Synergistic Effect of Cigarette Smoke and Bacterial Induced Chronic Obstructive Pulmonary Disease Type Airway Inflammation on Promotion of K-ras Mutant Lung Cancer

Ramos-Castaneda MA[1], Daga N[1], Umer M[1], Castro S[1], Gutierrez BA[1], Khosravi N[1], Moghaddam SJ[1].

Department of Pulmonary Medicine[1], The University of Texas MD Anderson Cancer Center, Houston, TX, USA,

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

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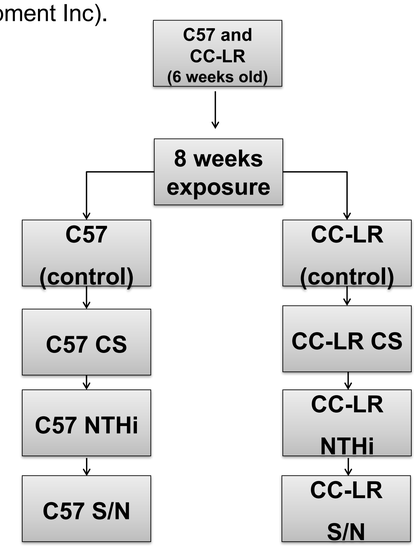
Introduction

Lung cancer, particularly K-ras mutant lung cancer, is the leading cause of cancer death worldwide, and cigarette smoking (CS) is its main cause. Epidemiologic studies had consistently revealed a strong association (3 to 9 fold increase) between lung cancer and COPD (chronic obstructive pulmonary disease), after controlling for CS exposure. COPD is an inflammatory disease of the airways with smoking being the main cause of it. Importantly, lung inflammation persists and lung function continues to deteriorate as does the increased risk of lung cancer even after cessation of cigarette smoking among former smokers with COPD. These facts suggest a strong link between COPD-related airway inflammation and lung cancer promotion independent of smoking. We have previously shown that weekly exposure to an aerosolized bacterial lysate of nontypeable *Haemophilus influenzae* (NTHi), induces lung inflammation with a profile of mediators and inflammatory cells similar to that observed in COPD patients excluding mucous metaplasia. NTHi is the most common colonizing bacteria in the lower respiratory tract of patients with COPD and could be a potential cause of perpetuating and promoting persistent airway inflammation after CS exposure in these patients. Therefore, we further studied the effect of combined CS and NTHi exposure in the induction of COPD phenotype and promotion of lung cancer in C57BL/6J and LSL-K-ras^{G12D}/CCSP^{Cre} (CC-LR) mice. The CS exposure was conducted by burning 3R4F reference cigarettes (University of Kentucky, Tobacco Research Institute), using an InExpose System (SCIREQ Scientific Respiratory Equipment Inc).



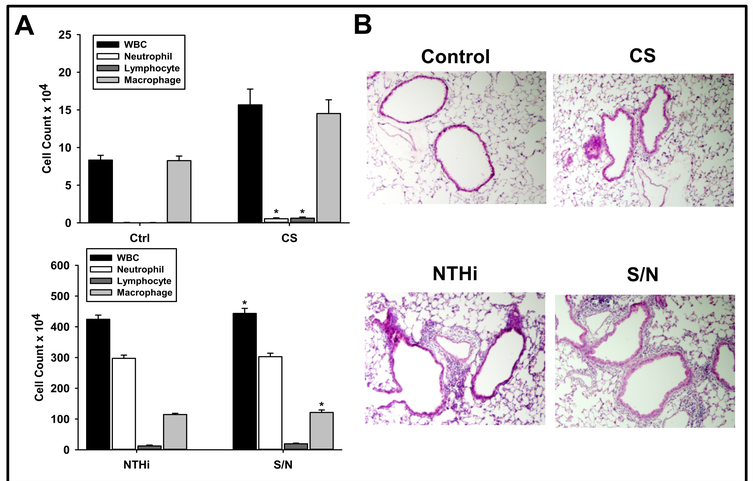


Figure 1: CS and NTHi exposure induce airway inflammation in C57 mice
(A) Bronchoalveolar lavage fluid (BALF) differential quantification of C57 mice demonstrate an increase inflammatory cellular macrophage component (CS, NTHi and S/N), and also a neutrophilic peak (NTHi and S/N).(B) Representative hematoxylin and eosin (H&E) photomicrographs of lung tissue (image shown at 10x magnification).

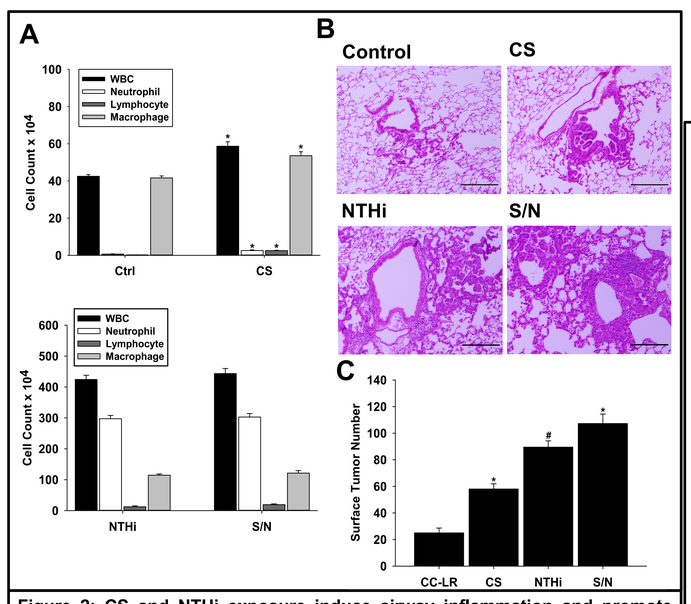


Figure 2: CS and NTHi exposure induce airway inflammation and promote tumorigenesis in CC-LR mice
(A) Bronchoalveolar lavage fluid (BALF) differential quantification of CC-LR mice demonstrate an increase inflammatory cellular macrophage component (CS, NTHi and S/N), and also a neutrophilic peak (NTHi and S/N).(B) Representative hematoxylin and eosin (H&E) photomicrographs of lung tissue (image shown at 10x magnification). (C) Lung surface tumor quantification shows and increase in tumor number by 2.3 fold change in CS mice compared to CC-LR, with NTHi and S/N increasing to 3.6 and 4.3 fold.

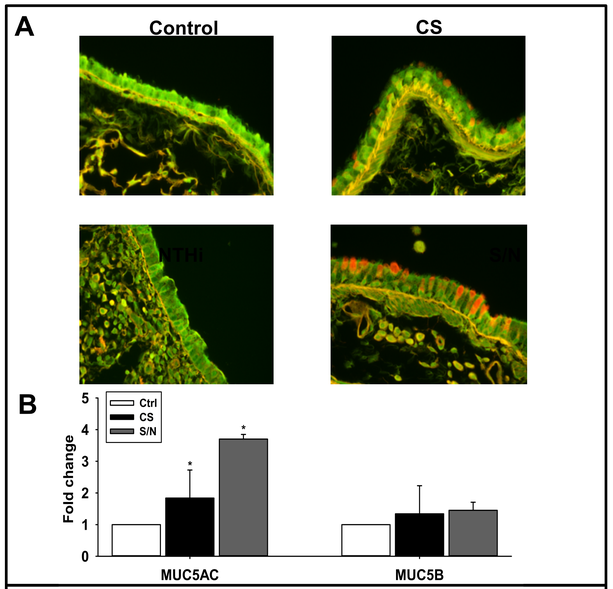


Figure 3: Effects of CS and NTHi exposure on lung epithelial mucin accumulation in C57 mice
(A) Periodic acid fluorescent Schiff (PAFS) representative photomicrographs of lung tissue show an increase in intracellular mucin accumulation after CS and S/N exposure but not after NTHi exposure (images shown at 40x magnification). (B) Quantitative RT-PCR (qPCR) of whole lung tissue for Munc5ac and Munc5b relative to control.

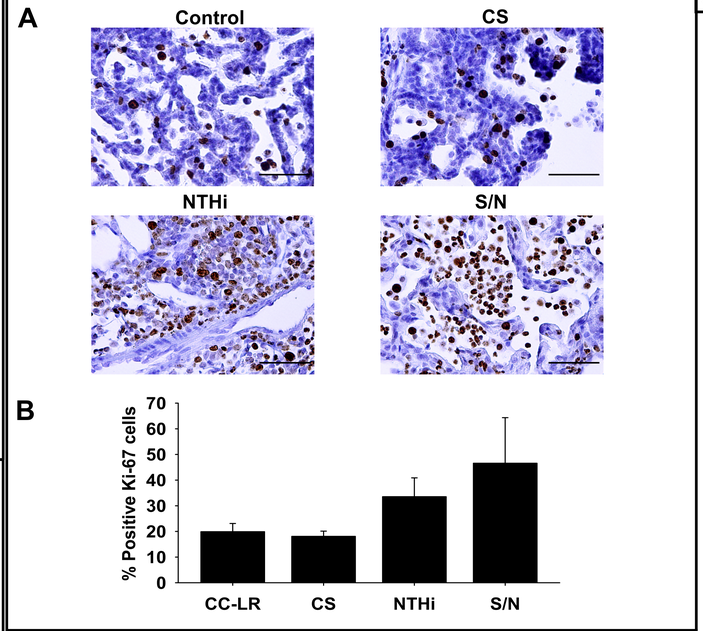


Figure 4: Effects of CS and NTHi exposure in tumor cell proliferation. (A) Representative photomicrograph (images shown at 40x magnification) of Ki-67 positive cells in lung tissue of CC-LR mice. (B) Quantification of Ki-67positive cells in tissue staining.

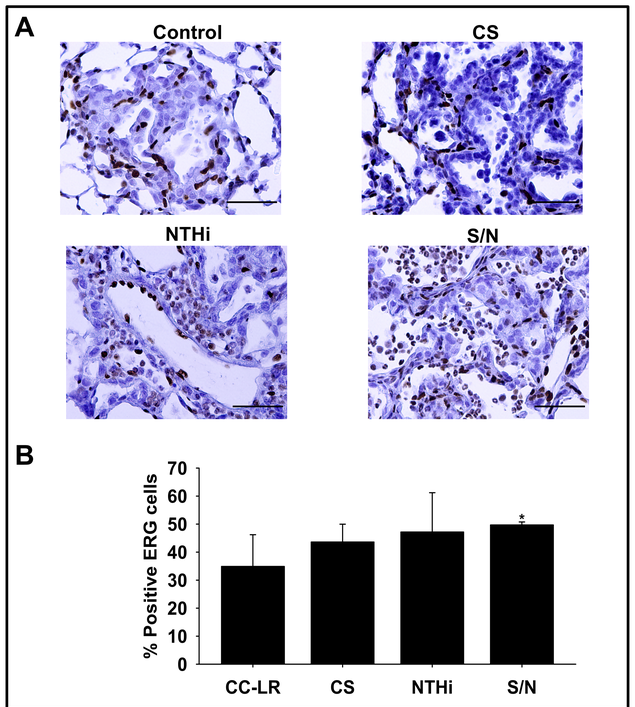


Figure 5: Effect of CS and NTHi exposure in tumor cell angiogenesis. (A) Representative photomicrograph (images shown at 40x magnification) of ERG positive cells in lung tissue of CC-LR mice. (B) Quantification of ERG positive cells in tissue staining.

Summary

- CS exposure alone causes a mild macrophage dominant airway inflammation and led to a 2.3 fold increase in lung tumor burden.
- Combined NTHi and CS exposure results in a robust neutrophilic lung inflammation and promotes K-ras mutant lung cancer by 4.3 folds (2 times more than CS alone).
- CS and combined NTHi and CS exposure promote intracellular mucin accumulation (mucous metaplasia) and increased gene expression.
- CS and NTHi alone or in combination promote tumor proliferation and angiogenesis.

Conclusion

Our results indicate that CS and colonization of smoke injured airways with NTHi induced a inflammatory and structural COPD-like phenotype and promote an inflammatory environment for K-ras mutant lung cancer development.

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Contact: smoghadd@mdanderson.org