human NSCLC tumor and normal adjacent lung samples with known loss of expression of Wnt7a in the tumor, and 6 (60%) had increased methylation of the Wnt7a promoter region in the tumors. Two NSCLC cell lines demonstrated to have methylated Wnt7a and loss of Wnt7a expression, H157, and H1299 were used to investigate methylation of the Wnt7a promoter. Treatment of these cell lines with 5-aza-2-deoxycytidine resulted in increased expression of Wnt7a and increased downstream activity of known targets of Wnt7a-Fzd9 signaling. Methylation of a Wnt7a promoter luciferase resulted in decreased activity of the luciferase compared with an untreated Wnt7a luciferase. NNK is a tobacco carcinogen known to attenuate DNMT1 degradation, leading to increased promoter methylation. Treatment of a nontransformed lung epithelial cell line with NNK resulted in decreased expression of Wnt7a and increased clonogenicity. Together, these results indicate that Wnt7a is frequently lost by methylation in NSCLC and that methylation of Wnt7a leads to decreased expression. Wnt7a could be methylated by exposure to tobacco carcinogens, leading to loss of expression and increased transformed lung cell growth. The clinical use of demethylating agents could restore Wnt7a activity, helping to decrease growth of NSCLC.

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The Effect of Toll-like Receptor 4 Deficiency on Emphysema in Smokers

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Smoking is the most important factor for chronic obstructive pulmonary disease (COPD), but only small portions of the smokers develop clinically significant COPD. These trends indicate that COPD is affected by other factors. In animal models, Toll-like receptor 4 (TLR4) deficiency causes emphysema by enhanced elastase activity and oxidant/antioxidant imbalance, but it has not yet been investigated in humans. In this study, we checked the association between TLR4 expression of lung and emphysema development among smokers.

All patients were eligible who had undertaken lung resection surgery for lung cancer from 2008 to 2010. Of this eligible group, we enrolled those with smoking history (> 10 pack-years). We measured TLR4 expression in lung lysate by Western blot and classified subjects into three groups: high expresser (TLR4/actin ≥ 1), intermediate expresser ($0.1 \leq \text{TLR4/actin} < 1$), and low expresser (TLR4/actin < 0.1). The presence of emphysema was confirmed on chest computed tomography. To determine whether the

development of emphysema itself could affect the level of TLR4 expression, we compared TLR4 expression between inbred Lewis rats with emphysema (induced by smoking for 16 weeks) and without emphysema (normal controls).

A total of 48 patients were enrolled. Fourteen subjects were grouped into the high expresser, 13 into the intermediate expresser, and 21 into the low expresser groups. There was no difference in age ($P = 0.65$), smoking amount ($P = 0.67$), or sex ($P = 0.44$) between groups. The proportions of subjects with emphysema were increased significantly as the expression of TLR4 decreased ($P = 0.045$); 4 (28.6%) of 14 high expressers had emphysema, 6 (46.1%) of 13 intermediate expressers had emphysema, and 15 (71.4%) of 21 low expressers had emphysema. FEV₁ per FVC was also decreased as the TLR4 expression decreased ($P = 0.046$); the mean of FEV₁/FVC was 0.62 ± 0.13 for high expressers, 0.68 ± 0.11 for intermediate expressers, and 0.70 ± 0.07 for low expressers. FEV₁ and FEV₁ per its predicted value were also decreased significantly as TLR4 expression was decreased ($P = 0.002$ and $P = 0.002$, respectively). There was no difference TLR4 expression level between inbred Lewis rats with and without emphysema.

Even with the same amount of smoking, humans with TLR4 deficiency seem to be more vulnerable to COPD development.

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Smoking-Altered DNA Methylation in Alveolar Macrophages Correlates with Gene Expression

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Rationale: Cigarette smoking is a leading cause of both emphysema and lung cancer, yet the mechanism of how smoking drives disease is unclear. Alveolar macrophages are key players in the development of smoking-related diseases. One important, but little studied, regulator of macrophage function is epigenetic modification of gene expression. **Methods:** Smokers with and without emphysema, and nonsmokers, underwent bronchoscopy with bronchoalveolar lavage to obtain alveolar macrophages. DNA and RNA were isolated using the Qiagen DNeasy kit (Qiagen, Valencia, CA) and MirVana kit (Life Technologies, Grand Island, NY) methods, respectively. Genome-wide methylation status of the samples was determined using Illumina Infinium 27K HumanMethylation array (Illumina, San Diego, CA). Macrophage mRNA expression was analyzed using GeneChip Human Exon 1.0 ST Arrays (Affymetrix, Santa Clara, CA). **Results:** The distribution of methylation in these probes differed significantly with respect to smoking status. There

is enrichment of differential methylation in genes from inflammatory pathways. Consistent with recent findings, significant methylation changes were particularly enriched in the areas flanking CpG islands. Analysis of matching gene expression data demonstrated a parallel enrichment for changes in inflammatory pathways. **Conclusion:** The alveolar macrophage data obtained from smokers, patients with emphysema, and nonsmokers demonstrates methylation changes that track with smoke exposure. The affected genes cluster in inflammation-linked pathways, and a significant number of the genes with differential methylation also have altered gene expression. This discovery suggests that epigenetic modification of alveolar macrophages contributes to smoking-related lung disease.

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Interleukin-15 and Influenza Virus Infection in a Mouse Model of Chronic Obstructive Pulmonary Disease

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Background: Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation with increased CD8 and NK cells, alveolar destruction, and fibrosis, where cigarette smoke (CS) and viral infection play an important role. Smokers with COPD have more severe responses to viral infections. Interleukin-15, a Th1 cytokine, is involved in lymphocyte homeostasis of CD8 and NK cells. IL-15 is increased in patients with COPD, but its role in pathogenesis is not known. **Hypothesis:** IL-15 plays a critical role in the augmented lung inflammation and remodeling caused by CS and viruses. **Methods:** We studied the effects of CS exposure and influenza virus on pulmonary IL-15 and the consequences of CS exposure and virus infection in wild-type (WT) and IL-15-deficient mice. We generated mice that overexpressed IL-15 in a lung-specific manner (IL-15Tg) and characterized the pulmonary inflammatory and remodeling responses during virus infection. **Results:** Mice exposed to CS and influenza manifest exaggerated inflammation, emphysema, and airway fibrosis. CS and viruses synergistically induced IL-15 production. The exaggerated tissue responses were diminished in IL-15-deficient mice. Unchallenged IL-15Tg mice did not have obvious phenotype. When compared with influenza-infected WT controls, influenza virus-infected IL-15Tg mice manifest exaggerated and more chronic lung inflammation, with heightened CD8⁺ and NK1.1⁺CD3⁺ cell accumulation and heightened alveolar destruction. **Conclusion:** CS and influenza

virus interact to induce IL-15 production, and IL-15 plays a key role in the pathogenesis of the heightened inflammation and remodeling caused by CS and virus. IL-15 in the tissues of patients with COPD is induced by CS and viruses, and contributes to the exaggerated viral responses seen in CS-exposed individuals.

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Nicotine-induced Fibronectin Expression Might Represent a Common Mechanism by which Tobacco Promotes Lung Cancer Progression and Obstructive Airway Disease

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Tobacco-related disorders like chronic obstructive pulmonary disease and lung cancer represent a tremendous burden to healthcare, with costs estimated in the billions of dollars yearly. We believe that fibronectin, a matrix glycoprotein implicated in tissue injury and repair and in embryogenesis, might play a role in the pathogenesis of both of these conditions. We reported that nicotine, an alkaloid present in tobacco, stimulates the expression of fibronectin in lung fibroblasts through activation of $\alpha 7$ nicotinic acetylcholine receptors (nAChR) (1). Importantly, we found that nicotine induces fibronectin expression in lung without promoting significant inflammation or changes in tissue architecture. These data suggested that nicotine-induced fibronectin may participate in the pathogenesis of tobacco-related disorders. Consistent with this, we found that fibronectin stimulates non-small cell lung cancer (NSCLC) growth through signals triggered by $\alpha 5 \beta 1$ integrin receptors expressed on the surface of tumor cells. Furthermore, silencing of $\alpha 5 \beta 1$ in lung carcinoma cells prevents growth and lung metastasis in animals (2). We also demonstrated that nicotine affects lung branching morphogenesis, and this, again, was mediated via $\alpha 7$ nAChRs and associated with alterations in the expression of fibronectin (3). This is intriguing, especially when considered together with reports showing that prenatal exposures increase the incidence of obstructive lung disorders (4). Based on the above, we hypothesize that nicotine exerts direct effects on lung cells through nAChRs. In smokers with lung cancer, nicotine-induced fibronectin may stimulate NSCLC growth and progression. In developing lungs, prenatal exposure to nicotine might affect lung branching morphogenesis, thereby increasing susceptibility to the development of obstructive airways disease. Further investigations are needed to determine the true role of this pathway that appears to provide at least one unifying mechanism linking tobacco-related disorders.