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COPD

The Effects of Smoking on the Developing Lung: Insights from a Biologic Model for Lung Development, Homeostasis, and Repair

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Abstract There is extensive epidemiologic and experimental evidence from both animal and human studies that demonstrates detrimental long-term pulmonary outcomes in the offspring of mothers who smoke during pregnancy. However, the molecular mechanisms underlying these associations are not understood. Therefore, it is not surprising that that there is no effective intervention to prevent the damaging effects of perinatal smoke exposure. Using a biologic model of lung development, homeostasis, and repair, we have determined that in utero nicotine exposure disrupts specific molecular paracrine communications between epithelium and interstitium that are driven by parathyroid hormone-related protein and peroxisome proliferator-activated receptor (PPAR)γ, resulting in transdifferentiation of lung lipofibroblasts to myofibroblasts, i.e., the conversion of the lipofibroblast phenotype to a cell type that is not conducive to alveolar homeostasis, and is the cellular hallmark of chronic lung disease, including asthma. Furthermore, we have shown that by molecularly targeting PPARγ expression, nicotine-induced lung injury can not only be significantly averted, it can also be reverted. The concept outlined by us differs from the traditional paradigm of teratogenic and toxicological effects of tobacco smoke that has been proposed in the past. We have argued that since nicotine alters the normal homeostatic epithelial-mesenchymal paracrine signaling in the developing alveolus, rather than causing totally disruptive structural changes, it offers a unique opportunity to prevent, halt, and/or reverse this process through targeted molecular manipulations.

Keywords Nicotine · Lung development · Tobacco · Smoking · Chronic obstructive pulmonary disease · Chronic lung disease

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Introduction

Tobacco smoking and exposure to second-hand smoke, biofuel smoke from cooking stoves, and breathed air pollutants are widely accepted to be causative factors for childhood asthma and chronic obstructive pulmonary disease (COPD) affecting nearly 3 billion people worldwide, predominantly in China, India, and Africa; however, over 15% of COPD occurs in nonsmokers [1]. Chronic obstructive pulmonary disease is irreversible and difficult to treat. Recent studies suggest that exposure of the developing fetal lung in smoking mothers increases the baby's susceptibility to childhood asthma and possibly COPD in later life. Because of the lack of direct experimental evidence, nicotine purportedly does not cause COPD [2]. However, recent data clearly demonstrate that nicotine is directly and specifically responsible for inducing differentiation of developing lung cells from the normal

to abnormal phenotype [3–5]. These studies, which focused on understanding the mechanistic effects of nicotine exposure on the developing lung, offer the hope that the compromised lung structure and functions in chronic lung diseases (CLDs) such as asthma and COPD could in the future be reversed to normalcy through pharmacological interventions of specific target molecules.

Barker Hypothesis for CLD

Tager et al. [6] had first shown that side-stream smoke affected fetal lung development in a landmark study of the effects of smoke exposure on neonatal pulmonary function. In a follow-up study it was shown that the levels of the nicotine metabolite cotinine in amniotic fluid correlated positively with the amount of cortisol, known to stimulate lung development [7]. This interrelationship was consistent with the burgeoning concept that antenatal factors could affect normal lung development—infection, hydramnios, hormones, and nutrients. Such thinking expedited Barker's hypothesis that chronic diseases have their origins in utero [8]. The then recent breakthrough success of antenatal glucocorticoids to prevent respiratory distress syndrome further supported the concept that the long-term consequences of intrauterine lung development could be corrected by the judicious use of physiopharmacologic agents [9].

Biologic Model of Lung Development, Homeostasis, and Repair

The molecular mechanisms of lung injuries due to a wide variety of perinatal insults such as barotrauma, oxotrauma, and infection have been recently elucidated by using a cellular/molecular model of normal lung development (Fig. 1) [10–13]. By experimentally determining how nicotine affects the integrated cell-cell signaling mechanism, this review describes the site and gene regulatory networks involved in nicotine's effects on lung structure and function, as a precursor of CLD.

Fostered by the seminal observation that glucocorticoids accelerate alveolar type II (ATII) cell surfactant synthesis by stimulating fibroblast synthesis and secretion of a low-molecular-weight peptide termed fibroblast pneumocyte factor [14], a paracrine growth factor model for the maturation of the pulmonary surfactant system, based on classic mesenchymal-epithelial interactions, has been developed (Fig. 1, steps 1–7). It had previously been shown that mesodermal development was under endocrine control and that early signals emanated from the epithelium to cause differentiation of the immature mesenchyme in the

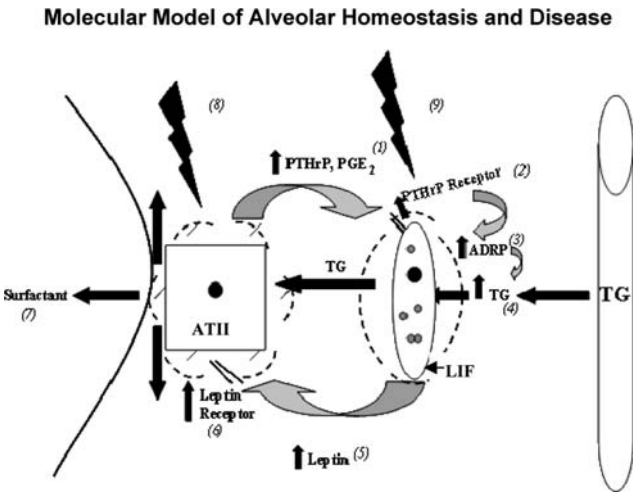

Fig. 1 Coordinating effects of stretch on alveolar type II (ATII) cell expression of PTHrP and prostaglandin E2 (PGE2) (step *1*), the expression of LIF PTHrP receptor (step *2*), its downstream target LIF ADRP (step *3*) and triglyceride uptake (step *4*), and the interaction between LIF-produced leptin (step *5*) and the ATII cell leptin receptor (step *7*), which stimulates de novo surfactant phospholipid synthesis by ATII cells (step *7*). We have also reported that stretching ATII cells in culture increases the production of PTHrP and leptin [1]. The effects of PTHrP and leptin are mutually exclusive, i.e., independent and unidirectional, since LIFs express the PTHrP receptor and leptin, but fetal ATII cells do not, although it should be noted that adult ATII cells do; conversely, ATII cells express PTHrP and the leptin receptor, but fibroblasts do not. Although it is difficult to compare the independent quantitative effects of stretch on PTHrP and leptin with their integrated effects on de novo surfactant phospholipid synthesis, the fold increase in each approximates the combined effect. Exposure to conditions such as hyperoxia, infection, nicotine, and/or fetal nutrient restriction can have molecular effects on the epithelial (step *8*) and mesenchymal (step *9*) cells

neighboring epithelium [15]. Furthermore, Brody's group originally showed that the developing lung fibroblast acquired an adipocyte-like phenotype termed the "lipid-laden fibroblast," leaving open the question as to whether these cells might be a source of lipid substrate for surfactant synthesis by the ATII cell [16]. Extending these observations, it was discovered that coculture of lipid-laden fibroblasts with type II cells resulted in the trafficking of the lipid from the fibroblast to the type II cell and its highly enriched incorporation into surfactant phospholipids, particularly when treated with glucocorticoids, suggesting a specific regulated mechanism for neutral lipid trafficking [17]. Interestingly, the fibroblasts take up neutral lipid but do not release it unless they are in the presence of type II cells; conversely, the type II cells are unable to take up neutral lipid. These observations led to the discovery that type II cell secretion of prostaglandin E_2 caused the release of neutral lipid from the fibroblasts, but the nature of the lipid uptake mechanism by the type II cells remained unknown [18]. However, it had been shown that the synthesis of pulmonary surfactant was an "on-demand"

system in which increased respiration resulted in increased surfactant production [19–21], suggesting a stretch-sensitive signal from the type II cell. This led to studying the role of parathyroid hormone-related protein (PTHrP) in lung development because (1) it was expressed in the embryonic endoderm [22]; (2) its receptor was present on the adepithelial mesoderm [23]; (3) it had been shown to be a stretch-regulated gene in the urinary bladder [24] and uterus [25], and distension of the lung was known to be of physiologic importance in normal lung development [26]; and (4) knockout of PTHrP caused stage-specific inhibition of fetal lung alveolarization in the transition from the pseudoglandular to the canalicular stage [27].

Early functional studies of PTHrP showed that it was a paracrine factor that stimulated surfactant phospholipid synthesis [28] and that it was stretch-regulated [29]. It was subsequently discovered that PTHrP stimulated neutral lipid uptake by the developing lung fibroblast (~lipofibroblasts) [30] by upregulating adipocyte differentiation-related protein (ADRP), a molecule shown to be necessary for lipid uptake and storage [31], which was subsequently found to be necessary for the transit of neutral lipid from the lipofibroblast to the ATII cell for surfactant phospholipid synthesis [32]. However, how PTHrP regulated lung surfactant via a lipofibroblast paracrine factor was still not known. Since lipofibroblasts are similar to adipocytes, it was hypothesized that lipofibroblasts would, like fat cells, express leptin, which would then bind to the type II cell and stimulate surfactant synthesis. Indeed, lipofibroblasts were shown to express leptin during rat lung development just prior to the onset of surfactant synthesis by type II cells [33]. Importantly, type II cells express the leptin receptor, thus providing a ligand-receptor signaling pathway between the lipofibroblast and the type II cell [34]. PTHrP was shown to stimulate leptin expression by fetal lung fibroblasts, thus providing a complete growth factor-mediated paracrine loop for the synthesis of pulmonary surfactant, as predicted by the PTHrP-based model of lung development [34].

Because the major effectors of normal lung development, such as barotrauma, oxotrauma, and infection, cause alveolar type II cell injury and damage, the effects of PTHrP deprivation on the lipofibroblast phenotype was next examined. It was discovered that in the absence of PTHrP, the lipofibroblast transdifferentiates to a myofibroblast, the cell-type that characterizes lung fibrosis [35]. Furthermore, myofibroblasts cannot sustain type II cell growth and differentiation whereas the lipofibroblast can, demonstrating the functional significance of these two fibroblast phenotypes for lung development. Importantly, when myofibroblasts are treated with a PPARγ agonist they revert back to the lipofibroblast phenotype, including their ability to promote type II cell growth and differentiation [35].

Effects of Nicotine on the Developing Lung

Tobacco smoke exposure of the developing infant in a pregnant woman who smokes begins in utero and continues throughout the fulminate period of lung development (up to age 8 years). There are well-documented short- and long-term effects of smoke exposure on lung physiology and pathophysiology that have life-long consequences. There is strong epidemiologic and experimental evidence that fetal exposure to maternal smoking during gestation results in detrimental long-term effects on lung growth and function (Table 1) [36–55]. Significant suppression of alveolarization, functional residual capacity, and tidal flow volume has been demonstrated in the offspring of women who smoked during pregnancy. It is important to emphasize that the main effects of in utero nicotine exposure on lung growth and differentiation are likely the result of specific alterations in late fetal lung development rather than its teratogenic or toxicological effects. These alterations in specific developmental and maturational programs may be subtle and thereby may explain significant long-term adverse pulmonary outcome with only minor immediate effects. The premise, therefore, is that nicotine exposure modifies physiologic development, i.e., its effects are part of the continuum of normal lung development, and therefore should be viewed as such and not as the traditional paradigm of teratogenic and toxicological effects of tobacco smoke. If this premise is valid, it allows for possible corrective treatment based on developmental and physiologic principles, whereas toxic, teratogenic effects would be less likely to be reversed since they lack an integrated, physiologic process. The underlying mechanisms and effector molecules involved in this process are not completely understood. However, it has been shown convincingly that in utero nicotine exposure disrupts specific molecular paracrine communications between epithelium and interstitium that are driven by PTHrP and PPARγ (see above), resulting in transdifferentiation of lung lipofibroblasts to myofibroblasts [3–5], i.e., the conversion

Table 1 Adverse effects of cigarette smoking during pregnancy on offspring pulmonary structure and function

1. Hypoplastic lungs with fewer air saccules [39–41]
2. Increased predisposition to both upper and lower respiratory tract infections [44–46]
3. Altered respiratory control and increased predisposition to sudden infant death syndrome [47, 48]
4. Persistently reduced pulmonary function [42, 43, 49, 50, 75]
5. Increased incidence and severity of pediatric asthma [51, 52]
6. Increased incidence of adult asthma and chronic obstructive pulmonary disease [53–55]

of the lipofibroblast phenotype to a cell type that is not conducive to alveolar homeostasis and is the cellular hallmark of CLD, including asthma [56]. We had previously clearly demonstrated that PPARγ expression is a key determinant of the lipofibroblast phenotype and that by molecularly targeting PPARγ expression, nicotine-induced lung injury can be significantly averted under both in vitro and in vivo conditions [3–5].

Evidence that Nicotine is the Main Agent that Causes Lung Injury in the Developing Fetus of the Pregnant Smoker

Although some of the effects of maternal smoking on the developing lung have been suggested to be stress-induced, the direct effects of maternal smoke on prenatal lung growth are constrained only by those components of maternal smoke that are transferred across the placenta. Although there are many agents in smoke that may be detrimental to the developing lung, there is evidence to support the idea that nicotine directly alters fetal lung development. Nicotine crosses the human placenta with minimal biotransformation to its metabolite cotinine [57]. It is accumulated in fetal blood, maternal milk, and amniotic fluid despite increased nicotine clearance during pregnancy, resulting in the fetus being exposed to even higher levels than those of the smoking mother [58–60]. Nicotine accumulates in several fetal tissues, including the respiratory tract [61], thereby suggesting nicotine as the likely agent that alters lung development in the fetus of the pregnant smoker. This is also supported by in vitro work done by others and by our data that show direct effects of nicotine on pulmonary ATII cells and fibroblasts isolated from the developing lung [61–63].

Level of Nicotine Exposure in Smokers

Although the dose of in vivo nicotine used in various studies to determine its systemic effects has ranged from 0.25 to 6 mg/kg, the range of nicotine intake in habitual smokers in one study was 0.16 to 1.8 mg/kg body weight [64, 65]. Because of this and the accelerated nicotine clearance during pregnancy in animal studies, the standard dose of nicotine used to mimic nicotine exposure in human smokers is 0.5 to 2.0 mg/kg per day, which is equivalent to an exposure to light (0.5 pack day^{-1}) to moderately heavy (2 pack day^{-1}) smoke exposure [64–68]. Typically, nicotine patches and gum deliver half to one quarter the nicotine dose of cigarette smoking, respectively [66].

Relationship of Maternal Smoking to Prenatal and Postnatal Lung Growth

There is extensive evidence from both animal and human studies of the reduction in both prenatal and postnatal lung growth in the offspring of mothers who smoke during pregnancy [6, 37–43, 67–77]. Arrested lung growth and lung hypoplasia have been reported after prenatal nicotine exposure in animal models [36, 39–41, 61, 65, 69–74, 77]. The hypoplastic fetal lungs of in utero smoke-exposed rat fetuses contain fewer and larger saccules that are more compliant and have reduced parenchymal tissue, septal crests, and markedly reduced surface area available for gas exchange [39]. It is important to realize that the mechanisms for prenatal and postnatal lung effects are likely to be different, and evidence suggests that prenatal exposure to tobacco smoke components may play a much greater role in altered lung function than postnatal or childhood exposures [75]. The pulmonary changes appear to occur early in pregnancy because respiratory function in premature infants of smoking mothers is significantly reduced compared to premature infants of nonsmokers [76].

Specific Cellular and Molecular Effects of Nicotine on the Developing Lung

Despite decades of research, specific cellular and molecular effects through which in utero nicotine exposure affects lung growth, development, and function remain incompletely understood [69]. Various investigators have pursued nicotine's effects on individual lung cell types or specific molecular pathways, but none of the models proposed so far explains the morphologic, molecular, and functional changes seen following in utero nicotine exposure completely. Alveolar type II cell hyperplasia and abnormal differentiation have been reported in rat and fetal monkey models of in utero nicotine exposure [61, 67, 74]. In the fetal monkey model, upregulation of α-7 nicotinic acetylcholine receptors in the lung and an increase in collagen and elastin deposition in airways were observed [67]. In the rat model, it was recently demonstrated that in utero nicotine exposure significantly stimulates ATII cell proliferation, differentiation, and metabolism [4]. Furthermore, it was shown that the nicotine-mediated stimulation of surfactant synthesis was by its direct effect on ATII cells, whereas ATII cell proliferation and metabolism were mediated via its paracrine effects on the adepithelial fibroblasts, permanently altering the "developmental program" of the developing lung. Nicotine's effects on lung fibroblasts have also been explored in rat and monkey models of perinatal nicotine exposure [67, 71–73]. Under in vitro conditions it was shown that nicotine exposure

disrupts epithelial-mesenchymal interactions and causes lipofibroblast-to-myofibroblast transdifferentiation [3, 5]. More importantly, in these studies, targeting specific alveolar interstitial fibroblast molecular intermediates effectively blocked nicotine's adverse effects on the developing lung. In addition to nicotine's effects on ATII cells and fibroblasts, its effects on pulmonary neuroendocrine cells via the activation of the paracrine serotonin pathway have also been described [78]. Therefore, prenatal nicotine exposure seems to alter lung development through multiple pathways, but a clear understanding of the underlying mechanisms involved and the mechanistic link between perinatal nicotine exposure and altered pulmonary structure and function are still not completely understood. Consequently, it is not surprising that there is no effective intervention to prevent the damaging effects of in utero nicotine exposure, though some strategies such as vitamin C and copper supplementation have been suggested as attractive options [70, 73]. However, the safety of these interventions is not established, the mechanisms underlying possible beneficial effects remain poorly understood, and the protection afforded is only partial and inconsistent. Given that despite enthusiastic antismoking campaigns, 12% of U.S. women still smoke during pregnancy, resulting in the birth of 450,000 smoke-exposed infants in 2002 [79]. Effective and safe interventions that are based upon a sound understanding of the molecular mechanisms involved in nicotine-induced lung injury are needed. Our studies have begun to precisely address these mechanisms and have already provided valuable and unique insights [3–5, 80, 81].

Barring some of the work from our group, reviewed above, no other studies seem to account for all of the pulmonary abnormalities seen following in utero nicotine exposure. For example, the paradox of advanced pulmonary maturity at birth and ultimate poor long-term pulmonary outcome is not explained by any of the previously proposed mechanisms. Since nicotine disrupts the specific homeostatic epithelial-mesenchymal pulmonary communications, inhibiting PTHrP/PPARγ signaling and stimulating Wnt signaling, culminating in lipofibroblast-to-myofibroblast transdifferentiation, this could potentially explain all of the known long-term pulmonary effects following in utero nicotine exposure, including the increased predisposition to childhood asthma [3–5, 80, 81]. Previous observations by others that there is decreased cellular lipid content and increased mitotic activity of fetal lung tissue in nicotine-exposed rat pups versus control pups are also consistent with the lipofibroblast-to-myofibroblast transdifferentiation following in utero nicotine exposure [35, 74]. The advanced pulmonary maturity at birth is explained by the direct pharmacologic effects of nicotine on ATII cells that lead to their pseudomaturation, which because of the breakdown of the underlying homeostatic epithelial-mesenchymal communications, ultimately fails, explaining both failed alveolarization and increased predisposition to asthma later in life in in utero smoke-exposed infants. Furthermore, the molecular basis for the increased generation of lung myofibroblasts, the key players in the pathophysiology of asthma and which contribute not only to tissue remodeling but also to airway inflammation, is clearly explained by the downregulation of PTHrP/PPARγ signaling and by the upregulation of Wnt signaling in nicotine-exposed lungs. Therefore, in addition to the abnormalities in lung structure, the increased generation of myofibroblasts in nicotine-exposed lungs explains the adverse long-term pulmonary outcomes in infants exposed to smoke during development [6, 37, 41, 75, 82, 83]. This paradigm provides a plausible and powerful unifying explanation for all of the nicotine-associated pulmonary morphometric, histologic, molecular, and functional abnormalities, setting the stage for not only effectively blocking but also possibly reversing these alterations by specific molecular targeting of lipofibroblast PTHrP/PPARγ and Wnt signaling intermediates (Table 2) [5].

Table 2 Specific cellular and molecular effects of nicotine on the developing lung

Specific cellular/molecular effect	Proposed intervention (PPARγ agonist ± Wnt antagonist)
Alveolar type II cell [4, 61, 74]	
Altered structure and increased proliferation	Normalized proliferation
Increased differentiation	Normalized differentiation
Increased surfactant synthesis	
Altered glucose and lipid metabolism	Normalized glucose and lipid metabolism
Alveolar interstitial fibroblast [3, 5]	
Decreased lipofibroblast differentiation	Increased lipofibroblast differentiation
Increased myofibroblast differentiation	Decreased myofibroblast differentiation
Epithelial-mesenchymal cross-talk [3–5]	
Decreased PTHrP/PPARγ signaling	Increased PTHrP/PPARγ signaling
Increased Wnt signaling	Decreased Wnt signaling

In summary, this review provides evidence for nicotine-induced lipofibroblast-to-myofibroblast transdifferentiation; alterations in ATII cell proliferation, differentiation, and metabolism; deleterious effects on pulmonary function; downregulation of the lipofibroblast PPARγ signaling and activation of Wnt signaling; and effective prevention of nicotine-induced effects on lipofibroblasts, ATII cells, and pulmonary function by targeting specific molecular intermediates of the PPARγ signaling pathway [3–5, 80, 81]. These findings, for the first time, provide a unifying mechanistic basis for various pulmonary morphologic and molecular features that are known to follow in utero nicotine exposure. Specifically, the downregulation of PPARγ signaling and the upregulation of Wnt signaling, resulting in lipofibroblast-to-myofibroblast transdifferentiation, are likely to be central molecular events in this process. The lipofibroblast-to-myofibroblast transdifferentiation, along with abnormal ATII cell proliferation and differentiation, explains the paradox of advanced pulmonary maturity at birth and increased predisposition to CLD in in utero nicotine-exposed infants.

Antenatal Steroid Administration for RDS as a Precedent for PPARγ Agonist Administration for CLD

Glucocorticoids have been used effectively to reduce the risk of respiratory distress syndrome (RDS) for over 30 years [9, 84]. This breakthrough in treating the fetus as a patient was preceded by extensive studies of the effects and mechanism of glucocorticoid action on normal lung development. The first double-blind clinical trial of glucocorticoid effects on lung development published in 1972 showed that it was safe and effective in lowering the incidence of RDS. However, there were aspects of the treatment that were of concern, namely, the lack of a statistically significant effect in males. A subsequent series of studies revealed that the male hormone inhibited the effect of glucocorticoids on the differentiation of the lung fibroblasts [85–87]. More recent studies of the mechanism of androgen action have shown that androgens stimulate the Wnt pathway by increasing β-catenin expression [88]; similar results have been obtained with nicotine [80, 81], suggesting a common pathway for androgen and nicotine blocking glucocorticoid-induced fetal lung development. However, PPARγ agonists can effectively block the inhibitory effect of nicotine on fetal lung fibroblast differentiation [3–5]. Bearing in mind that antenatal glucocorticoids have a less than optimal effect on fetal lung development, testing the effects of antenatal and postnatal PPARγ agonists on this process is worthwhile and in progress [89]. Promising preliminary data show that PPARγ agonists accelerate lung development and that its effect is non-gender-dependent, calling for a clinical trial of this therapy in the near future.

Conclusion

Given that more than 400,000 infants are exposed to maternal smoke per annum in the US alone, maternal smoking is a huge worldwide public health problem. And given that the cost of advertising for smoking increased by over one billion dollars from 2001 to 2002, it is unlikely that the problem of smoking during pregnancy will go away any time soon. Therefore, the organ-specific mechanisms for the harmful effects of in utero smoke exposure need to thoroughly understood before there is any real chance of its prevention. For example, with the approaches adopted so far, the mechanism for the 40% increase in clinically impaired lung function of the in utero smoke-exposed infants on follow-up is not known. As reviewed here, in utero nicotine exposure disrupts the homeostatic alveolar interaction between the alveolar lung fibroblast PPARγ and Wnt signaling pathways, offering a unique mechanistic perspective and an exceptional translational opportunity. This concept differs from the traditional paradigm of the teratogenic and toxicological effects of tobacco smoke that has been proposed to underlie nicotine-related pulmonary damage in the past. Since nicotine seems to alter the normal homeostatic epithelial-mesenchymal paracrine signaling in the developing alveolus, rather than causing totally disruptive structural changes, there is a distinct opportunity to prevent, halt, and/or reverse this process through targeted molecular manipulations, e.g., PPARγ administration. And because of the relatively recent exposure of humans to cigarette smoke, it is likely that elucidation of the deleterious effects of nicotine on the lung will help in understanding other chronic lung diseases due to failed cell-cell signaling as well.

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