Aerosol delivery of urocanic acid–modified chitosan/programmed cell death 4 complex regulated apoptosis, cell cycle, and angiogenesis in lungs of K-ras null mice

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Abstract

The low efficiency of conventional therapies in achieving long-term survival of patients with lung cancer calls for development of novel treatment options. Although several genes have been investigated for their antitumor activities through gene delivery, problems surrounding the methods used, such as efficiency, specificity, and toxicity, hinder application of such therapies in clinical settings. Aerosol gene delivery as nonviral and noninvasive method for gene therapy may provide an alternative for a safer and more effective treatment for lung cancer. In this study, imidazole ring-containing urocanic acid–modified chitosan (UAC) designed in previous study was used as a gene carrier. The efficiency of UAC carrier in lungs was confirmed, and the potential effects of the programmed cell death protein 4 (PDCD4) tumor suppressor gene on three major pathways (apoptosis, cell cycle, and angiogenesis) were evaluated. Aerosol containing UAC/PDCD4 complexes was delivered into K-ras null lung cancer model mice through the nose-only inhalation system developed by our group. Delivered UAC/PDCD4 complex facilitated apoptosis, inhibited pathways important for cell proliferation, and efficiently suppressed pathways important for tumor angiogenesis. In summary, results obtained by Western blot analysis, immunohistochemistry, and terminal deoxynucleotidyl transferase–mediated nick end labeling assay suggest that our aerosol gene delivery technique is compatible with in vivo gene delivery and can be applied as a noninvasive gene therapy. [Mol Cancer Ther 2006;5(4):1041–9]

Introduction

The low efficiency of conventional therapies in achieving long-term survival of patients with lung cancer calls for development of novel treatment options. Viral and nonviral vectors have been used as gene delivery systems (1). Despite their usefulness in gene delivery, existing viral vectors have their down sides. To date, recombinant adenoviral vectors have been used for gene delivery because of their high affinity for airway epithelium and transfection efficiency for pulmonary cells (2). However, the degree of toxicity (3) and the subject’s immune response against such vectors upon repeated administration limited application of such systems in practice (4, 5). Moreover, viral vectors are difficult to produce on a large scale (6). Nonviral vectors, on the other hand, have been increasingly proposed as safer alternatives to viral vectors for their potential to be given repeatedly with minimal host immune response. In addition, nonviral vectors have high specificity, targetability, and stability in storage and simplicity in large-scale production (7). Therefore, we suggest that gene delivery through inhalation, a nonviral, noninvasive treatment option with numerous advantages over viral, invasive modes of gene delivery, may be useful in treatment of a wide range of pulmonary disorders.

Several studies have shown that binding of DNA with cationic polymers, such as polylysine, polyethylenimine, protamine, and histones, may improve transfection efficiency both in vitro and in vivo (8). These polymers enhance transfection via condensing DNA into nanoparticles, protecting DNA from enzymatic degradation, facilitating
cellular uptake of DNA complexes, and aiding in endosomal escape (9), as well as contributing to stability during nebulization (10). For nonviral vectors to be used as an effective tool for gene delivery, their guaranteed transfection efficiency is necessary. Multiple steps are involved in transfection, such as DNA complexation, cellular uptake of the complexes, release of DNA or complexes from endosomes, release of DNA from the carriers, and DNA transfer into the nucleus (11, 12). Chitosan is a good candidate for gene carrier owing to its proven biocompatibility, biodegradability, low toxicity, and high cationic potential (13). However, inefficient release of chitosan/DNA complexes from endocytic vesicles into the cytoplasm is one of the primary causes of poor gene delivery (14). To overcome such obstacle, in a previous study, we have designed imidazole ring-containing urocanic acid–modified chitosan (UAC) and proved the efficiency of UAC for gene delivery in vitro by enhancing the release of UAC/DNA complexes following the endosomal rupture through proton sponge mechanism (15). In this study, UAC was adopted as a gene carrier for aerosol gene delivery in attempt to develop a safer and more efficient system for in vivo lung cancer therapy.

Approximately 30% of human tumors carry ras gene mutations. Of the three members of the ras-family (K-ras, N-ras, and H-ras), K-ras is found to be the most frequently mutated member in human tumors, including lung adenocarcinomas (25–50%; ref. 16). Mice carrying such mutations are highly predisposed to a range of tumor types and exhibit short latency and high penetrance (17). In this study, K-ras null mice, a laboratory animal model of non–small cell lung cancer (NSCLC), was used for in vivo assessment of the role of programmed cell death protein 4 (PDCD4) in lung tumorigenesis.

The inhibitor of translation initiation PDCD4 is a novel candidate for cancer preventive intervention, having recently been validated for prevention of tumorigenesis and tumor progression in transgenic mouse model of skin carcinogenesis (18). In addition, a recent study indicated that the loss of PDCD4 expression is linked to tumor progression and poor prognosis in humans (19). To investigate whether PDCD4 prevents or inhibits lung tumorigenesis in vivo, PDCD4 with UAC was delivered into K-ras null mice through inhalation. Thus, aerosol delivery of PDCD4 facilitated apoptosis and suppressed pathways important for cell cycle and tumor angiogenesis in the lungs of K-ras null mice. Our results support the possibility that PDCD4 may suppress pulmonary carcinogenesis as a tumor suppressor gene and suggest that PDCD4 gene delivery may be a promising approach for lung cancer prevention.

Materials and Methods

Materials

Water-soluble chitosan (molecular weight = 100 kDa, 80% degree of deacetylation) was kindly donated by Prof. Nah (Sunchon National University, Sunchon, Korea), and the method for free amine chitosan preparation was described in a previous study (20). pGL3 construct containing SV40 promoter with high expression level of luc+ was purchased from Promega (Madison, WI). pcDNA3.1-GFP (6.1 kb) was purchased from Invitrogen (Carlsbad, CA). Monoclonal PDCD4 antibody was raised using a general method described elsewhere. BAD, BAX, Bcl-2, Bcl-Xl, Apaf-1, p53, p27, p21, fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), cyclin D1, cyclin-dependent kinase 4, and proliferating cell nuclear antigen antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Glyceraldehyde-3-phosphate dehydrogenase antibodies were obtained from BD Biotechnology (San Jose, CA).

Preparation of UAC and UAC/DNA Complexes

UAC was prepared as previously described (15). Briefly, chitosan was coupled with urocanic acid (70 mole %) via an active ester intermediate using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (Peptide Institute, Osaka, Japan). Free amino groups of UAC were determined by ninhydrin assay using glucosamine standard, and the composition of UAC was verified by nuclear magnetic resonance spectroscopy (600 MHz; Bruker BioSpin, Munich, Germany). With careful and extensive preliminary studies, UAC/DNA complexes at charge ratio of 30 were chosen as the most efficient condition for aerosol gene delivery. Briefly, self-assembled UAC/DNA complexes were initiated in distilled water by adding 1 mg of DNA plasmid to UAC, drop by drop, under gentle vortexing, and the final volume was adjusted to 50 mL. The complexes were then incubated at room temperature for 30 minutes before use.

Evaluation of Cytotoxicity

Cytotoxicity of chitosan and UAC at different concentrations was determined by using CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) following the manufacturer’s protocol. Human lung carcinoma A549 cells were seeded at a density of 1 × 10^4 per well in a 96-well plate and incubated for 24 hours before replacing media with fresh serum-free DMEM containing polymers at various concentrations. Cells were incubated with the polymers for 48 hours before treated with CellTiter 96 AQueous One Solution Reagent. Polymer-untreated cells in media were used as a control.

In vivo Aerosol Delivery of UAC/PDCD4 Complexes

Experiments were carried out on 12-week-old male ICR and K-ras null mice. ICR mice for in vivo gene transfection efficiency were purchased from Joongang Laboratory Animal (Seoul, Korea). Breeding K-ras null mice were obtained from Human Cancer Consortium-National Cancer Institute (Frederick, MD) and kept in the laboratory animal facility with temperature and relative humidity maintained at 23 ± 2 °C and 50 ± 20 °C, respectively, and were kept on a 12-hour light/dark cycle. All methods used in this study were approved by the Animal Care and Use Committee at Seoul National University and conform to the NIH guidelines (NIH publication no. 86-23, revised 1985). Mice were placed in nose-only exposure chamber (Dusturbo, Seoul, Korea) and exposed to the aerosol based on the method used previously (6). Briefly, aerosol was generated using...
the patented nebulizer (patent no. 20304964), which was designed to minimize sample loss as well as shearing force. For the delivery efficiency, ICR mice were exposed to aerosol containing UAC/PDCD4 complex solution (50 mL) of contained 1 mg of pcDNA3.0-PDCD4 plasmid DNA. K-ras null null mice were placed in nose-only exposure chamber and were allowed to inhale the aerosolized UAC/DNA complexes from the nebulizer for 30 minutes. For delivery efficiency, ICR mice were exposed to aerosol containing UAC/green fluorescent protein (GFP) complex with chitosan/GFP complex as a control under same condition. Two days after exposure, the mice were sacrificed, and the lungs were collected for further analysis.

**Western Blot Analysis**

Protein concentration of the homogenized lysates was measured using Bradford kit (Bio-Rad, Hercules, CA), and equal amounts (50 μg) of protein were separated on SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were then blocked for 1 hour in TTBS containing 5% skim milk, and immunoblotting was done by incubating the membranes overnight at 4°C with corresponding primary antibodies in 5% skim milk and then with secondary antibodies conjugated to horseradish peroxidase for 3 hours at room temperature or overnight at 4°C. After washing, the bands of interest were analyzed by luminescent image analyzer LAS-3000 (Fujifilm, Tokyo, Japan).

**Immunohistochemistry**

Formalin-fixed, paraffin-embedded tissue sections were cut at 5 μm and transferred to plus slides (Fisher Scientific, Pittsburgh, PA). The tissues were deparaffinized in xylene and rehydrated through alcohol gradient. The tissue sections were incubated in 200 μL proteinase K and then washed and incubated in 0.3% hydrogen peroxide (ApplyChem, Darmstadt, Germany) for 30 minutes to quench endogenous peroxidase activity. After washing in PBS, the tissue sections were incubated with 5% bovine serum albumin in PBS for 1 hour at room temperature to block unspecific binding sites. Primary antibodies were applied on tissue sections overnight at 4°C. On the following day, tissue sections were washed and incubated with secondary horseradish peroxidase–conjugated antibodies for 1 hour at room temperature. After careful washing, tissue sections were counterstained with Mayer’s Hematoxylin (DAKO, Carpinteria, CA) and washed with xylene. Cover slips were mounted using Permount (Fisher Scientific), and the slides were viewed under a light microscope (Carl Zeiss, Thornwood, NY).

**Terminal Deoxynucleotidyl Transferase–Mediated Nick End Labeling Assay**

Formalin-fixed, paraffin-embedded lung tissue slides were deparaffinized in xylene and rehydrated through alcohol gradient. The slides were washed with PBS, and nicked DNA ends were labeled by terminal deoxynucleotidyl transferase–mediated nick end labeling method using in situ cell death detection kit (Roche, Basel, Switzerland) following the manufacturer’s protocol. As a final step, tissue sections were counterstained with methyl green (Trevisgen, Gaithersburg, MD).

**Data Analysis**

Quantification of Western blot analysis was done by using Multi Gauge version 2.02 program (Fujifilm). All results are given as means ± SD. The results were analyzed by Student’s t test (GraphPad software, San Diego, CA). *, P < 0.05 was considered significant, and **, P < 0.01 was highly significant compared with corresponding control values. #, P < 0.05 was considered significant, and ##, P < 0.01 was highly significant compared with corresponding values from vector control.

**Results**

**UAC, with Its Low Toxicity and Good DNA Carrying Capacity, Is a Suitable Carrier for Gene Delivery**

Nuclear magnetic resonance spectra confirmed successful synthesis of UAC and the substitution degree obtained of urocanic acid coupled with chitosan was ~37.4 mole % of amine groups in chitosan, a value similar to that of UAC70, which had the highest transfection efficiency in a previous study (15). The synthesized UAC exhibited a high capacity for condensing DNA as well as protecting DNA from DNase I attack (data not shown). The cell viability of human lung carcinoma A549 cells treated with a wide range of concentrations of chitosan is shown in Fig. 1A. Chitosan exhibited low toxicity, and chitosan coupled with urocanic acid showed no adverse effect on cell viability. Average cell viability of ≥90% was obtained from both chitosan and UAC groups. As mentioned earlier, UAC was adopted for enhanced gene delivery efficiency through proton sponge mechanism. Gene delivery efficiency of GFP and PDCD4, a reporter gene and a target gene, respectively, was tested. Immunohistochemical analysis of mouse-specific intracellular antigen against macrophage and monocyte showed that delivered UAC/GFP complexes successfully avoided the attack of alveolar macrophage. Efficient transfection of delivered UAC/GFP complexes into lung cells was confirmed. As shown in Fig. 1B, the green signal of GFP is dominant (c) compared with the red signal of mouse-specific intracellular antigen against macrophage and monocyte (d). Green and red signals in control chitosan/GFP–treated lungs (a and b) were negligible. Please note that dominant green signals represent the expression of GFP protein escaped from digestion by alveolar macrophage and monocyte shown with red color. Western blot analysis of PDCD4 protein in the lungs of mice exposed to aerosol UAC/PDCD4 complexes confirmed a significant increase in the PDCD4 protein level compared with that of control and vector control (Fig. 1C). Immunohistochemical analysis further confirmed successful transfection and expression of aerosol-delivered PDCD4 in the lungs (Fig. 1D). Together, these results indicate that UAC aerosol has low cytotoxicity as well as good DNA carrying capacity with high transfection efficiency.

**Aerosol Delivery of UAC/PDCD4 Complexes Facilitates Apoptosis in the Lungs of K-ras Mice**

As mentioned earlier, overexpression of PDCD4 induces apoptosis in breast cancer cells. To determine whether...
aerosol-delivered PDCD4 would induce apoptosis in the lungs of K-ras null mice with pulmonary cancer. Western blot assay was done to quantify protein expression levels of representative apoptosis signals. Aerosol-delivered PDCD4 significantly increased proapoptotic signals, such as BAD and Apaf-1, whereas BAX protein level remained unchanged. On the other hand, aerosol-delivered UAC/PDCD4 complexes significantly suppressed antiapoptotic Bcl-2 activity with no changes in that of Bcl-XL (Fig. 2A). In addition to densitometric analysis (Fig. 2B), terminal deoxynucleotidyl transferase–mediated nick end labeling assay clearly showed that aerosol-delivered UAC/PDCD4 complexes facilitated apoptosis in the lungs of K-ras null mice because apoptotic signals (dark blue) are distinctive compared with control and vector control (Fig. 2C).

**Aerosol-Delivered PDCD4 Suppressed Lung Cancer Cell Growth by Controlling the Pathways Important for Cell Cycle and Angiogenesis**

Recently, a study showed repression of colon cancer growth by PDCD4 and indirect suppression of tumor growth in lungs by PDCD4 expression (21). To determine aerosol-delivered PDCD4’s capacity for controlling lung cancer growth through cell cycle regulation, expression...
levels of cell cycle proteins were examined. Aerosol-delivered PDCD4 significantly increased the pulmonary protein expression levels of p27 and p21, signal molecules associated with cell cycle arrest, with no significant change of p53 protein in the lungs of K-ras null mice treated with aerosol-delivered PDCD4 compared with those of control and vector control (Fig. 3A and C). In addition, the protein level of cyclin D1 remained unchanged, whereas suppression of cyclin-dependent kinase 4 and proliferating cell nuclear antigen proteins was observed (Fig. 3B and D). In particular, immunohistochemical analysis showed that the expression of proliferating cell nuclear antigen was significantly suppressed in the lungs of K-ras null mice treated with aerosol-delivered PDCD4 compared with control and vector control (Fig. 3E). Above results suggest that aerosol-delivered PDCD4 is capable of inducing cell cycle arrest in the lungs of K-ras null mice. VEGF and FGF-2 are known to stimulate angiogenesis in both in vitro and in vivo animal models and have been widely studied and may serve as prototypes of proangiogenic drugs (22). Therefore, changes in protein expression of VEGF and FGF-2 in the lungs of group treated with aerosol-delivered PDCD4 were investigated by Western blotting analysis, VEGF and FGF-2 expression levels significantly decreased compared with untreated control and vector control.

Figure 2. Western blot analysis of aerosol-delivered PDCD4-induced apoptosis in lungs. A, lysates from the lungs of K-ras null mice treated with aerosol-delivered PDCD4 were analyzed for protein levels of BAD, BAX, Apaf-1, Bcl-2, and Bcl-XL by Western blot. B, bands of interest were further analyzed by densitometer. *, P < 0.05, considered significant. **, P < 0.01, highly significant compared with corresponding control. #, P < 0.05, considered significant. ##, P < 0.01, highly significant compared with corresponding vector control. Data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Columns, mean (n = 3); bars, SE. C, terminal deoxynucleotidyl transferase-mediated nick end labeling assay. Apoptotic signals (dark blue) were clearly detected in the lungs of K-ras null mice treated with aerosol-delivered PDCD4 compared with vector control. Please also note that some expression of apoptotic signals was even observed in vector control. CON, control; VEC, vector control; PDCD4, aerosol PDCD4-delivered lungs. Magnification, ×200. Bar, 100 μm.
groups (Fig. 4A and B). Immunohistochemical analysis further confirmed that expression of FGF-2 protein was significantly suppressed in the lungs of K-ras null mice treated with aerosol-delivered PDCD4 compared with control and vector control lungs (Fig. 4C). Taken together, our results suggest that aerosol delivery of PDCD4 also result in the inhibition of tumor angiogenesis.

Discussion

The low efficiency of conventional therapies in achieving long-term survival of patients with lung cancer calls for development of novel treatment options. Substantial efforts have been made to develop an efficient method for delivery of a wide range of therapeutic agents to lungs through inhalation. As mentioned earlier, nonviral
Vectors for gene therapy have been increasingly proposed as safer alternatives to viral vectors. Nonviral vectors manifest considerable advantages over their viral counterparts. However, nonviral vector systems have their own downsides, such as low transfection efficiency, short duration of target gene expression, and limited targeting (23). To overcome such obstacles, numerous attempts have been made for modifications of existing carriers.

We previously developed modified chitosan and employed it as a gene carrier in this study. Chitosan has been widely used in pharmaceutical research and industry for drug delivery. For example, chitosan is used as biomedical materials for artificial skin construction and wound-healing bandage applications for its nonimmunogenicity, excellent biocompatibility, and low toxicity (24). However, practical application of chitosan is limited due to its high molecular weight, resulting in low solubility in aqueous media. Moreover, it has low transfection efficiency, resulting from inefficient release of chitosan/DNA complex from endocytic vesicles into cytoplasm (14). To take advantage of the benefits chitosan offers while minimizing its negative effects, we previously developed UAC, a modified chitosan, as a novel gene carrier and showed its biocompatibility and gene delivery efficiency (15). In this study, we confirmed low cytotoxicity of UAC (Fig. 1A) and its ability to escape from the attack of alveolar macrophage (Fig. 1B) and showed successful delivery and expression of the gene of interest as a result of aerosol delivery using UAC as a carrier. It can be concluded from these results that UAC is an excellent candidate for a gene carrier for aerosol gene delivery in vivo.

Efficient inhibition of growth and apoptosis of cancer cells are important factors in prevention and treatment of lung cancer. Given such facts, the most efficient way to counter lung cancer would be to induce apoptosis and cell cycle arrest in cancer cells. Accumulating evidences indicate that mitochondria play a pivotal role in apoptotic process in mammalian cells. Disruption of mitochondrial membranes indicates mitochondrial damage and is generally defined as the early stage of apoptosis followed by the efflux of small molecules, such as Apaf-1 (25). Our results also showed that aerosol delivery of PDCD4 significantly increased the protein expression of Apaf-1 (Fig. 2A and B), thus suggesting that mitochondria-mediated pathway might be involved in PDCD4-triggered apoptosis in the lung of K-ras null mice. This observation is consistent with the finding of a recent study about the apoptotic effect of PDCD4 in breast cancer cell line (26). Our results also indicated that delivered PDCD4 suppressed the protein expression of Bcl-2, whereas Bcl-XL level remained unchanged (Fig. 2A and B). Both Bcl-2 and Bcl-XL are located mainly in the outer membranes of mitochondria. They play a small role in prevention of apoptosis by preventing the release of cytochrome c into the cytoplasm. The decrease in the protein expression of Bcl-2 suggests that
aerosol-delivered PDCD4 partly induces apoptosis by inhibiting antiapoptotic Bcl-2 protein. On the other hand, the role of Bcl-X\textsubscript{L} in PDCD4-mediated apoptosis may be somewhat different. The unchanged protein expression level of Bcl-X\textsubscript{L} may be related to multidrug resistance. In fact, NSCLC often shows intrinsic multidrug resistance, one of the most serious problems in cancer chemotherapy, because anticancer drugs exert at least part of their cytotoxic effect by triggering apoptosis. A recent line of evidence indicated that Bcl-X\textsubscript{L} is required for resistance response to cisplatin-induced apoptosis in NSCLC (27). In this study, K-ras null mice, models of NSCLC, were used, thus suggesting that aerosol-delivered PDCD4 could facilitate apoptosis by inhibiting antiapoptotic Bcl-2 and partially suppressing the activity of multidrug resistance–related Bcl-X\textsubscript{L} protein and by increasing the levels of proapoptotic proteins, such as BAD and Apaf-1 (Fig. 2A and B). Interestingly, aerosol-delivered PDCD4 increased the levels of BAD protein, whereas no changes in BAX protein levels were observed (Fig. 2A and B). Both BAD and BAX proteins are involved in apoptosis. Therefore, the change in the protein expression level of only one of the proteins suggests that each of the proteins might possess different modes of action. This hypothesis is further confirmed by the recent report that BAX- and BAD-mediated apoptosis can be controlled in different manners by cooperation of amphiregulin and insulin-like growth factor through protein kinase C–dependent pathways in NSCLC (28). Further studies on the relative roles of BAX and BAD in PDCD4-induced apoptosis are in progress. As for the proapoptotic effect of UAC, UAC alone seemed to increase apoptosis in the vector control group. Such effect of UAC on apoptosis could be observed by terminal deoxynucleotidyl transferase–mediated nick end labeling assay shown in Fig. 2C. Such results suggest that UAC alone might induce apoptosis. In fact, a recent study showed induced apoptosis by water-soluble chitosan through activating caspase (29). Together, the inherited proapoptotic capacity of UAC and the efficient induction of pathways important for apoptosis strongly suggest that UAC/PDCD4 complex is an excellent candidate for lung cancer gene therapy.

In the last decade, many studies have focused on the correlation between cell cycle control and lung carcinogenesis. Just as apoptosis is controlled by highly conserved machinery, cell cycle is also a highly conserved mechanism by which eukaryotic cells proliferate. In fact, cell cycle control is closely associated with apoptosis. In this study, the effects of aerosol-delivered PDCD4 on cell cycle control in lungs of K-ras null mice were investigated. Initiation of cell cycle control via extracellular signals induces the transcription of several proteins, including cyclin D, which, when complexes with cyclin-dependent kinase 4, moves into the next cell cycle (30). In our study, aerosol-delivered PDCD4 may regulate lung cancer growth through increasing the cell cycle inhibitors, such as p21 and p27, while suppressing cell proliferation proteins, such as cyclin-dependent kinase 4 and proliferating cell nuclear antigen (Fig. 3A-E). Interestingly, the effect of aerosol-delivered PDCD4 on p53 was insignificant. However, cell growth may be regulated by p53 target gene p21 because some research reports showed that p53 could bind to specific DNA sequence and transactivate a group of target genes, including p21, thereby inhibiting cell proliferation and promoting apoptosis (30).

Aerosol-delivered PDCD4 induced inhibition of pathways important for tumor angiogenesis (Fig. 4A-C). Angiogenesis is a prerequisite for tumor growth because tumors cannot grow beyond several mm\(^3\) when new vessel formation is blocked (31). VEGF has been recognized as the most important growth factor involved in angiogenesis because it provides the tissue surrounding the tumor with nutrients and enhances vascular permeability and is also known to induce tumor metastasis (32). Many researches have shown a close connection between VEGF and tumor vascular growth, and resistance to therapy, and poor prognosis has been reported for patients with NSCLC (33). In addition, FGF is released by proteolytic enzymes from extracellular matrix, which in turn increases the expression of other proteolytic molecules. It is known to be proangiogenic and increase tumor growth (34). Our results clearly showed that aerosol-delivered PDCD4 suppressed both of abovementioned proangiogenic growth factors, thus suggesting that aerosol-delivered PDCD4 is an important agent for lung cancer prevention as well as treatment.

Through this study, we emphasize the importance of developing effective and selective preventive agents for lung cancer through an extensive research into the therapeutic effects of noninvasive biomarkers primarily involved the three major pathways mentioned above. In conclusion, aerosol-delivered UAC/PDCD4 complex efficiently regulated the three major pathways important for apoptosis, cell cycle, and angiogenesis. The results of our study strongly suggest that aerosol gene delivery may provide an effective noninvasive model of gene delivery, and PDCD4 may be effective in targeting translation initiation for lung cancer prevention as well as treatment.

References


Correction: Aerosol Delivery of Urocanic Acid–Modified Chitosan/Programmed Cell Death 4 Complex Regulated Apoptosis, Cell Cycle, and Angiogenesis in Lungs of K-ras Null Mice

In this article (Mol Cancer Ther 2006;5:1041–1049), which was published in the April 2006 issue of Molecular Cancer Therapeutics (1), the authors incorrectly described the use of Kras null mice in their experiments; they actually used the KrasLA1 model. The authors regret this error.

Reference


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