Lycopene Inhibits Smoke-Induced Chronic Obstructive Pulmonary Disease and Lung Carcinogenesis by Modulating Reverse Cholesterol Transport in Ferrets

Jelena Mustra Rakic1,2, Chun Liu1, Sudipta Veeramachaneni1, Dayong Wu2,3, Ligi Paul2,4, C.-Y. Oliver Chen2, Lynne M. Ausman1,2, and Xiang-Dong Wang1,2

Abstract

Chronic obstructive pulmonary disease (COPD) and lung cancer share the same etiologic factor, cigarette smoking. Higher consumption of dietary lycopene has been associated with lower risks of COPD and lung cancer in smokers. We investigated whether lycopene feeding protects against COPD and lung cancer in ferrets, a nonrodent model that closely mimics cigarette smoke (CS)-induced chronic bronchitis, emphysema, and lung tumorigenesis in human. We also explored whether the protective effect of lycopene is associated with restoring reverse cholesterol transport (RCT), a key driver in persistent inflammation with CS exposure. Ferrets (4 groups, n = 12–16/group) were exposed to a combination of tobacco carcinogen (NNK) and CS with or without consuming lycopene at low and high doses (equivalent to 30 and 90 mg lycopene/day in human, respectively) for 22 weeks. Results showed that dietary lycopene at a high dose significantly inhibited NNK/CS-induced chronic bronchitis, emphysema, and preneoplastic lesions, including squamous metaplasia and atypical adenomatous hyperplasia, as compared with the NNK/CS alone (P < 0.05). Lycopene feeding also tended to decrease the lung neoplastic lesions. Furthermore, lycopene feeding significantly inhibited NNK/CS-induced accumulation of total cholesterol, and increased mRNA expression of critical genes related to the RCT (PPARα, LXRα, and ATP-binding cassette transporters ABCA1 and ABCG1) in the lungs, which were downregulated by the NNK/CS exposure. The present study has provided the first evidence linking a protective role of dietary lycopene against COPD and preneoplastic lesions to RCT-mediated cholesterol accumulation in lungs.

Introduction

Despite the efforts toward smoking prevention and cessation, one fifth of the population continues to smoke in the United States, resulting in more than 400,000 premature deaths each year (1). Cigarette smoke (CS) is the single most important risk factor for many diseases, including lung diseases, such as chronic obstructive pul-

1Nutrition and Cancer Biology Lab, Jean Mayer USDA-Human Nutrition Research Center on Aging (HNRC) at Tufts University, Boston, Massachusetts.
2Biochemical and Molecular Nutrition Program, Friedman School of Nutrition Science and Policy, Tufts University, Boston, Massachusetts.
3Nutritional Immunology Lab, JM USDA-HNRCA at Tufts University, Boston, Massachusetts.
4Department of Developmental, Molecular and Chemical Biology, Tufts University School of Medicine, Boston, Massachusetts.

Corresponding Author: Xiang-Dong Wang, Nutrition and Cancer Biology Laboratory, JM USDA-HNRCA at Tufts University, 711 Washington Street, Boston, MA 02111. Phone: 617-556-3150; Fax: 617-556-3344; E-mail: xiang-dong.wang@tufts.edu
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potent lung carcinogen (10). Both CS and the carcinogen NNK are known to cause inflammation in lung in vivo (2, 13, 14). Accumulating data from both in vitro and in vivo studies have implied that lycopene has important biological properties, including antioxidant and anti-inflammatory effect (13, 15–17). We have previously demonstrated that lycopene was protective against NNK-induced pulmonary neoplastic lesions and CS-induced squamous metaplasia in ferrets (13, 18). Recently, Campos and colleagues reported lycopene to be protective against CS-induced emphysema in mice (16). All of this emphasizes the importance of understanding the common molecular mechanism(s) driving these two devastating diseases together and the importance of discovering dietary lycopene that can convincingly prevent or delay the progression of COPD and lung cancer.

The significance of cholesterol homeostasis has been recognized in lung disease development (19, 20). Cholesterol plays a critical role in cell morphology and function. Cholesterol intracellular accumulation results in dysregulated inflammatory response (19), and cholesterol overload can amplify inflammation in macrophages (21). A role of the reverse cholesterol transport (RCT), which mediates the removal of excess cholesterol from peripheral tissue, has been shown in the persistent lung inflammation and emphysema after CS exposure in mice (22). Reports from human studies indicated the adverse effect of CS on plasma lipid profile, including decrease of high-density lipoprotein cholesterol (HDL-C) (23), which is a main mediator in RCT (24). Furthermore, animal studies have reported that mice deficient in ATP-binding cassette (ABC) transporters ABCA1 and ABCG1, key transporters of the RCT, have cholesterol overload with abnormal pulmonary morphology and physiology and proinflammatory phenotype (25, 26). Although the role of lycopene in regulation of cholesterol metabolism has been demonstrated (27, 28), little is known about the role of lycopene in CS exposure–related cholesterol accumulation and pulmonary pathophysiology, such as COPD and lung cancer.

Liver X Receptor (LXRα), a nuclear hormone receptor, regulates expression of many genes involved in RCT, including ABCA1 and ABCG1 transporters (29, 30), which are highly expressed in macrophages and pneumocytes (31, 32). In addition, expression of ABC transporters can be regulated by peroxisome proliferator-activated receptor (PPARα), possibly through the LXRα-dependent pathway (33). Recently, anti-inflammatory properties of lycopene have been demonstrated after acute CS exposure in mice (16). Furthermore, new evidence has shown that an LXR agonist can restore altered RCT and suppress inflammation after CS exposure in mice (22). Studies have shown that lycopene can modulate RCT by inducing LXRα and PPARα in cell cultures (17, 27, 34); however, there is no in vivo evidence available as to whether lycopene could restore ABCA1/G1 expression by upregulating LXRxα and PPARα after CS exposure in lung.

Choosing appropriate animal models is important for potential translational value of the study results to human. Exposure of ferrets to the CS with or without NNK has been shown as a useful model for studying inflammation (14), COPD (35), and lung carcinogenesis (18, 36). Although mice are widely used in animal models for CS exposure studies, CS-exposed mice do not develop all the characteristics of COPD seen in people, such as chronic bronchitis (37). In contrast, CS-exposed ferrets develop both, emphysema and chronic bronchitis, because their lung structure and submucosal gland distribution are similar to those of humans (35, 37). We have previously shown that ferrets closely mimic NNK or CS-induced lung preneoplastic and neoplastic lesions seen in humans (13, 14, 18). Additionally, ferrets are an excellent nonrodent animal model for studying lycopene intervention due to their similarities to humans in carotenoid absorption, metabolism, and tissue accumulation (13). In the present study, we used the ferret model to investigate the potential protective effects of different doses of lycopene achievable through diet against NNK/CS-induced COPD and carcinogenesis, and to explore the involvement of RCT in these effects.

Materials and Methods
Animals, diet, and study design
The animal experimental protocol, including exposure to smoke, was approved by the Institutional Animal Care and Use Committee at the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University. Fifty-four adult male ferrets (1.2–1.5 kg) from Marshall Farms were housed individually in cages in an American Association of Accreditation of Laboratory Animal Care (AAALAC)–accredited animal facility at the HNRCA at Tufts University. The animals were randomly assigned to four groups (n = 12–16/group) and received the following treatments, respectively, for 26 weeks: (i) control + sham (C); (ii) NNK + smoke-exposed (N + S); (iii) NNK + smoke-exposed + low-dose lycopene (N + S + LL); (iv) NNK + smoke-exposed + high-dose lycopene (N + S + HL). Ferrets were fed a semipurified ferret diet (D90001 Research Diets) ad libitum with free access to water throughout the study. The ferrets were fed lycopene or placebo starting three weeks prior to the NNK injections (as a dietary prevention model) and continued until the end of the study. Body weights were recorded weekly. All ferrets were euthanized by terminal exsanguination under deep isoflurane (Isothesia; Butler Schein) anesthesia, and tissues were harvested and processed for future analyses.
NNK treatment and smoke exposure
The procedures for both NNK treatment and CS exposure were as described (36). Briefly, for lung tumorigenesis, ferrets were given intraperitoneal (i.p.) injections of NNK (Toronto Research Chemicals) at a dose of 50 mg/kg body weight (BW) once a month for 4 consecutive months (total dose of 200 mg/kg BW), as described previously (13). Control animals were given an injection of normal saline. One week after first NNK injection, ferrets were exposed to CS from nonfiltered cigarettes (Standard Research Cigarettes, Type 2R4F, Tobacco-Health Research Institute, University of Kentucky, Lexington, KY) by placing them in a chamber connected to a smoking device for 30 minutes in the morning (10 cigarettes over a 30-minute period), 5 days/week for 22 weeks. The smoke exposure received by the animals is equivalent to a human smoking 1/4 package of cigarettes/day based on the urinary cotinine equivalent (36).

Lycopene supplementation
Lycopene beadlets containing 10% w/w lycopene or placebo beadlets (BASF Inc.) were mixed with 0.5 g peanut butter (Skippy creamy peanut butter) to provide lycopene at two doses, a dose of 2.2 mg/kg BW/day or 6.6 mg/kg BW/day. Ferrets spontaneously eat peanut butter, so there was no need to gavage. The lower dose (2.2 mg/kg BW/day) and the higher dose (6.6 mg/kg BW/day) are equivalent to a daily intake of 30 and 90 mg, respectively, for a man weighing 70 kg (13, 18).

Lung histopathologic examinations
Four-micrometer sections of formalin-fixed and paraffin-embedded lung tissue were stained with hematoxylin and eosin (H&E) for histopathologic examination. The sections were examined under light microscopy by two independent investigators who were blinded to the treatment groups. Lung histopathology of lesions was evaluated and classified as preneoplastic lesions (atypical adenomatous hyperplasia and squamous metaplasia) and neoplastic lesions (including dysplasia, adenocarcinoma, squamous cell carcinoma, adenocarcinoma and squamous cell carcinomas, and bronchioalveolar carcinomas) as described previously (13). The quantification of emphysema in each histologic section of the lung was determined by the mean linear intercept (Lm) in micrometers from observed microscopic fields. The measurement of Lm was performed by using a 100 × 100 μm grid that was randomly positioned over each field in the lung section. The total length of each line of the grid divided by the number of alveolar intercepts yielded the average distance between alveolated surfaces (Lm in each field). For each animal, 20 fields at a magnification of 20× were measured, and the mean of the 20 fields was taken as the Lm for each animal. The degree of severity of lung inflammation was graded (0 to 4) according to the magnitude of peribronchial/bronchiolar infiltrates of inflammatory cells and alveolar septal infiltrates of inflammatory cells as described previously (14).

Lung cholesterol quantification
Total cholesterol (TC) concentrations were quantified using a commercial cholesterol kit (Cell Biolabs Inc). Lung tissue (10 mg) was used to determine TC following the manufacturer's instructions. Final data were presented as mg cholesterol/g lung tissue.

High-performance liquid chromatography (HPLC) analysis
Liver (100 mg) tissue, lung (200 mg) tissue, and plasma (1 mL) were analyzed by the HPLC method to determine concentrations of all-trans and cis isomers (5-cis, 13-cis, and 9-cis) of lycopene as previously described (13).

RNA extraction and RT-qPCR
Total RNA was isolated from frozen lung tissue using TriPure Isolation Reagent (Roche Applied Science) following the manufacturer's instructions. RNA was checked for purity using the NanoDrop spectrophotometer (NanoDrop 1000). Complementary DNA (cDNA) was synthesized by reverse transcription using M-MLV (Invitrogen) using a thermocycler (Bio-Rad PTC 200, GMI) following the manufacturer's instructions. Quantitative real-time PCR was done using specific primers and FastStart Universal SYBR green Master (Roche) with the real-time PCR machine (Applied Biosystems). The relative mRNA expression was calculated as 2−ΔΔCT, with β-actin used as the endogenous control.

Protein isolation and immunoblotting
The nuclear fraction from whole lung cell lysate was prepared as described (38). The protein concentration of the nuclear fraction was determined by Coomassie Plus protein quantification method (Thermo Fisher Scientific). Equal amounts of protein from each sample (30 μg) were denatured by boiling with 3× SDS sample buffer with 2-mercaptoethanol in a 100°C water bath for 7 minutes and resolved by loading into wells of 7% or 10% SDS-polyacrylamide gel. After electrophoresis with 1× tris-glycine-SDS buffer, proteins were transferred to Immunoblot-P membranes (Millipore) overnight at 4°C and blocked with 5% nonfat dried milk in Tris-buffered saline with 0.2% Tween-20 (TBST) for 1 hour at room temperature. Membranes were incubated with selected primary antibodies LXRα (Abcam) and PPARα (Abcam) overnight at 4°C. After 3× washing in TBST, horseradish peroxidase–conjugated secondary antibodies (Bio-Rad) in 5% nonfat milk were used for protein detection. The protein bands were visualized by a SuperSignal West Chemiluminescent Substrate Kit (Pierce) following the manufacturer’s instructions. The intensities of the specific relative band were quantified by using a densitometer (GS-710 Calibrated...
Imaging Densitometer, Bio-Rad). Protein β-actin was used as loading control (Sigma-Aldrich).

Statistical analyses
SAS 9.4 software was used to perform linear and logistic regression. GraphPad Prism 7 was used for all other analyses. The incidence of neoplastic or neoplastic lesions was evaluated using the Fisher exact test. The grading of inflammation was evaluated by the Kruskal–Wallis test, followed by the Dunn multiple comparison tests for comparing groups. One-way ANOVA followed by the Tukey multiple comparison tests was performed for mean values of the multiple groups. Linear or logistic regressions were used to analyze the correlation between emphysema, chronic bronchitis, neoplastic lesions, and TC in lungs. The results are expressed as means ± SEMs. Differences were considered significant at \( P < 0.05 \).

Results
Lycopene feeding inhibited NNK/CS-induced chronic bronchitis, emphysema, and preneoplastic lesions
Exposure of ferrets to CS (equivalent to humans who smoke 1/4 package per day) in combination with the four injections of NNK (50 mg/kg BW) for 22 weeks resulted in chronic bronchitis with typical peribronchial/bronchiolar infiltrates of inflammatory cells and alveolar septal infiltrates of inflammatory cells determined by inflammation grades from 0 (normal) to 4 (severe inflammation; Fig. 1A). There were significant differences in the inflammation grades between the N + S group (mean grade, 2.9) and the control group (mean grade, 0.6). Group fed high lycopene dose, N + S + HL, had significantly lower inflammation results (mean grade, 1.5) compared with the N + S group. A nonsignificant decrease \( (P = 0.095) \) in inflammation was observed in

Figure 1.
Histopathologic examination of COPD in ferrets exposed to NNK/CS for 22 weeks. A, The representative images of inflammation grades with H&E staining at 40× used for the evaluation of chronic bronchitis. B, Distribution of inflammation grade in groups. C, The representative images of emphysema with H&E staining at 10×. D, Distribution of alveolar diameter expressed as mean linear intercept (Lm, μm) in groups. Groups: C, control; N + S, NNK + CS exposure; N + S + LL, NNK + CS exposure + low-dose lycopene; N + S + HL, NNK + CS exposure + high-dose lycopene. Values are means ± SEMs (n = 12–16 per group). Statistical significance for emphysema was determined by one-way ANOVA. The grading of inflammation was analyzed by Kruskal–Wallis test. Data not sharing a common letter are significantly different from each other (\( P < 0.05 \)).
the N + S + LL group (mean grade, 1.8) compared with the N + S group (Fig. 1B).

NNK/CS exposure significantly induced emphysema as determined by the average distance between alveolated surfaces (alveolar Lm, μm; Fig. 1C) compared with the control group. Both lycopene groups significantly decreased emphysema compared with the N + S group (Lm 81.5 μm in N + S + LL and Lm 74.2 μm in N + S + HL vs. Lm 97.9 μm in N + S, \( P < 0.001 \)). The alveolar Lm in the N + S + HL group was not statistically different from that in the control group (Lm 66.1 μm) (Fig. 1D).

The incidence of preneoplastic lesions in the N + S group (75%) was significantly greater than that in the control group (16.7%). Compared with the N + S group, the incidence of preneoplastic lesions was significantly lower in the N + S + HL group (30.8%), whereas the incidence in the N + S + LL group (46.2%) was non-significantly decreased. There was no significant difference in the incidence of preneoplastic lesions between the control and the N + S + HL groups (Fig. 2B). The histopathology results showed significantly higher incidence of neoplastic lesions in the N + S group (81.3%) compared with the control group (8.3%). Animals in both lycopene groups had lower incidence of neoplastic lesions, by ~20% in the N + S + LL (61.5%) and by ~28% in the N + S + HL (53.9%), compared with the N + S group, but the differences were not statistically significant (Fig. 2F).

Lycopene feeding inhibited NNK/CS-induced accumulation of TC in both plasma and lungs

There was no significant difference in BW among all groups at the end of the study (Table 1). Pulmonary TC levels were significantly higher in the N + S group compared with the control group (Table 1). Animals receiving lycopene tended to have lower pulmonary TC levels after NNK/CS exposure compared with those exposed to NNK/CS alone. However, only the TC level in the N + S + HL group was significantly lower than that in the N + S group (\( P = 0.01 \)), but not significantly different from that in the control group (Table 1). In addition, the pulmonary TC level was significantly correlated with emphysema (linear regression), chronic bronchitis, and preneoplastic lesions (logistic regression) in lungs (\( n = 12-16 \)/group; \( P = 0.01, P < 0.01, \) and \( P = 0.02 \), respectively). In plasma, TC levels were significantly higher in the ferrets exposed to NNK/CS compared with the control animals (Table 1). Animals fed lycopene tended to have lower TC in plasma, despite NNK/CS exposure, but the difference was not significantly different compared with the N + S group. However, plasma TC levels were also not significantly different between N + S + HL and the control groups. Plasma HDL-C was significantly higher in the N + S group compared with the control. Groups that received lycopene had insignificantly lower HDL-C relative to the N + S group. Plasma levels of TG, LDL-C, and VLDL-C were not significantly different between the groups.

Lycopene feeding increased expressions of critical genes related to the biomarkers of RCT

In the lung tissues, mRNA expression and protein levels of genes related to reverse cholesterol transport were determined by RT-qPCR and immunoblotting, respectively (Fig. 3A and B). Results showed a significant downregulation of the mRNA ABCA1 transporter in the N + S group compared with the control group (\( P < 0.0001 \); Fig. 3A). In the N + S group, we also found a trend of decrease in mRNA expression of the ABCG1 transporter. Furthermore, mRNA expression of both \( LXRa \) and \( PPARa \) was significantly decreased in the N + S group compared with the control group. Relative mRNA expressions of \( LXRa, PPARa, ABCA1, \) and \( ABCG1 \) in the N + S + HL group and also mRNA of \( LXRa \) in the N + S + LL group were significantly higher, compared with those in the N + S group. There were no significant differences in mRNA expression of \( LXRa, PPARa, ABCA1, \) and \( ABCG1 \) between N + S + HL and the control group (Fig. 3A). Although we did not observe significant decreases in nuclear \( LXRa \) and \( PPARa \) protein levels after NNK/CS exposure, lycopene feeding increased both \( LXRa \) and \( PPARa \) protein levels with high-dose lycopene reaching significance compared with the N + S group (Fig. 3B).

Lycopene feeding resulted in accumulation of lycopene in both plasma and liver of ferrets

Lycopene was detected in the plasma and livers (Table 2), but not in the lungs of ferrets fed lycopene. Animals in the high-dose lycopene group had higher total lycopene concentration than the low-dose lycopene group in plasma and livers, but the difference was not significant. The high-dose lycopene group had higher plasma and liver accumulation of all-trans isomer compared with the low-dose lycopene group, but only the difference in plasma was significant. We observed a nonsignificant increase in 5-cis and 9-cis in high lycopene group relative to low lycopene group in both, plasma and liver, whereas the increase in 13-cis isomers reached significance in plasma, but not liver. There was more trans-isomer in plasma than cis-isomer in low-dose and high-dose lycopene groups (ratios of \( transciscis \) isomers, 72:28; 73:27, respectively). The ratios of \( transciscis \) isomers in liver were 55:45 and 58:42 in low-dose and high-dose lycopene groups, respectively (Table 2).

Discussion

The present study strongly suggests that dietary lycopene can protect lung from the detrimental effects of NNK/CS exposure by maintaining cholesterol homeostasis through
We demonstrated that ferrets exposed to the combination of NNK and CS resulted in chronic bronchitis and emphysema, accompanied with preneoplastic and neoplastic lesions in the lungs of ferrets, indicating ferrets as an excellent model for studying COPD and lung cancer in preclinical settings. Importantly, we

Figure 2. Histopathologic examination of lung preneoplastic and neoplastic lesions in ferrets exposed to NNK/CS for 22 weeks. A, Representative images of atypical adenomatous hyperplasia and squamous metaplasia with H&E staining at 10×. B, Quantification of positive animals with preneoplastic lesions in each group expressed as a percentage (%). C, Tumors visible on lung surface (arrows). D, The representative image of lung squamous cell carcinomas with H&E staining at 10×. E, Representative images of lung adenocarcinoma and lung squamous cell carcinomas with H&E staining at 40×. F, Quantification of positive animals with neoplastic lesions in each group expressed as a percentage (%). Groups: C, control; N+S, NNK+CS exposure; N+S+LL, NNK+CS exposure+low-dose lycopene; N+S+HL, NNK+CS exposure+high-dose lycopene. Values are means ± SEMs (n = 12–16 per group). The incidence of preneoplastic and neoplastic lesions was evaluated using Fisher exact test. Data not sharing a common letter are significantly different from each other (P < 0.05).
demonstrated that lycopene feeding, starting 3 weeks prior to NNK/CS exposure, resulting in lycopene accumulation in the circulation and liver, significantly inhibited NNK/CS-induced chronic bronchitis, emphysema, and preneoplastic lesions, and tended to decrease neoplastic lesions for up to 28%. The observed protective effect was strongly associated with the attenuation of TC accumulation in lungs and plasma. Furthermore, lycopene was effective in upregulating critical genes related to the RCT pathway (LXRα, PPARα, ABCA1, and ABCG1), which were downregulated by the NNK/CS exposure, resulting in restored TC concentrations in lung and plasma to normal.

Many studies indicated the importance of cholesterol in lung disease development, including COPD and lung carcinogenesis (19, 20, 22, 25, 39, 40). We observed that ferrets had plasma TC levels comparable with humans, which is in contrast to most other laboratory animals that have lower blood TC levels (41). This suggests that the ferret is an ideal model for studying effect of CS on plasma TC in humans. Interestingly, a significant accumulation of TC in lungs of ferrets after NNK/CS exposure, which was associated with both COPD and lung carcinogenesis, suggests that the alteration of RCT accompanied by TC accumulation may mediate the development of NNK/CS-induced COPD and lung cancer development. Our

**Table 1.** Body weight (kg), lung (mg/g), and plasma (mg/dL) lipids

<table>
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<th></th>
<th>C</th>
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<th>N + S + LL</th>
<th>N + S + HL</th>
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<td>175.7 ± 6.26</td>
<td>174 ± 9.03</td>
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**NOTE:** Values are means ± SEMs (n = 12–16 per group). One-way ANOVA was used to examine the difference between the overall groups. Data not sharing a common letter are significantly different from each other (P < 0.05).

Abbreviations: TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol. Groups: C, control; N + S, NNK + CS exposure; N + S + LL, NNK + CS exposure + low-dose lycopene; N + S + HL, NNK + CS exposure + high-dose lycopene.

Figure 3.
The effect of NNK/CS and lycopene on the RCT biomarkers. A, Relative mRNA expression of the RCT biomarkers (ABCA1, ABCG1, LXRα, and PPARα) in lung tissue lysate. B, Relative protein levels of nuclear hormone receptors LXRα and PPARα in lungs. Groups: C, control; N + S, NNK + CS exposure; N + S + LL, NNK + CS exposure + low-dose lycopene; N + S + HL, NNK + CS exposure + high-dose lycopene. Values are expressed as means ± SEMs (n = 10–12 per group). Statistical difference was determined by the one-way ANOVA. Data not sharing a common letter are significantly different from each other (P < 0.05).
results are in agreement with previous studies where alterations in RCT led to cholesterol overload, abnormal structure and function in lungs of mice (25, 26, 39). Although NNK and CS are known to induce oxidative stress and inflammation in lungs (13, 16), cholesterol accumulation through impairment of RCT after NNK/CS exposure may lead to excessive inflammation and progression of COPD and lung lesions. Indeed, CS exposure of macrophages resulted in reduced expression of ABCA1/G1 and impairment of cholesterol efflux, which were correlated with upregulation of genes involved in inflammation and emphysema development (22), suggesting that alteration of RCT may be one of the initial events leading to a chronic inflammation (22), a common feature of COPD and lung cancer (29, 42). In addition, lung analysis of COPD patients showed lower relative expression of ABCA1/G1 compared with non-COPD patients (22). In our study, we found that NNK/CS downregulated pulmonary ABCA1 and ABCG1 transporters, which is consistent with the previous studies reporting that acute and chronic CS exposure decreased relative expression of ABCA1 in bronchoalveolar lavage fluid of mice, and in isolated mouse alveolar macrophages after acute CS exposure (22). Furthermore, the downregulation of ABCA1 and ABCG1 led to cholesterol overload after NNK/CS exposure, which resulted in excessive inflammation and promoting COPD and lung lesions development.

ABCA1 and ABCG1 are the direct target genes of LXRα. PPARα can enhance RCT (43). We found a lower mRNA expression of LXRα, PPARα, and ABCA1/G1 followed by TC accumulation in the NNK/CS group compared with the control group in lungs. Although the mechanism(s) underlying lower expression of PPARα, LXRα, and its target genes in lungs after NNK/CS exposure remains unclear, previous studies reported an increased expression of micro-RNAs (e.g., miRNA-21, miRNA-155, and miRNA-20), which target PPARα/LXRα and ABCA1/G1, after CS exposure (44, 45). These findings could help us to explain the downregulation of mRNA expression of PPARα/LXRα and ABCA1/G1 after NNK/CS exposure, but future studies (such as miRNA analysis) are required.

Craig and colleagues reported increased TC, VLDL-C, LDL-C, and TG and decreased HDL-C in plasma of smokers (46). The adverse effect of CS exposure on RCT and plasma HDL-C has been shown in cholesteryl ester transfer protein transgenic mice (47). We found significantly higher plasma TC in the NNK/CS group compared with a control, which is consistent with human data (46). Moreover, we found a similar trend for VLDL-C, LDL-C, and TG levels after NNK/CS exposure, though the changes were not significant. Interestingly, HDL-C concentrations were increased, which was opposite from what we expected. This could be due to the fact that ferrets are HDL animals, meaning >50% of overall cholesterol in plasma is HDL-C (48). In addition, HDL-C animals do not have cholesteryl ester transfer protein, which transfers cholesteryl esters from HDL-C to VLDL-C, and LDL-C, allowing additional cholesterol delivery to liver (48). LXRα and PPARα are known to regulate expression of scavenger receptor class B member 1 (SR-B1), which is responsible for the HDL-C uptake in the liver (49). Increase of HDL-C in ferret plasma could be explained by the possibility that NNK/CS downregulate LXRα/PPARα and consequently SR-B1 in liver. Future studies are warranted to confirm the speculation.

In the present study, both the low and high doses of lycopene significantly prevented NNK/CS-induced emphysema. The inhibitory effect of lycopene on NNK/CS-induced chronic bronchitis and preneoplastic lesions appeared to be dose dependent as the trend of this effect was seen in the low-dose group, whereas it became significant in the high-dose group. Lycopene feeding at both doses decreased the lung neoplastic lesions by 20% to 28%, but this effect did not reach a significant difference. Possibly, the significant effect of lycopene for the neoplastic lesions would have been seen with a larger sample size.

Previously, we and other investigators showed that lycopene and lycopene metabolites are effective in inducing antioxidant/detoxification enzymes by activating Nrf2 transcription factor and inducing Nrf2-mediated pathway (50). Very recently, it has been reported that lycopene administration decreases lipid peroxidation and DNA damage, and increases the activities of SOD, CAT, and GSH content (16), supporting the role of lycopene as an antioxidant and anti-inflammatory agent in mice exposed to CS. However, lycopene has been shown to regulate transcription factors in recent years, such as nuclear
Lycopene, COPD, and Lung Carcinogenesis

hormone receptors. In the present study, we propose that protective effects of lycopene against NNK/CS-induced COPD and precancerous lesions are through regulating PPARs/LXRs. This notion was supported by previous two in vitro studies showing that lycopene can increase expression of LXRα, ABCA1, cholesterol efflux, and reduce intracellular cholesterol (28, 51). Indeed, lycopene upregulated LXRα, its target gene ABCA1, and decreased cholesterol and cell proliferation in prostate cancer cells, which was attenuated by the addition of LXRα antagonist (GGPP) and by si-RNA LXRα knockdown (28). Moreover, we showed that lycopene metabolite can induce PPAR and inhibit cancer cell migration and angiogenesis (52). Interestingly, Sonett and colleagues demonstrated the protective effect of an LXR antagonist against CS-induced inflammation and emphysema progression by restoring ABC transporters in vitro and in vivo (22), indicating the importance of identifying agents that can rescue these pathways and shield lungs from CS-induced inflammation and emphysema development. In our study, we observed that lycopene was effective in upregulating critical genes (LXRα, PPARα, ABCA1, and ABCG1) related to the RCT pathway, which were downregulated by the NNK/CS exposure, resulting in restored TC concentrations in lung and plasma to normal, which provided supporting evidence that lycopene protective effect may be due to the modulation of transcription systems, such as PPARα/LXRα.

The efficacy of lycopene doses used in our ferret model can be translational to human chemoprevention of lycopene in lung cancer development with comorbidity of COPD. The average intake of lycopene in the U.S. general population is estimated to be ~10 mg/day (53), and lycopene doses used in dietary supplements are 10 to 50 mg/day on market. The doses used in this study are 3 and 9 times of the average intake of lycopene in the United States, but they are achievable through regular diet; for example, the lower dose is achievable by eating ~2/3 cup of tomato sauce (54). These doses should be considered safe because up to 120 mg/day of lycopene was used in prostate cancer clinical trials without adverse effects (55, 56). Previous studies have shown that plasma and liver concentrations of lycopene in lycopene fed ferrets without NNK or CS treatment are similar to those seen in human subjects (13, 18). In this study, we detected lycopene in plasma and livers, but not in lungs, of ferrets after the low-dose and the high-dose lycopene feeding and NNK/CS exposure. However, lycopene in circulation would be systemically distributed to a majority of tissues, including lungs. Lycopene content in liver was within the range reported in humans (0.1–20.7 nmol/g liver) (13). The concentrations of total lycopene in plasma at 18.0–31.2 nmol/L are ~10 times lower than what was reported in humans (290–350 nmol/L) (18). The lower plasma concentrations, and undetectable levels of lycopene in the lungs, may be attributed to CS exposure. In a previous study, we showed that CS exposure for 9 weeks decreased lycopene ~40% in plasma, and ~90% in lungs, compared with the control group in ferrets (18). The longer CS exposure in the present study (22 weeks) could lead to the additional decrease in plasma and lung lycopene concentrations. Treatment with NNK alone reduced plasma lycopene levels by 33% and 56% in ferrets fed low-dose and high-dose lycopene, respectively (13). The fact that both NNK and CS can decrease lycopene levels in plasma and lungs of ferrets suggests that lycopene degradation could be a reason why we detected lower lycopene concentrations in plasma compared with our previous study (13, 18). This may also explain why we were unable to detect lycopene in lungs of ferrets after NNK/CS exposure, despite lycopene feeding.

In humans, cis isomers cover 50% to 79% of total lycopene in plasma (57). In our previous studies, ferrets fed the same lycopene doses had plasma concentrations of cis-isomers similar to human results (52%; ref. 13). Interestingly, in the present study, all-trans-isomer was a dominant isomer (72%–73%) when ferrets were exposed to NNK/CS and fed lycopene. Although the mechanism is not clear, it is possible that lycopene feeding could upregulate carotenoid cleavage enzyme, β-carotene-9’,10’-oxygenase (BCO2), which cleaves only cis-isomer of lycopene (58). Therefore, cis isomers of lycopene may be metabolized at a higher rate than trans-isomers in NNK/CS-exposed ferrets. However, further studies are needed to elucidate this mechanism.

In conclusion, we have provided strong evidence to suggest a protective effect of the dietary lycopene against NNK/CS-induced COPD and lung carcinogenesis in a ferret model. This effect of lycopene is associated with maintaining a pulmonary cholesterol homeostasis, through the regulation of the impaired RCT after NNK/CS exposure.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Authors’ Contributions
Conception and design: J. Mustra Rakic, C. Liu, S. Veeramachaneni, X.-D. Wang
Development of methodology: J. Mustra Rakic, C. Liu, S. Veeramachaneni
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Mustra Rakic, C. Liu, S. Veeramachaneni, X.-D. Wang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Mustra Rakic, C. Liu, D. Wu, I. Paul, L.M. Ausman, X.-D. Wang
Writing, review, and/or revision of the manuscript: J. Mustra Rakic, C. Liu, D. Wu, I. Paul, C.-Y.O. Chen, L.M. Ausman, X.-D. Wang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Mustra Rakic, C. Liu
Study supervision: X.-D. Wang

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Jelena Mustra Rakic, Chun Liu, Sudipta Veeramachaneni, et al.


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