three polyamines were reduced to very low levels. After a comparison of Ad-ODC-AdoMetDCas- and Ad-ODCas-infected cells, both spermidine and spermine were significantly reduced ($P < 0.05$).

Table 1 Effects of Ad-ODCas and Ad-ODC-AdoMetDCas on polyamine content (mol/g protein) in A-549 cells

Cell lines and treatment	Polyamine pools /(nmol·g^{-1})		
	Put	Spd	Spm
A-549	540.2±12.0	1 390.0±16.1	1 425.0±21.0
Ad-GFP	485.0±9.1	1 275.3±14.0	1 470.0±18.2
Ad-ODCas	235.3±5.2[1)]	1 160.0±10.2[1)]	1 215.0±13.3
Ad-ODC-AdoMetDCas	46.2±6.0[1)]	675.0±15.2[1)]	2 135.2±26.1[1)]

[1)] $P < 0.05$.

2.3 Ad-ODC-AdoMetDCas inhibits lung cancer cell proliferation

After confirming the suppression of ODC and AdoMetDC gene expression and polyamine reduction by adenoviral gene transfer, we then asked whether these inhibitory effects could be translated into

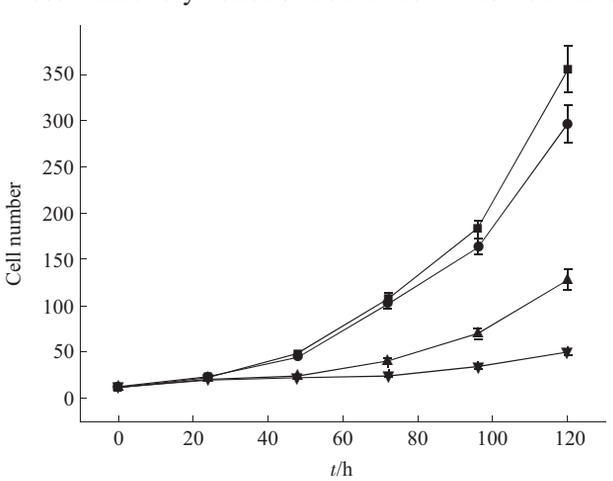

Fig. 2 Effects of Ad-ODCas and Ad-ODC-AdoMetDCas on proliferation of A-549 cell

Cells were seeded at 5 × 10^4 cells/well and allowed to attach for 24 h. Viable cells were counted daily by trypan blue exclusion on days 0～5 after infection with Ad-GFP, Ad-ODCas and Ad-ODC-AdoMetDCas at an MOI of 50 and compared with uninfected cells. ■—■: PBS; ◆—◆: Ad-GFP; ▲—▲: Ad-ODCas; ▼—▼: Ad-ODC-AdoMetDCas.

inhibition of cell growth. We used viable cell counts to determine rates of tumor cell proliferation. The results in Figure 2 demonstrate significant inhibition of cell proliferation in lung cancer cell lines treated with either Ad-ODCas or Ad-ODC-AdoMetDCas (P<0.05) compared with control cells treated with either Ad-GFP or PBS. This inhibition of cell growth was maintained for 7 days (data not shown). Significant differences in the inhibitory effects existed between Ad-ODCas- and Ad-ODC-AdoMetDCas-mediated transduction (P < 0.05). When compared with Ad-ODCas, Ad-ODC-AdoMetDCas was shown to be more effective in inhibiting proliferation of A-549 cells.

2.4 TUNEL assay for apoptosis

To examine the mechanism by which Ad-ODC-AdoMetDCas might retard lung cancer cell growth *in vitro*, we used TUNEL to detect the effect of the Ad-ODC-AdoMetDCas on cell apoptosis at 48 h (Figure 3) and 72 h after infection. As shown in Table 2, the rate of apoptosis in cells infected by Ad-ODC-AdoMetDCas was significantly higher than in cells infected by Ad-GFP or no virus-treated cells (P < 0.05).

Table 2 Apoptosis rate of A-549 lung cancer cells

Cell line and treatment	48 h	72 h
A-549 cell	5.1 ± 0.7	7.6 ± 0.7
Ad-GFP	8.7 ± 0.9	11.3 ± 0.7
Ad-ODCas	23.1 ± 1.5*	56.4 ± 2.3*
Ad-ODC-AdoMetDCas	26.5 ± 1.8*	65.7 ± 3.4*

*P < 0.05.

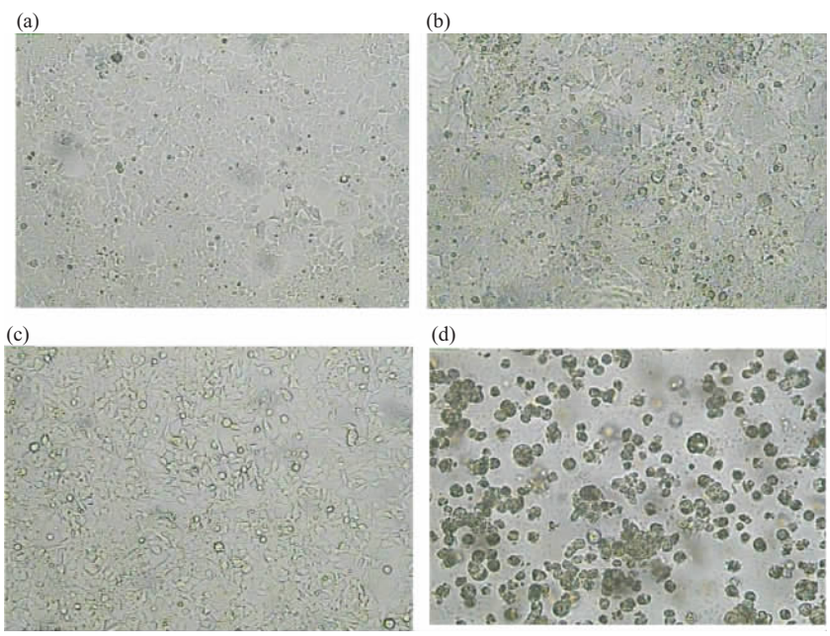

Fig. 3 The effect of Ad-ODC-AdoMetDCas on the apoptosis of A-549 cells 72 h after infection

Cells were observed under 100× microscope.(a) PBS. (b) Ad-ODCas. (c) Ad-GFP. (d) Ad-ODC-AdoMetDCas.

2.5 Ad-ODC-AdoMetDCas impairs tumor invasiveness *in vitro*

The Matrigel assay is a widely used protocol to evaluate tumor invasiveness *in vitro*. We therefore performed the Matrigel assay to evaluate whether either Ad-ODCas or Ad-ODC-AdoMetDCas could decrease tumor invasiveness in addition to their anti-proliferative effects reported above. A-549 cells (5×10^4 cells per insert) were allowed to invade the Matrigel-coated membrane. The numbers of invading cells were represented as the average of five randomly selected microscopic fields on the underside of the membrane (Figure 4a). As shown in Figure 4b, only (8 ± 3) cells in the Ad-ODCAdoMetDCas condition and (19 ± 5) cells in the Ad-ODCas condition passed through the membrane. In comparison, (48 ± 7) cells in the PBS condition and (47 ± 9) cells in the Ad-GFP condition passed through the filter (P < 0.01). In

addition, only 20% of Ad-ODC-AdoMetDCas-infected tumor cells successfully passed through the membrane. These results clearly demonstrate that Ad-ODC-AdoMetDCas significantly decreased tumor invasiveness *in vitro*.

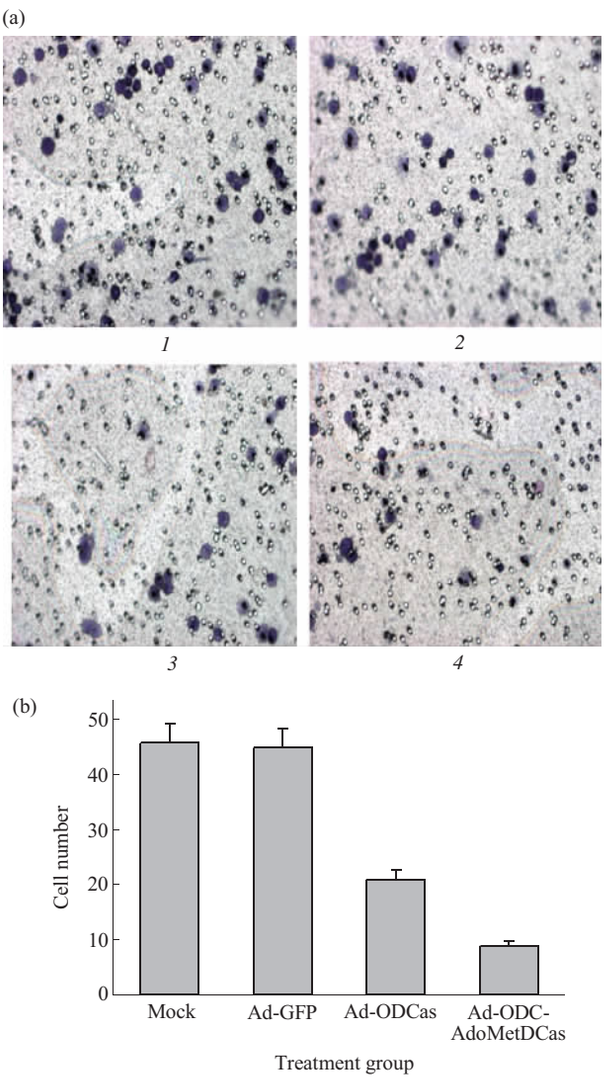

Fig. 4 Ad-ODC-AdoMetDCas inhibited A-540 cell invasion (a) and the numbers of cells that invaded through the Matrigel-coated inserts (b)

A-549 cells were treated with recombinant adenovirus at an MOI of 50 for 72 h and then allowed to invade transwell inserts (8 μm pores) coated with Matrigel for 24 h. The cells that invaded through the inserts were stained, counted, and photographed under light microscopy at 200× magnification. The data are presented as the ($\bar{x} \pm s$) for three separate experiments from each group. *1*: PBS; *2*: Ad-GFP; *3*: Ad-ODCas; *4*: Ad-ODC-AdoMetDCas.

2.6 Anti-tumorigenic effect of Ad-ODC-AdoMetDCas in nude mouse xenograft model

The potential antitumorigenicity of Ad-ODC-AdoMetDCas was evaluated by using a A-549 xenograft model in nude mice. Expression of antisense ODC and AdoMetDC inhibited the growth of A-549 cells *in vivo*, as evidenced by the reduction in tumor incidence and tumor sizes, when compared with those of Ad-GFP treated or no virus-treated tumors (Figure 5a). Mice that received Ad-ODC-AdoMetDCas -treated cells did not develop tumors during a 6-week observation period. The tumor growth rates, calculated by using an exponential curve, were 7.5 mm^3/day and 33.73 mm^3/day for the Ad-GFP-treated and no virus-treated tumors (Figure 5b).

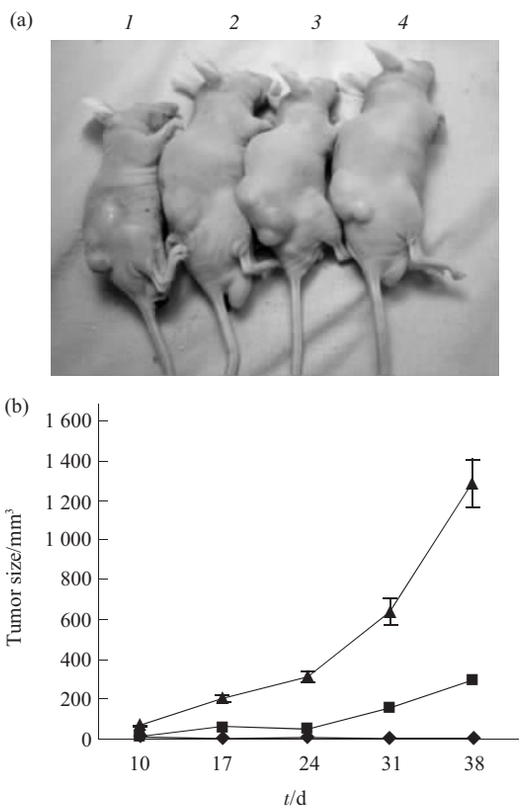

Fig. 5 The picture of mice in four groups (a) and time course of estimated mean tumor volume of either Ad-ODCas and Ad-ODC-AdoMetDCas (b)

1: Ad-GFP treated group; *2*: Ad-ODC-AdoMetDCas treated group; *3*: No virus-treated group; *4*: Ad-ODCas. 2×10^6 A-549 cells infected with adenoviruses or not were injected subcutaneously into nude mice which were observed for 6 weeks. ◆—◆: Ad-ODC-AdoMetDCas; ■—■: Ad-ODCas; ▲—▲: No virus-treated. n=8. Bars represent standard deviation.

3 Discussion

It has been known for many years that normal cell growth is regulated in a cyclical manner by the increase and decrease of cyclins and cyclin-dependent kinases (cdks). Furthermore, there are also changes in

polyamine, ODC and AdoMetDC concentrations during the cell cycle. Both ODC and AdoMetDC mRNA levels and polyamine concentration are doubled during the cell cycle. Elevated levels of ODC and AdoMetDC activity were found in various cancers[17], such as, breast and colorectal cancer, and are related to cancer recurrence[18~20]. Our recent work has proven that inhibition of ODC activity by recombinant antisense ODC adenovirus has had antitumor effects on human lung cancer [1,2]. This adenovirus, however, did not inhibit AdoMetDC, a critical enzyme that is normally elevated in tumor cells. We speculate that double inhibition of ODC and AdoMetDC might be a more effective way to suppress tumor growth. Our *in vitro* study demonstrated more robust antitumor effects by dual inhibition of both ODC and AdoMetDC activities compared to inhibition of ODC activity alone. Double inhibition by Ad-ODCAdoMetDCas infection significantly reduced ODC and AdoMetDC protein levels more than 50% A-549 cells compared to controls. A substantial decrease in ODC and AdoMetDC expression levels also causes a reduction of polyamine biosynthesis. Ad-ODC-AdoMetDCas infection depresses three types of polyamines. In contrast, only putrescine and spermidine were shown to be decreased after Ad-ODCas infection. Ad-ODCas treatment of tumor cells did not elicit a statistical difference in spermine content compared with control treatment. We speculate that the inability of Ad-ODCas to block AdoMetDC activity might be responsible for this observation, consistent with results reported by other researchers who demonstrated that the ODC inhibitor, difluoromethylornithine (DFMO), had no effect on spermine levels in tumor cells. Spermine, however, plays an equally important role in carcinogenesis as do the other polyamines. Furthermore, high levels of spermine also contribute to cellular resistance to apoptotic cell death[21]. The inability of Ad-ODCas to decrease intracellular spermine levels therefore represents an inherent drawback in its potential antitumor effects.

To examine the mechanism of antisense ODC and AdoMetDC inhibiting the growth of lung cancer cells, we demonstrated Ad-ODCAdoMetDCas infection can contribute significantly to cell apoptosis in comparison to Ad-GFP infected or no virus-treated cells by TUNEL. In the last years, some studies had demonstrated the inhibition of ODC could lead to induction of apoptosis of some cancer cells[22,23]. So, our previous study indicated the induction of apoptosis was the mechanism of antisense ODC inhibiting the growth of lung cancer cells.

We also assessed the effects of the two antisense constructs in the context of tumor invasiveness. Both Ad-ODCAdoMetDCas and Ad-ODCas reduced the invasiveness of A-549 cells compared with vector controls. Furthermore, the data also showed that Ad-ODC-AdoMetDCas was superior in inhibiting lung cancer cell invasion compared with Ad-ODCas infection. Overexpression of ODC has been suggested to confer an invasive phenotype on cells. Kubota reported in 1997 [24] that overexpression of ODC in mouse 10T1/2 fibroblasts induced not only cell transformation and anchorage-independent growth in soft agar, but also invasiveness through a Matrigel-coated filter. Similar work had been done by this same group [25] that compared the invasiveness of mouse mammary carcinoma FM3A and EXOD cell lines that overexpress ODC and found that EXOD cells showed more than a 5.6-fold increase in invasiveness compared with FM3A cells by Matrigel assay. Inhibition of ODC by DFMO reduced invasiveness in breast cancer cells significantly[26]. Our previous work in which ODC levels were reduced using the adenovirus-delivered antisense ODC found that lower ODC levels also inhibited tumor invasion in lung cancer [1]. ODC, however,is not the sole enzyme responsible for olyamine biosynthesis or tumor invasion. AdoMetDC was also proven to strongly correlate with progression of tumor invasiveness. Overexpression of AdoMetDC alone has been reported to be sufficient to transform NIH 3T3 cells and induce highly invasive tumors in nude mice [27]. High expression levels of AdoMetDC may compensate for and strengthen the activity of ODC through different molecular pathways[28]. Therefore, we simultaneously targeted both these critical enzymes and obtained superior inhibition of lung cancer invasion.

Finally, the ability of Ad-ODC-AdoMetDCas to suppress the growth of tumor xenografts was investigated in an *in vivo* nude mouse model. Intratumoral injection of Ad-ODC-AdoMetDCas prevented growth of established A-549 tumors compared with injection of control vectors. The combination of the anti-proliferative and anti-invasive effects of Ad-ODC-AdoMetDCas may be the primary causes for the growth inhibition of subcutaneous

tumors *in vivo*.

In summary, we provide evidence that polyamine reduction by antisense techniques that targeted ODC and AdoMetDCas suppresses lung cancer cell growth and invasiveness *in vitro* and leads to significant growth suppression of established tumors *in vivo*. Synergistic inhibition of both ODC and AdoMetDC activities by gene therapy approaches therefore might represent a novel treatment option for lung cancer.

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ODC, AdoMetDC 双反义腺病毒对肺癌增殖和侵袭抑制作用的体外和体内研究 *

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摘 要　鸟氨酸脱羧酶(ODC)和 S- 甲硫氨酸脱羧酶(AdoMetDC)是多胺体内合成的 2 个关键酶. 研究腺病毒 Ad-ODC-AdoMetDCas 介导的 ODC 和 AdoMetDC 反义 RNA 对肺癌多胺合成，细胞增殖以及侵袭的抑制作用. 用活细胞计数和流式细胞术分别检测 Ad-ODCas 和 Ad-ODC-AdoMetDCas 对肺癌 A-549 细胞增殖的影响，蛋白质印迹和 HPLC 方法分别检测腺病毒对肺癌 A-549 细胞中 ODC 和 AdoMetDC 蛋白表达以及胞内多胺含量的抑制作用，TUNEL 标记检测法观察 Ad-ODC-AdoMetDCas对肺癌细胞凋亡的影响，Matrigel 侵袭实验分析腺病毒对肺癌 A-549 细胞侵袭活性的改变，裸鼠皮下移植瘤模型研究 Ad-ODC-AdoMetDCas 对体内肺癌生长的抑制作用. 实验结果显示， Ad-ODC-AdoMetDCas 明显抑制肺癌 A-549 细胞的增殖，导致细胞凋亡，显著降低肺癌 A-549 细胞的体外侵袭能力，肺癌 A-549 细胞感染 Ad-ODC-AdoMetDCas 后细胞内 3 种多胺含量都明显降低，Ad-ODC-AdoMetDCas 对已形成的裸鼠皮下移植瘤具有明显的抑制作用. 实验表明，ODC 和 AdoMetDC 双反义腺病毒具有显著抑制肺癌增殖和侵袭的作用，对于肺癌的防治研究具有一定的前景.

关键词　鸟氨酸脱羧酶，S- 甲硫氨酸脱羧酶，多胺， 肺癌，基因治疗
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