Lung cancer is the number one cause of cancer death and the second most frequent cancer reported each year in the United States (1). Despite recent advances in therapeutic modalities, the severe mortality of lung cancer has not improved, and the need to develop novel therapeutic approaches for this disease is urgent. In addition to developing effective therapeutic modalities, researchers have made extensive efforts to discover effective drug delivery systems. Carrier-assisted, localized drug delivery delivery enables drugs to avoid first-pass metabolic degradation in the liver and increases their efficacy and anticancer effects. Liposomes have been proposed and used as a good carrier of chemicals and drugs due to the simplicity of preparation and its high biocompatibility (2, 3). The greatest potential advantage to using a liposome-encapsulated agent to prevent or treat lung cancer is the ability to deliver the agent directly to the lungs through natural or device-assisted inhalation of the aerosol remedy (4, 5).

Given the complexity of the signaling pathways present in most cancers, small-molecule inhibitors, such as deguelin, which can alter multiple aberrant signaling components, may provide better therapeutic outcomes than agents commonly used in cancer prevention and therapy. Deguelin, a natural product isolated from several plant species, including *Mundulea serica* (*Leguminosae*), has emerged as an effective agent for a variety of cancer types, including skin, mammary, colon, prostate, head and neck, and lung cancers (6–12). A potential barrier to the use of deguelin, however, is the possible risk of a Parkinson’s disease–like syndrome, which has been reported in rats treated with a high dose of deguelin (4). Based on the hydrophobicity of the drug, we hypothesized that a selective drug delivery system by liposomal delivery of deguelin could increase its bioavailability to desired targets and reduce the effective dose of deguelin, leading to decrease in the risk of the side effect.

In the present study, we sought to determine the chemopreventive and therapeutic activities of deguelin in a liposomal
formulation in vitro and in vivo. We found that liposomal deguelin inhibited proliferation of premalignant and malignant human bronchial epithelial (HBE) and non–small cell lung cancer (NSCLC) cell lines by inducing apoptosis with a greater potency than deguelin did in certain cell lines. We also found that organ-to-plasma distribution of deguelin in mice was better with nasal administration of liposomal deguelin than with oral administration with deguelin. Furthermore, liposomal deguelin in intranasal or intratracheal administration revealed effective antitumor activities in two different mouse models with lung tumors induced either by a tobacco carcinogen or by the oncogenic K-ras (13). Overall, our results suggest that delivery of deguelin via liposomal formulation is an effective strategy for lung cancer chemoprevention and therapy.

Materials and Methods

Preparation of liposomal deguelin

Liposomal deguelin was formulated as previously reported for liposomal 9-nitrocamptothecin (14). Briefly, stock solutions of dilaurylphosphatidylcholine and deguelin were prepared in t-butanol. Aliquots of deguelin and dilaurylphosphatidylcholine (1:10) were mixed and frozen at −70°C. The frozen solution was then lyophilized overnight, stored at −20°C, and freshly reconstituted at room temperature in sterile PBS before use.

Western blot analysis

Whole-cell lysates were prepared by incubating cell pellets in lysis buffer [50 mmol/L Tris (pH 7.5), 1% NP40, 0.25% sodium deoxycholate, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L Na2VO4, 1 mmol/L NaF with protease inhibitor cocktail (Roche)] for 20 min on ice. After centrifugation at 12,000 rpm for 20 min, the supernatant fractions were harvested carefully and the protein concentration was measured using a bichinchoninic acid protein assay kit (Pierce). Aliquots of protein (50 μg) were electrophoresed through 10% SDS-polyacrylamide gel (Bio-Rad) and transferred to a polyvinylidene difluoride membrane (Bio-Rad). After blocking with TBS containing 0.05% Tween 20 (TBST) and 5% nonfat powdered milk, the membrane was incubated with the primary antibody solution at 4°C overnight. The following antibodies and working dilutions were used for the Western blots: anti–phosphorylated AKT (1:1,000), anti–AKT (1:1,000), anti–phosphorylated glycogen synthase kinase 3β (GSK3β; 1:1,000), anti–mitogen-activated protein/extracellular signal-regulated kinase (MEK) 1 (1:1,000), and anti–p85 (1:1,000) antibodies (Cell Signaling Technology); anti–cyclin-dependent kinase 4 (CDK4; 1:1,000) and anti–actin (1:2,000) antibodies (Santa Cruz Biotechnology, Inc.); anti–hypoxia-inducible factor (HIF)-2α (1:1,000) antibody (Novus Biologicals); and anti–endothelial nitric oxide synthase (eNOS; 1:1,000) antibody (BD Biosciences). After overnight incubation with the primary antibody, the membrane was washed with TBST and incubated with the appropriate horseradish peroxidase–conjugated secondary antibody for 1 h at room temperature. The protein–antibody complexes were detected by enhanced chemiluminescence kit in accordance with the manufacturer’s directions (Amersham).

Cell proliferation assay

NSCLC cell lines (H1299, A549, H460, and 226B) were maintained in RPMI 1640 with 10% fetal bovine serum in a humidified environment with 5% CO2. Normal HBE (NHBE) cells were purchased from Cambrex and cultured according to the manufacturer’s recommended protocol. BEAS-2B cells (NHBE immortalized with a hybrid adenovirus/SV40; ref. 15) were cultured in keratinocyte serum-free medium with recommended supplements. One premalignant (1198) and one malignant (1170) cell line derived from BEAS-2B cells by exposure to beeswax pellets containing cigarette smoke condensate (15, 16) were cultured in keratinocyte serum-free medium with 3% fetal bovine serum. The cells were plated on 96-well cell culture plates (Griner Bio-One) at a density of 4,000 per well. The next day, cells were treated with 100 nmol/L deguelin or liposomal deguelin in complete medium with control cells receiving DMSO or liposome vehicle. After 96 h, viable cells in each well were measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell growth assay as described previously (17) and as per manufacturer’s protocol (Sigma).

Anchorage-dependent colony formation assay

NSCLC cell lines were seeded in a six-well cell culture plate at a density of 5 × 103 per well. On the second day, cells were treated with 100 nmol/L deguelin or liposomal deguelin in complete medium. After 3 d, cells were harvested and reseeded into a six-well plate at a density of 500 per well in complete medium without drugs. Two weeks later, plates were stained with hematoxylin and the colony number was counted.

Assessment of apoptosis in HBE cells and NSCLC cells by annexin V/propidium iodide double-staining assay

For detection of apoptotic cells, expression of Annexin V-FITC and exclusion of propidium iodide (PI) were simultaneously detected using two-color flow cytometry. The HBE and NSCLC cells were seeded into wells of a six-well cell culture plates and allowed to attach overnight. Cells were treated with a 100 nmol/L concentration of liposomal deguelin or deguelin in complete medium. After 36 h of treatment, cells were harvested by trypsinization. Cell pellets were suspended in 100 μL of 1× binding buffer, to which was then added 3 μL of FITC-conjugated Annexin V and 5 μL of PI (Santa Cruz Biotechnology). After incubation at room temperature for 10 min, 400 μL of 1× binding buffer were added, and cells were immediately analyzed by flow cytometry (Becton Dickinson).

Measurement of caspase-3 activity

Caspase-3 activity was determined using the Caspase-3 Colorimetric Assay (R&D Systems) according to the manufacturer’s protocol. Briefly, the cell lysate was tested for protease activity by the addition of a caspase–specific peptide conjugated to the color reporter molecule p-nitroanilide. Cleavage of the peptide by caspase releases the chromophore p-nitroanilide, which is quantitated spectrophotometrically at a wavelength of 405 nm. The caspase-3 activity was compared in fold changes by setting the control reading as 1.

Evaluation of therapeutic effects of liposomal deguelin in the A/J and K-rasLAC57B16/129/sv F1 mutant mice

To assess the chemopreventive effects of liposomal deguelin for tobacco-induced tumorigenesis, 6-wk-old female A/J mice (The Jackson Laboratory) were treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK) and benzo(a)pyrene (BaP; 3 mmol/L each in 0.1 mL cottonseed oil) once a week for 8 wk. After the first treatment, mice were randomly divided into four groups. One group of mice received liposomal deguelin (0.4 mg/kg), the second group received 4 mg/kg deguelin, the third group received vehicle alone, and the fourth group of mice was kept untreated. Deguelin and DMSO control were administered by oral gavage, and liposomal deguelin and empty liposome were administered nasally. Deguelin, liposomal deguelin, and vehicle were administered once a week for 16 wk. After 20 wk, all mice were sacrificed, and the lungs were removed and fixed in a 10% formalin-PBS solution. Twelve-week-old K-rasLAC57B16/129/sv F1 mutant mice were used as a spontaneous lung tumor model and treated with either PBS or the liposomal deguelin (0.4 mg/kg) via intratracheal instillation once a week for 8 wk. At the end of the...
9th week, the mice were sacrificed, and the lungs were removed and fixed in a 10% formalin-PBS solution. As an indicator of drug efficacy, the total number of tumor nodules in each lung was counted under a dissecting microscope. The tissue was submitted for histologic evaluation with H&E staining, and tumor multiplicity, volume, and load were quantitated. The tumor volume was calculated as volume (mm$^3$ = $\frac{1}{2} \times$ long diameter $\times$ short diameter$^2$). The tumor load was calculated as tumor number multiplied by tumor volume.

**Deguelin concentration analysis**

Fifty ICR outbred tumor-free mice were randomized into two groups for pharmacokinetic assays. One group received liposomal deguelin at 0.4 mg/kg via intranasal instillation. The other group received deguelin at 4 mg/kg via oral gavage. Mice were sacrificed using isoflurane for blood collection and harvesting of organs at the following time points: pretreatment and 1, 4, and 24 h after drug administration. Blood samples were collected through the orbital plexus and allowed to clot. Serum was isolated by centrifugation at 5,000 × g at 4°C for 5 min. Serum samples were stored at −70°C until analysis.

The organs were rapidly excised, weighed, frozen, and stored in liquid nitrogen until analyzed. All samples were processed and analyzed by high-performance liquid chromatography with mass spectrophotometry detection as previously described (18).

**Immunohistochemistry**

After serial deparaffinization and rehydration steps, antigen was retrieved in citrate buffer (10 mmol/L, pH 8.0) by boiling in a microwave for 10 min and by trypsin digestion (1 μg/mL in PBS) for 20 min at 37°C. After incubation in 3% H$_2$O$_2$ for 5 min, the tissue sections were blocked with 10% fetal bovine serum for overnight at 4°C. Then, tissues were incubated at 25°C with the primary rabbit phosphorylated AKT (Thr308) antibodies (Santa Cruz Biotechnology) at 1:100 dilution in the antibody diluent (Invitrogen Co.). After washing thrice for 5 min, the tissues were incubated using Vectastain ABC Elite kit (Vector Laboratories, Inc.) and stained using 3,3′-diaminobenzidine substrate kit (Vector Laboratories) as the manufacturer’s manuals. Positive nuclear staining was counted for each node in lung tissue sections, and the total counted nuclei were divided by the number of total nodes for each group.
Statistical analysis

Colony formation number, lung tumor multiplicity, and positive nuclear staining for phosphorylated AKT were compared among treatment groups using unpaired, two-tailed Student’s t-test, and P < 0.05 was considered statistically significant for all tests.

Results

Inhibition of in vitro growth and colony formation of pre-malignant, malignant, and NSCLC cell lines by liposomal deguelin

The effect of liposomal deguelin on cell proliferation was assessed in primary cultured normal (NHBE), immortalized (BEAS-2B), pre-malignant (1198), and malignant (1170-1) HBE cells and a subset of NSCLC cell lines (H1299, H460, 226B, and A549; Fig. 1). Liposomal and parental deguelin showed similar levels of dose-dependent antiproliferative activities in 1198, 1170-1, H1299, A549, and H460 cells. However, neither compound showed any significant cytotoxicity on NHBE and BEAS-2B cells at doses of 50 and 100 nmol/L (Fig. 1A). We next examined the effect of the drugs on NSCLC cell survival. Liposomal deguelin significantly inhibited the anchorage-dependent colony-forming abilities of H1299, H460, and A549 cells with a greater potency than parental deguelin (Fig. 1C). MTT and colony formation assays showed a ~80% growth inhibition in H1299 cell lines after treatment with 100 nmol/L liposomal deguelin (Fig. 1B and C).

Induction of apoptosis in pre-malignant, cancerous, and NSCLC cells by liposomal deguelin

Because apoptosis is one of the main biological events caused by deguelin, we investigated whether liposomal deguelin would also induce apoptosis in 1198, 1170-1, H1299, H460, and A549 cells. Figure 2A shows the density plots of PI fluorescence versus Annexin V-FITC fluorescence obtained from control (untreated), deguelin-treated, or liposomal deguelin–treated cells. Apoptotic cell death was obviously increased after the treatment with deguelin or liposomal deguelin; approximately 11.1%, 31.8%, 45.7%, 31.0%, and 20.6% of deguelin-treated versus 8.0%, 38.5%, 50.0%, 48.2%, and 24.4% of liposomal deguelin–treated 1198, 1170-1, H1299, H460, and A549 cells, respectively, underwent apoptosis (Fig. 2A). Apoptotic cell death was further confirmed by detecting increased caspase-3 activities following treatment with either parental or liposomal deguelin in the cell lines (Fig. 2B and C). These results show that liposomal deguelin and parental deguelin have apoptotic activities in pre-malignant and malignant HBE and NSCLC cells.
Effect of liposomal deguelin on the phosphatidylinositol 3-Kinase/AKT pathway and heat shock protein 90 client proteins

The phosphatidylinositol 3-kinase (PI3K)/AKT pathway is important in regulating cell survival and proliferation (19–21). We previously showed that deguelin down-regulates heat shock protein 90 (HSP90) client proteins, including AKT, HIF-1α, HIF-2α, CDK4, and eNOS in vitro and in vivo in pre-malignant and malignant HBE cells (22, 23) and/or NSCLC cells (10, 24). Consistent with previous results, we observed down-regulation of total and phosphorylated AKT as well as phosphorylated GSK3β, a direct downstream target of the PI3K/AKT pathway, by liposomal deguelin in 1198, 1170-1, H1299, A549, and H460 cell lines to a degree comparable with those observed with the parental drug (Fig. 3A and B). Furthermore, we observed decreases in the expression of client proteins of HSP90, including CDK4, MEK1/2, HIF-2α, and eNOS, in 1198 and H1299 cell lines after treatment with 100 nmol/L of liposomal deguelin or deguelin (Fig. 3C). All protein levels were down-regulated to a similar degree by liposomal deguelin and parental deguelin (Fig. 3A and B). As a whole, the results of the tissue culture studies suggest that liposome-encapsulated deguelin has the same efficacy as the parental compound on cell proliferation, apoptosis, and down-regulation of the HSP90 function.

Comparison of biodistribution and availability of deguelin and liposomal deguelin

We attempted to examine the chemopreventive and therapeutic activities of deguelin and liposomal deguelin in vivo. We first evaluated the serum and tissue distribution of deguelin in ICR mice treated with deguelin or liposomal deguelin orally or intranasally. Figure 4 shows the concentration-time curves of deguelin in serum and various organs after oral gavage or intranasal single-dose administration of 0.4 or 4 mg/kg of deguelin. A peak concentration was detected from 1 to 4 hours after the administration with 4 mg/kg deguelin, and deguelin was distributed more to lung, spleen, and brain (Fig. 4A). In contrast, mice treated with liposomal deguelin showed different patterns of organ biodistribution. Oral...
were measured by high-performance liquid chromatography. Collected as indicated in Materials and Methods and drug concentrations in mice and sacrificed at 0, 1, 4, and 24 h later. Sera and organs were treated by oral gavage, and liposomal deguelin was administered using either oral gavage (B) and nasal instillation (C) on ICR outbred tumor-free mice and sacrificed at 0, 1, 4, and 24 h later. Sera and organs were collected as indicated in Materials and Methods and drug concentrations were measured by high-performance liquid chromatography.

The effectiveness of intranasal administration of liposomal deguelin shows a rapid increase in distribution in the lungs at 1 hour, suggesting the intranasal administration of liposomal deguelin also showed increased accumulation of deguelin in most organs, but the distribution was more confined to lung, heart, and spleen. Interestingly, the liposomal formulation showed 2-fold increase in the accumulation of deguelin at 4 hours in the lung compared with deguelin, whereas the level of accumulation in the brain seemed to be unchanged. As indicated by Fig. 4C, the intranasal administration of liposomal deguelin shows a rapid increase in distribution in the lungs at 1 hour, suggesting the effectiveness of intranasal administration of liposomal deguelin for delivery to the lungs.

Chemopreventive effects of liposomal deguelin in the A/J mouse/NNK/BaP model

To assess the chemoprevention activity of liposomal deguelin, we used the A/J mouse and NNK/BaP system as described in Materials and Methods and Fig. 5A because NNK and BaP have been shown to effectively induce lung tumor formation in laboratory animals, including mice, rats, and hamsters, regardless of the route of administration (25). The animals receiving NNK/BaP (n = 13) showed much higher tumor incidence and tumor multiplicity compared with the control animals (Fig. 5B). However, liposomal deguelin (n = 13) or parental deguelin (n = 13) significantly reduced tumor multiplicity, volume, and load compared with NNK/BaP. The tumor multiplicity was reduced to 34 ± 27% at a dose of 0.4 mg/kg of liposomal deguelin administered by oral gavage and to 41 ± 30% at a dose of 4.0 mg/kg of deguelin administered by oral gavage when compared with that of the NNK/BaP-treated control. The tumor volume was significantly also decreased markedly by both liposomal deguelin (66 ± 42%) and parental deguelin (54 ± 28%; Fig. 5C). The tumor load was also notably decreased by liposomal deguelin and parental deguelin to 48 ± 52% and 34 ± 32%, respectively, in comparison with the NNK/BaP control (Fig. 5C). We measured body weight thrice over the period of this experiment and found no significant change. Other signs of toxicity were also absent in all groups of mice.

Therapeutic efficacy of liposomal deguelin in the K-rasLAC57Bl6/129/sv F1 mutant mouse model

The K-rasLAC57Bl6/129/sv F1 mutant mice develop lung tumors spontaneously with 100% incidence rate (13). In this current study, we used this model to assess the therapeutic efficacy of liposomal deguelin. We began treating the animals with the liposomal deguelin when they reached 3 months of age. The drug was administered once a week for 8 weeks by the intratracheal instillation (0.4 mg/kg in 100 μL PBS). Liposomal deguelin showed remarkably high efficacy in reducing tumor burden in the mouse lung (Fig. 6). There was a 40% reduction in the number of tumor nodules in the mice group treated with liposomal deguelin in comparison with the control (P < 0.05; Fig. 6A and B). In addition, total tumor volume was dramatically reduced by 80% in the liposomal deguelin–treated animals compared with the control group (P < 0.05; Fig. 6B). Furthermore, the liposomal deguelin reduced phosphorylated AKT (Thr308) levels by half compared with the vehicle-treated tumors (P < 0.05; Fig. 6C). In spite of the significant reduction of tumor burden by liposomal deguelin, there was no significant weight loss or behavioral abnormality observed.

Discussion

In this study, we have shown that a liposomal formulation of deguelin, a well-studied chemopreventive and therapeutic agent, leads to an improved pharmacokinetic activity, resulting in significant antitumor activities similar to the levels induced by a 10 times higher dose of the parent drug. These results are important because there is a concern about potential side effects of deguelin (26). We and others have shown important preclinical evidence of the cancer chemopreventive and therapeutic activities of deguelin in a variety of cancer types (10, 11, 23, 27), implicating deguelin as an effective lung cancer chemopreventive and therapeutic agent.

However, the possibility that deguelin inhibits NADH:ubisemiquinone oxidoreductases and thus induces cardiotoxicity,
respiratory depression, and nerve conduction blockade at high doses (a dose that is lethal to 50% of those exposed = 10-100 g in humans; ref. 26) could be a concern for its use in cancer chemoprevention and treatment. Caboni and coworkers (4) observed a Parkinson’s disease–like syndrome in the rat brain after treatment with 6 mg/kg deguelin but not after treatment with 3 mg/kg. We have not observed behavioral neurotoxicity in our previous and current studies with 4 mg/kg. However, our data concern an animal model of lung cancer development and, as such, are similar but do not mirror human lung cancer, implying the limitations about translation of an animal model to practical use in human lung cancer prevention. The possible side effects from the drug treatment emphasize the need to develop a better delivery system for the drug.

Given the fact that liposomal delivery could provide increased accumulation of the encapsulated drug in the target organ, we hypothesized that delivery of deguelin in a liposomal formulation through lung-targeted administration would achieve effective chemopreventive and therapeutic outcomes with a reduced dose and minimize potential side effects. Hence, we attempted to test our hypothesis in vitro in normal, immortalized, premalignant, and malignant HBE cells and a subset of NSCLC cells and in vivo in two mouse models in which lung tumors were established by treatment with NNK/BaP or by mutant K-ras (G12D).

When tested in vitro in premalignant (1198) and malignant (1170-1) HBE cells and NSCLC cell lines (H1299, H460, and A549), liposomal deguelin decreased viability of these cells and induced apoptotic activities with no detectable cytotoxic effects on NHBE (primary culture) or BEAS-2B HBE (immortalized) cells. Moreover, we also confirmed that liposomal deguelin was also able to inhibit the functions of HSP90 in the similar manner and degree as deguelin did. Of note, liposomal deguelin tended to have greater apoptotic activities in certain cell lines than did deguelin. This cell-specific sensitivity may be associated with differences in a cellular chemical metabolism context (4) or the delivery of the encapsulated drug.

Given these promising in vitro findings and data supporting the benefits of liposomal drug delivery system (2, 3), we anticipated that liposomal encapsulation would increase delivery and distribution of deguelin in vivo. We have shown that administration of 4 mg/kg deguelin by oral gavage exerts effective lung cancer chemopreventive and therapeutic activities in the A/J and xenograft mouse models (10, 11, 23, 27). In the current study, we show that intranasal administration of deguelin in liposomal formulation at 10-fold lower dose (0.4 mg/kg) resulted in more effective delivery of the drug

![Fig. 5. Evaluation of the chemoprevention effect of liposomal deguelin in a tobacco-induced tumor model. A, diagram showing treatment protocol for deguelin or liposomal deguelin and NNK/BaP. The NNK/BaP was administered orally once each week. Animals also received liposomal deguelin (0.4 mg/kg through nasal), deguelin (4 mg/kg through oral gavages), or vehicle (DMSO through oral gavages) once each week. After 20 wk, all mice were sacrificed and lungs were removed and examined. B, photographs of lungs removed from A/J mice treated with NNK/BaP and liposomal deguelin, deguelin, or vehicle control. C, effects of liposomal deguelin and deguelin on lung tumor induction by NNK/BaP in A/J mice. Graphs show tumor number per slide, tumor number per animal, average tumor volume (mm³ = 1/2 × long diameter × short diameter²), and tumor load (tumor load = tumor number × tumor volume). D, body weight of NNK/BaP-treated A/J mice inoculated each drug. *, P < 0.05; **, P < 0.01; *** P < 0.001.](image-url)
Our results suggest that liposomal encapsulation could protect deguelin from metabolic degradation, allowing sustained pulmonary release and causing more efficient intracellular delivery. Although the mechanism by which the concentration of deguelin is increased remains to be explored, we hypothesized that the improved pharmacokinetics of deguelin could allow lowering the therapeutic dose of deguelin. Aerosol delivery of liposomal cancer drugs to patients has been successfully done (28). In this instance, liposomal deguelin resuspended in water will be nebulized using a commercially available nebulizer such as the Aerotech II flowing 10 L of air/min and delivered through a facemask or mouthpiece. The patient, breathing normally, will receive a dose of deguelin proportional to his/her minute volume/kg body weight.

Led by this speculation, we sought to determine the antitumor activities of the lower dose of liposomal deguelin in two animal models, including the NNK/BaP-treated A/J and K-rasLAC57Bl6/129/sv F1 mutant mice. Indeed, we were able to confirm that treatment with 0.4 mg/kg of liposomal deguelin once a week effectively reduced lung tumor burdens in these two mouse models. These results support the use of liposome for drug delivery into the pulmonary tissues as discussed in the preceding report (14). Dilauroylphosphatidylcholine liposome tended to remain in the lung for a prolonged period, which extended drug efficacy. Therefore, it is plausible to suggest that deguelin treatment in liposomal formulation permits a sharp reduction in therapeutic dose, leading to reduce the risk of potential side effects of the drug treatment while maintaining the high drug efficacy.

We recently showed that the antitumor activities of deguelin involve its binding to the ATP-binding pocket of HSP90, which suppresses HSP90 function leading to degradation of a variety of HSP90 client proteins carrying important roles in cancer cell homeostasis (29–31) and survival under stress.

**Fig. 6.** Therapeutic effects of liposomal deguelin on lung tumorigenesis in K-rasLAC57Bl6/129/sv F1 mutant mice. Liposomal deguelin (0.4 mg/kg) or vehicle was administered via tracheal instillation once a week for 8 wk. The mice were sacrificed at the end of the 9th week and lungs were excised for tumor evaluation. A, photographs of lungs from either vehicle-treated or liposomal deguelin-treated mice. B, number of tumor nodes on the lungs as counted under a dissecting microscope. For H&E staining and histlogic evaluation, the lungs were fixed and paraffin blocked. Each block was sectioned at 5-μm intervals, and the 1st, 21st, and 41st sections were mounted on a glass slide. Tumors were measured on sections carrying the highest number of tumors as seen under a dissecting microscope and the volumes were calculated as described in the text. C, the lung tissue sections were immunostained against phosphorylated AKT (Thr308) as described in Materials and Methods and the representative results are shown. Arrows, positive nuclear staining. The positive nuclear stainings were counted for each tumor node and compared by the average number of positive nuclei per node. *, P < 0.05.
conditions (21, 22, 32–36). In the current study, we showed the ability of liposomal deguelin to regulate the expression of HSP90 client proteins as effectively as parental deguelin. These findings confirm that liposomal encapsulation enhances the therapeutic potency of deguelin without affecting the biochemical activity of the drug. Therefore, the direct administration of deguelin in liposomal formulation would be a preferred method for controlling human lung cancer.

Taken together, our preclinical results present the possibility of using deguelin at a reduced dose and frequency through a direct route of administration for lung cancer chemoprevention and therapy. These findings provide a strong rationale for testing the toxicity and efficacy of liposomal deguelin in clinical trials of lung cancer chemoprevention and therapy. Finally, our results suggest that a smart delivery system targeting tumors specifically may enhance the efficacy of deguelin even in smaller amount and reduce the putative side effects associated with deguelin.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**

Liposomal Encapsulation of Deguelin: Evidence for Enhanced Antitumor Activity in Tobacco Carcinogen–Induced and Oncogenic K-ras–Induced Lung Tumorigenesis

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