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# Adenovirus-mediated Expression of both Antisense Ornithine Decarboxylase and S-adenosylmethionine Decarboxylase Inhibits Lung Cancer Cell Growth

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**Abstract** Polyamine biosynthesis is controlled primarily by ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC). Antisense sequences of ODC and AdoMetDC genes were cloned into an adenoviral vector (named Ad-ODC-AdoMetDCas). To evaluate the effects of recombinant adenovirus Ad-ODC-AdoMetDCas that can simultaneously express both antisense ODC and AdoMetDC, the human lung cancer cell line A-549 was infected with Ad-ODC-AdoMetDCas or the control vector. Viable cell counting, determination of polyamine concentrations, cell cycle analysis, and Matrigel invasion assays were carried out to assess the properties of tumor growth and invasiveness. Our study showed that adenovirus-mediated antisense ODC and AdoMetDC expression inhibits tumor cell growth through blocking the polyamine synthesis pathway. Tumor cells were arrested at the $G_1$ phase after gene transfer and the invasiveness was reduced. It suggested that the recombinant adenovirus Ad-ODC-AdoMetDCas might be a new anticancer reagent in the treatment of lung cancers.

**Keywords** ornithine decarboxylase; S-adenosylmethionine decarboxylase; polyamine; lung cancer; gene therapy

Polyamines including spermidine, spermine, and their diamine precursor, putrescine [1] are naturally occurring aliphatic polycations in almost all living organisms. Polyamines have critical physiological functions in cell growth and differentiation. In mammalian cells, the intracellular polyamine biosynthetic pathway is primarily regulated by two rate-limiting enzymes. One is ornithine decarboxylase (ODC), the first key enzyme required for polyamine synthesis, decarboxylating ornithine to produce putrescine [2]; the other is S-adenosylmethionine decarboxylase (AdoMetDC), which generates the aminopropyl donor, decarboxylated S-adenosylmethionine, by decarboxylating adenosylmethionine. The decarboxylated S-adenosylmethionine donates its propylamine moiety for the formation of spermidine and spermine through 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 induce a depletion of intracellular polyamines, providing an effective anticancer treatment strategy. Previous work 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 cancers [8]. AdoMetDC inhibitors, such as methylglyoxalbisguanylhydrazone, have also been shown to inhibit tumor growth [9]. SAM486A is a new AdoMetDC

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inhibitor that has been shown to possess antiproliferative activity in both tissue culture cells and preclinical animal studies [10].

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

In the present study, we constructed a replication-deficient recombinant adenovirus (named Ad-ODC-AdoMetDCas) containing antisense sequences of both ODC and AdoMetDC genes to down-regulate their gene expression levels simultaneously. Our data showed that the down-regulation of these two key enzymes by Ad-ODC-AdoMetDCas could significantly inhibit lung cancer cell growth and tumor invasiveness *in vitro*. The tumor cells were arrested in the $G_1$ phase of the cell cycle. Polyamine levels were significantly decreased in Ad-ODC-AdoMetDCas-treated cells compared with controls.

## Materials and Methods

### Cell culture and reagents

Human lung cancer cell line A-549, obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China), was maintained in RPMI 1640 supplemented with 10% (*V*/*V*) heat-inactivated bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. HEK293 cells (transformed human embryonic kidney cells), also purchased from the Institute of Biochemistry and Cell Biology, were grown in Dulbecco's modified Eagle's medium (Invitrogen, Grand Island, USA) containing 10% fetal bovine serum. All cells were cultured in a 5% $CO_2$ incubator at 37 ºC. The polyamine standards (putrescine, spermidine, and spermine) and dansyl chloride for HPLC were purchased from Sigma (St. Louis, USA). An anti-ODC mouse monoclonal antibody and the anti-AdoMetDC mouse polyclonal antibody were prepared in our laboratory. An anti-p21 (sc-6246) mouse monoclonal antibody and an anti-actin (sc-1616) goat polyclonal antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Matrigel and Transwell plates were obtained from BD Biosciences (Bedford, USA) and Costar (Cambridge, USA), respectively.

### Construction of Ad-ODC-AdoMetDCas

The construction of the adenoviral vector, rAd-ODC/EX3as, containing the antisense ODC sequence with both a cytomegalovirus promoter and a green fluorescent protein (GFP) gene, was reported previously [11]. To construct an adenoviral vector harboring an 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 using specific primers and 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′-GGCTCGAGTAAGCTTCCTGCTTGTCAGT-3′. The resulting clone, pAd-ODC-AdoMetDCas, was confirmed by sequencing, 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 Lipofectamine 2000 (Invitrogen) for the isolation of recombinant adenovirus. Recombinant viral plaques were identified and amplified by polymerase chain reaction to verify ligation success. The recombinant virus particles were purified by CsCl ultracentrifugation [13] and a standard plaque assay was carried out to measure the titer of the purified viral stock. The control virus, Ad-GFP, contained no gene insertion in the multiple cloning site.

### Analysis of gene transduction efficiency *in vitro*

The efficiency of adenovirus-mediated gene transfer was assessed by detection of GFP. A-549 cells ($3\times10^5$ cells/well) seeded in 6-well plates were infected with Ad-GFP at different multiplicities of infection (MOI) of 5, 10, 20, 50 and 100. GFP expression was analyzed at 48 h after infection using flow cytometry (Beckman Coulter, Miami, USA).

### Western blot analysis

The A-549 cells were treated with phosphate-buffered saline (PBS), Ad-GFP, Ad-ODCas, or Ad-ODC-AdoMetDCas for 72 h, and total cell lysates were prepared in extraction buffer containing 50 mM Tris (pH 8.0), 1% NP-40, 1 μg/ml aprotinin, 0.1% sodium dodecyl sulfate, 0.02% sodium azide, 150 mM NaCl, and 100 μg/ml phenylmethylsulfonyl fluoride. Protein concentrations were quantified using the bicinchoninic acid protein assay. After electrophoresis, samples were transferred onto nitrocellulose membranes (Millipore, Bedford, USA). After incubation with appropriate antibodies in PBS containing 5% nonfat dry milk and 0.02% Tween-20, the membranes were incubated with horseradish peroxidase-conjugated second-

ary antibodies, developed using the Western blotting luminol reagent (Santa Cruz Biotechnology), and exposed to X-ray film (Kodak, Shantou, China).

### Measurement of polyamine content

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

### 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 $5\times10^4$ cells/well. After 24 h, tumor cells were treated with Ad-GFP, Ad-ODCas, and Ad-ODC-AdoMetDCas at an MOI of 50 or with PBS as a control. Cells in each group were plated in triplicate and cultured for 6 d, then treated with trypsin and harvested every 24 h and subsequently stained with 0.4% trypan blue (Gibco, Gaithersburg, USA) for the identification of dead cells. Viable cells were then counted using a hemocytometer.

### Cell cycle analysis

A-549 cells were seeded at a density of $3\times10^5$ cells/well in 6-well plates and treated with Ad-GFP, Ad-ODCas, or Ad-ODC-AdoMetDCas at an MOI of 50 or with PBS as a control. Three days following treatment, cells were harvested as described above, washed once with cold PBS, and fixed with 70% ethanol. Cells were then washed with ice-cold PBS and treated with RNase. DNA was subsequently stained with propidium iodide. Cell cycle phases were analyzed using FACScan (Becton Dickinson, San Joes, USA).

### Matrigel invasion assay

A-549 cells were infected with Ad-GFP, Ad-ODCas, or Ad-ODC-AdoMetDCas at an MOI of 50 for 2 d. Invasiveness was measured by counting cells that had traveled through Matrigel-coated Transwell inserts. Transwell inserts (6.5 mm) with an 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. Cell suspension (100 μl) containing $5\times10^4$ 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 with magnification of 200×.

### Statistical analysis

Data are reported as the mean±standard deviation. Statistical differences between control and treated cells were evaluated using Student's *t*-test. A value of $P<0.05$ was considered significant.

## Results

### 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 [15]. As the coxsackie and adenovirus receptor status in lung cancer cells are 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 showed 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, or Ad-ODC-AdoMetDCas at an MOI of 50 for 72 h. Proteins extracted from adenoviral vector-treated cells or controls were probed with antibodies against ODC and AdoMetDC. **Fig. 1** showed that Ad-ODC-AdoMetDCas induced a greater than 60% decrease of both ODC and AdoMetDC proteins in A-549 cells compared with Ad-GFP-infected or uninfected cells. Not surprisingly, ODC protein levels dropped more than 50% 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 uninfected cells.

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

After showing 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 transferring adenoviral genes into these tumor cells. Polyamines in adenovirus-

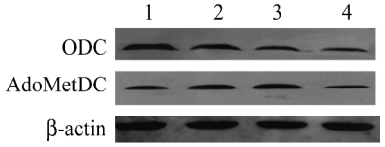


**Fig. 1 Western blot analysis of ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC) gene expression in A-549 lung cancer cells**

Total protein was extracted 3 d after infection with adenoviruses Ad-GFP, Ad-ODCas, or Ad-ODC-AdoMetDCas at a multiplicity of infection of 50. Each lane was loaded with 50 μg protein and electro-transferred onto a nitrocellulose membrane. The blot was probed with either an ODC monoclonal antibody or an AdoMetDC polyclonal antibody. 1, phosphate-buffered saline; 2, Ad-GFP; 3, Ad-ODCas; 4, Ad-ODC-AdoMetDCas.

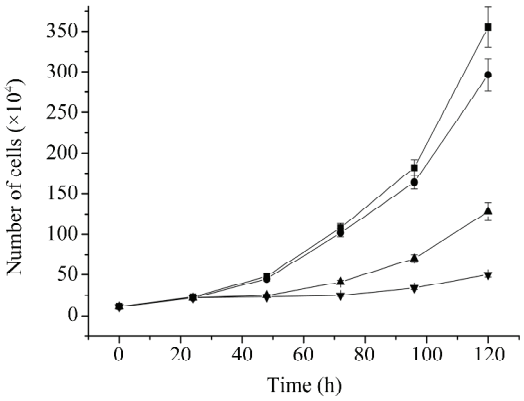


**Fig. 2 Effects of adenoviruses Ad-ODCas and Ad-ODC-AdoMetDCas on proliferation of A-549 lung cancer cells**

Cells were seeded at $5\times10^4$ 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 a multiplicity of infection of 50 and compared with uninfected cells. ■, phosphate-buffered saline

infected or uninfected lung cancer cells were separated by ion-paired, reversed-phase HPLC. As shown in **Table 1**, both Ad-ODCas and Ad-ODC-AdoMetDCas decreased the polyamine content of A-549 cells, correlating with the down-regulation of polyamine biosynthesis. **Table 1** also shows that incubation with Ad-ODCas alone caused a drop in putrescine content in A-549 cells. 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 ($P<0.05$).

## Ad-ODC-AdoMetDCas inhibits lung cancer cell proliferation

We counted viable cell numbers to determine the rate of tumor cell proliferation. The results in **Fig. 2** show a significant inhibition of cell proliferation in lung cancer cell lines treated with either Ad-ODCas or Ad-ODC-AdoMetDCas compared with control cells treated with either Ad-GFP or PBS ($P<0.05$). This inhibition of cell growth was maintained for 7 d (data not shown). Significant differences in the inhibitory effects existed between Ad-ODCas- and Ad-ODC-AdoMetDCas-mediated infection ($P<0.05$) (analysis was done at day 5). Compared with Ad-ODCas, Ad-ODC-AdoMetDCas was more effective in inhibiting proliferation of A-549 cells.

**Table 1 Effects of adenoviruses Ad-ODCas and Ad-ODC-AdoMetDCas on polyamine content (mmol/mg protein) in A-549 lung cancer cells**

| Cell line and treatment | Polyamine pools (pmol/mg protein) | | |
|---|---|---|---|
| | Putrescine | Spermidine | Spermine |
| A-549 | 540±12 | 1390±16 | 1425±21 |
| + Ad-GFP | 485±9 | 1275±14 | 1470±18 |
| + Ad-ODCas | 235±5* | 1160±10* | 1215±13 |
| + Ad-ODC-AdoMetDCas | 46±6* | 675±15* | 235±26* |

Cells were seeded at a density of $1\times10^6$ cells/cm$^2$ and infected at a multiplicity of infection of 50 with Ad-GFP, Ad-ODCas, or Ad-ODC-AdoMetDCas. After 3 d of infection, cells were collected and prepared for HPLC analysis. Results are presented as the mean±SD of three separate experiments. *$P<0.05$ vs. Ad-GFP or uninfected cells.

## Ad-ODC-AdoMetDCas arrests lung cancer cell cycles in $G_1$ phase

We further analyzed cell cycle profiles of adenovirus-infected tumor cells. A-549 cells were treated with PBS, Ad-GFP, Ad-ODCas, or Ad-ODC-AdoMetDCas at an MOI of 50 for 72 h. Cells were then harvested by treatment with trypsin. Propidium iodide staining was used to detect changes in DNA concentrations in different phases of the cell cycle (**Fig. 3**). Results in **Table 2** show that Ad-ODC-AdoMetDCas and Ad-ODCas caused more A-549 cells to arrest compared with controls ($P< 0.05$). A-549 cells were arrested in $G_0$–$G_1$ phase (68%±3.5% in Ad-ODC-AdoMetDCas- and 56%±2.5% in Ad-ODCas-treated cells) compared with 48%±3.5% in PBS-treated cells and 50%±4.5% in Ad-GFP-treated cells. Statistical analysis also revealed a significant difference between Ad-ODC-AdoMetDCas-treated and Ad-ODCas-treated A-549 cells ($P<0.05$) and a greater number of A-549 cells were arrested by Ad-ODCAdoMetDCas.

The cell cycle regulatory protein, $p21^{WAF1/CIP1/SDI1}$ (p21),

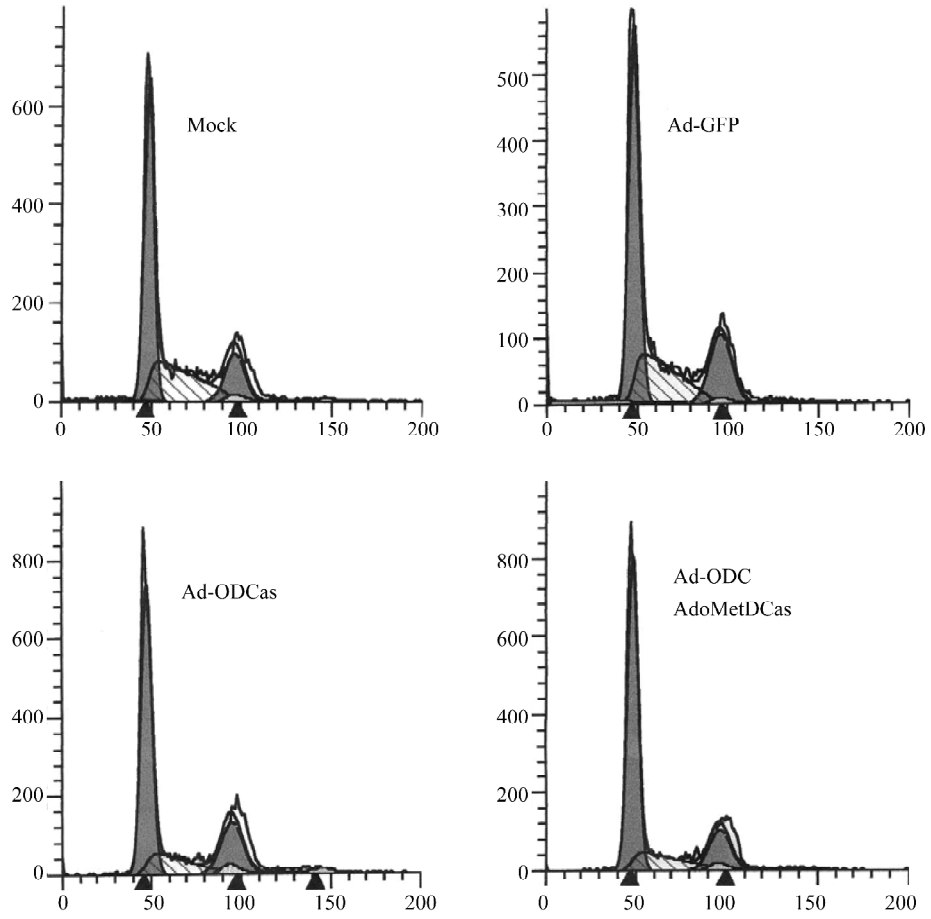


**Fig. 3 Effects of adenoviruses Ad-ODCas and Ad-ODC-AdoMetDCas on A-549 lung cancer cell cycle**

Cells were treated with a multiplicity of infection of 50 of Ad-GFP, Ad-ODCas, Ad-ODC-AdoMetDCas or phosphate-buffered saline (Mock) as a control for 3 d, then collected and dyed with propidium iodide for cell cycle analysis. The data are representative of three separate experiments

**Table 2 $G_0$–$G_1$ cell cycle phase distribution of A-549 lung cancer cells**

| Group | Percent of $G_0$–$G_1$ cells in total cells |
|---|---|
| PBS | 48±3.5 |
| Ad-GFP | 50±4.5 |
| Ad-ODC | 56±2.5* |
| Ad-ODC-AdoMetDCas | 68±3.5* |

*$P$<0.05 vs. cells treated with Ad-GFP or phosphate-buffered saline (PBS). Data were represented as mean±SD.

is known to regulate the $G_1$–S transition [16]. We further analyzed whether p21 gene expression was altered after adenoviral gene transfer and whether it correlated with cell cycle arrest. Expression of p21 in A-549 cells was detected by Western blot analysis. After 3 d of incubation, p21 increased up to threefold in Ad-ODC-AdoMetDCas treated cells (**Fig. 4**). Our data indicated that Ad-ODC-AdoMetDCas treatment arrests tumor cells in the $G_0$–$G_1$ phase. This cell cycle arrest correlates with an increased level of p21 expression.

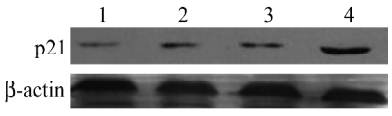


**Fig. 4 Western blot analysis of p21 expression levels in A-549 lung cancer cells**

Total protein was extracted 3 d after infection at a multiplicity of infection of 50. Each lane was loaded with 80 μg of protein and probed with a p21 monoclonal antibody. 1, phosphate-buffered saline-treated cells; 2, Ad-GFP-infected cells; 3, Ad-ODCas-infected cells; 4, Ad-ODC-AdoMetDCas-infected cells.

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

The Matrigel assay is widely used to evaluate tumor invasiveness *in vitro*. We carried out 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 ($5\times10^4$ 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 [**Fig. 5(A)**]. As shown in **Fig. 5(B)**, only 8±3 cells in the Ad-ODC-AdoMetDCas 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 showed that Ad-ODC-AdoMetDCas significantly decreased tumor invasiveness *in vitro*.

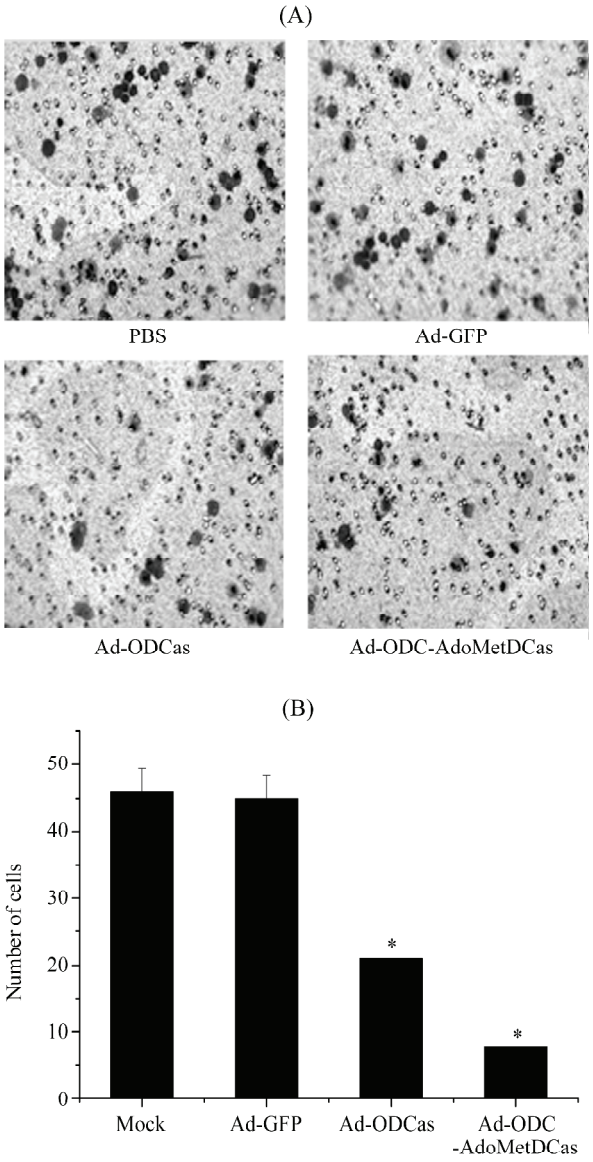


**Fig. 5 Evaluation of effects of adenoviruses Ad-ODCas and Ad-ODC-AdoMetDCas on tumor invasiveness using Matrigel assay**

(A) Adenovirus Ad-ODC-AdoMetDCas inhibited A-549 cell invasion. A-549 cells were treated with recombinant adenovirus at a multiplicity of infection of 50 for 72 h, 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. PBS, phosphate-buffered saline. Magnification, 200×. (B) The numbers of cells that invaded through the Matrigel-coated inserts. The data are presented as the mean±SD for three separate experiments from each group. *$P$<0.05 vs. Ad-GFP or uninfected cells.

## 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. There are also changes in polyamine, ODC and AdoMetDC concentrations during the cell cycle. Both ODC and AdoMetDC mRNA levels and polyamine concentrations are doubled during the cell cycle. Elevated levels of ODC and AdoMetDC activity were found in various cancers, such as prostate, breast, and colorectal cancers, and are related to cancer recurrence [17–19]. Our recent work has proven that inhibition of ODC activity by recombinant antisense ODC adenovirus 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 showed more robust antitumor effects by dual inhibition of both ODC and AdoMetDC activities compared to inhibition of ODC activity alone. Double inhibition by Ad-ODC-AdoMetDCas infection significantly reduced ODC and AdoMetDC protein levels by more than 50% in 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 showed that the ODC inhibitor, 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 [20]. The inability of Ad-ODCas to decrease intracellular spermine levels therefore represents an inherent drawback in its potential antitumor effects.

To further understand the underlying mechanism of tumor cell growth inhibition, the cell cycle and cell cycle related proteins were examined. Previous studies have shown that DFMO arrested a broad spectrum of tumor cell types, such as IEC-6, Hep-2, MKN45, and HL-60, in the $G_1$ phase [21]. Our recent work also showed that treatment of A-549 cells with Ad-ODCas causes lung cells to accumulate in $G_0/G_1$ [1]. In agreement with these findings, we showed that both Ad-ODC-AdoMetDCas and Ad-ODCas decreased the rate of DNA synthesis of lung cancer cells and blocked the cell cycle at the $G_1$/S boundary. This result also suggests that synergistic inhibition of ODC and AdoMetDC activities might be more effective in inducing cell cycle arrest and halting cell growth than a single blockade of ODC activity. These data are in agreement with a report that treatment of MALME-3M cells with either the ODC inhibitor, DFMO, or the AdoMetDC inhibitor, MDL-73811, slowed cell growth but failed to induce cell cycle arrest, and treatment with a combination of both inhibitors halted cell growth and caused a significant $G_1$ arrest [22].

Furthermore, it also showed that depletion of the polyamine pool, achieved by specific biosynthetic enzyme inhibitors, was associated with an increase in p21 gene expression. The induction of p21 expression was also observed in Ad-ODC-AdoMetDCas-infected lung cancer cells but not in Ad-ODCas-infected cells (**Fig. 4**). p21 was reported to dephosphorylate retinoblastoma protein and subsequently inhibit E2F transcription factors, which act as positive regulators of many genes involved in cell proliferation. Thus, p21 and p21-mediated pathways might be the main mechanisms through which Ad-ODC-AdoMetDCas mediates more significant effects on cell cycle arrest and cell growth inhibition than does Ad-ODCas.

We also assessed the effects of the two antisense constructs on tumor invasiveness. Both Ad-ODC-AdoMetDCas 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. Kubota *et al.* [23] reported 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 by this same group [24] showed that the ODC-overexpressing EXOD mouse mammary carcinoma cell line was 5.6-fold more invasive in the Matrigel assay than the FM3A mouse mammary carcinoma cells that did not overexpress ODC. Inhibition of ODC by DFMO reduced invasiveness in breast cancer cells significantly [25]. 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 polyamine 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 NIH3T3 cells and induce highly invasive tumors in nude mice [26]. High expression levels of AdoMetDC might compensate for and strengthen the activity of ODC through different molecular pathways [27]. Therefore, we simultaneously targeted both these critical enzymes and obtained superior inhibition of lung cancer invasion.

In summary, we provided evidence that polyamine reduction by antisense techniques that targeted ODC and AdoMetDCas suppresses lung cancer cell growth and invasiveness *in vitro*. 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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