Past as Prologue: Vaping Effects on the Developing Lung

It is well established that maternal exposure to systemic nicotine interferes with airway and airspace development in animal models (1, 2). Recent efforts have further characterized transplacental effects of maternal nicotine and e-cigarette vapor on fetal lung development and introduced the concept of susceptibilities to injury in various compartments that may persist into adulthood (3–5). How lung fibroblast subpopulations participate in *in utero* responses to maternal nicotine is largely unresolved.

The designation of reparative lipofibroblasts and profibrotic myofibroblasts, although simplistic, describes cellular "skill sets" that contribute to lung homeostasis or dysfunction (6). The basic paradigm, informed by use of fate mapping approaches in experimental fibrosis models, invokes fibroblast subgroups that toggle between lipofibroblasts (lipogenic) and myofibroblasts (myogenic) with the ultimate fate and phenotype, enabling repair or promoting fibrosis, respectively (7). Lipofibroblasts reside in the airspace compartment near alveolar type II epithelial cells presumably providing peroxisome proliferator-activated receptor γ-mediated paracrine maintenance functions. Although *in vitro* and *in vivo* studies show that neonatal lipofibroblasts exposed to nicotine transition to a myofibroblast phenotype, little is known about effects of e-cigarette vapor with and without nicotine (8, 9).

Whether the neonatal milieu is especially hospitable to this switch and whether this fate is durable is specifically relevant to the current study. In this issue of the *Journal*, Wang and colleagues (pp. 794–805) report on their exploration of selected fibroblast markers and matrix proteins in adult offspring mice after maternal exposure to e-cigarette vapor (10). The examination of markers of lipogenic versus myogenic fibroblasts in whole lung preparations, although indirect, provides evidence of different remodeling programs triggered by maternal exposure to either propylene glycol/vegetable glycerin (PG/VG) or PG/VG plus nicotine during fetal development. Unfortunately, the markers are not coordinately regulated by PG/VG or PG/VG with nicotine. By contrast, perinatal subcutaneous nicotine promotes clear myogenic differentiation of lung fibroblasts in adult offspring mice (3). Thus, e-cigarette vapor delivery of nicotine and humectants to pregnant mice may result in substantially different systemic dosing, placental uptake, and fetal cell exposures compared with systemic administration and manifest in different offspring outcomes. Humectant (PG/VG) effects that either modulate nicotine responses or operate in isolation afford added complexity to the fibroblast readouts. Although no fully coherent matrix phenotype associated with early e-cigarette exposure can be discerned from this survey, the findings may guide future efforts. To build on prior knowledge and inform clinical management, maternal e-cigarette vapor exposures should not only reflect current device use patterns but also fully describe efficiency of transplacental transport of e-cigarette vapor components and the resultant levels in fetal tissues.

Nicotine triggers fibrotic programs known to contribute to preclinical and clinical fibrosis (11–13). Maternal nicotine exposure also increases neonatal susceptibility to both perinatal and adult lung fibrosis in experimental models (14, 15). The mechanisms are complex and developmental stage dependent but likely involve the activation of the widely expressed nAChRs (alpha7 nicotinic acetylcholine receptor). No studies have yet demonstrated a clear profibrotic effect of e-cigarette vapor (PG/VG with or without nicotine) or evidence of altered susceptibility to fibrotic triggers. In Wang and colleagues, PG/VG shows divergent effects in male versus female offspring, inducing fibronectin and collagen 1a in female offspring but reducing fibronectin in males when compared with offspring of maternal room air controls. Surprisingly, PG/VG plus nicotine reduces fibronectin but increases profibrotic PAI-1 in male mice associated with a reduction in TGFβ and psmad2 lung staining and improved Ashcroft fibrosis scores compared with room air and PG/VG controls. In a prior publication, the authors found enhanced smooth muscle actin expression with PG/VG in male mice with and without nicotine and elevated fibronectin expression with PG/VG without nicotine but used a different vapor delivery system (16). This complex constellation of responses that may be developmental stage dependent invites future studies with consistent exposure protocols to better characterize the profibrotic and antifibrotic effects of e-cigarette vapor on the fetal or postnatal lung.

Wang and colleagues focus on quantitative changes in subpopulations of lipofibroblasts and profibrotic myofibroblasts in lungs of adult mice exposed to *in utero* PG/VG ± nicotine; however, it is unknown whether these changes correlate with adult lung function in a sex-dependent manner. The effects of nicotine on small airway growth have been highlighted in several preclinical studies. Nicotine can readily cross the placenta from the maternal bloodstream (17) and is associated with increased levels of oxidative stress (18) and airway remodeling associated with high levels of fibroblast nAChRs (18). Additionally, airway narrowing, airway wall thickening, dysynaptic lung growth, and reduced forced expiratory flows have been associated with *in utero* nicotine exposure (17, 19). The long-term effects of maternal vaping during pregnancy on adult lung function are unknown. Nevertheless, the study by Wang and colleagues suggests that sex differences in structural genes are involved in alveolar scaffolding and development, although they do not report functional studies. Together, their findings indicate that sex differences in extracellular matrix genes involved in alveolar structure may be affected by exposure to e-cigarette vapors, potentially linking their findings to airflow obstruction ± inflammation in later life.

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Early-life exposures that confer late-life apparently acquired disease is an area of great interest to lung biologists but remains mechanistically obscure and complex. Sex-disparate effects of exposures are also an emerging aspect of lung pathophysiology. Of greater public health concern is the rapidly increasing use of e-cigarettes by young adults, especially pregnant women. This study is a creative foundational approach to assessing mesenchymal consequences of fetal e-cigarette exposures with all three mandates in mind. The lack of broad matrix panels, direct mesenchymal cell characterizations, pulmonary function tests, or time course analyses precludes a unifying picture of meaningful anatomic or functional perturbations. Future studies should observe the following requirements: *1*) time course analyses to resolve whether adult perturbations reflect persistent disturbances or late-onset developments; *2*) highly powered studies to facilitate the generation of coherent expression and cell functional themes; *3*) analyses of sex partitioning that include sex hormone measurements and maneuvers targeting sex hormone status; *4*) studies to examine changes in mesenchymal cell subpopulations and lung function with e-cigarette exposures; and *5*) three-dimensional imaging studies to detect subtle architectural changes in the lung.

Finally, the relevance of preclinical studies as they relate to human exposures remains unresolved. The changing landscape of e-cigarette use, products, and technology can prevent accurate modeling of the human condition. The recent popularity of nicotine salt over nicotine base products, the use of flavors, vaping of unregulated liquids, and new disposable pod-based e-cigarette technologies can create barriers for designing studies that accurately reflect the health effects of e-cigarette exposures on lung growth and function throughout the lifespan. ■

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References

1. Maritz GS, Dennis H. Maternal nicotine exposure during gestation and lactation interferes with alveolar development in the neonatal lung. *Reprod Fertil Dev* 1998;10:255–261.
2. Sekhon HS, Jia Y, Raab R, Kuryatov A, Pankow JF, Whitsett JA, *et al*. Prenatal nicotine increases pulmonary alpha7 nicotinic receptor expression and alters fetal lung development in monkeys. *J Clin Invest* 1999;103:637–647.
3. Sakurai R, Liu J, Gong M, Bo J, Rehan VK. Perinatal nicotine exposure induces myogenic differentiation, but not epithelial-mesenchymal transition in rat offspring lung. *Pediatr Pulmonol* 2016;51: 1142–1150.
4. Noël A, Hansen S, Zaman A, Perveen Z, Pinkston R, Hossain E, *et al*. In utero exposures to electronic-cigarette aerosols impair the *Wnt* signaling during mouse lung development. *Am J Physiol Lung Cell Mol Physiol* 2020;318:L705–L722.
5. McAlinden KD, Naidu V, Sohal SS, Sharma P. *In utero* exposure to nicotine containing electronic cigarettes increases the risk of allergic asthma in female offspring. *Am J Physiol Lung Cell Mol Physiol* [online ahead of print] 12 Aug 2020; DOI: 10.1152/ajplung.00230.2019.
6. McGowan SE, Torday JS. The pulmonary lipofibroblast (lipid interstitial cell) and its contributions to alveolar development. *Annu Rev Physiol* 1997;59:43–62.
7. El Agha E, Moiseenko A, Kheirollahi V, De Langhe S, Crnkovic S, Kwapiszewska G, *et al*. Two-way conversion between lipogenic and myogenic fibroblastic phenotypes marks the progression and resolution of lung fibrosis. *Cell Stem Cell* 2017;20:571.
8. Rehan VK, Wang Y, Sugano S, Romero S, Chen X, Santos J, *et al*. Mechanism of nicotine-induced pulmonary fibroblast transdifferentiation. *Am J Physiol Lung Cell Mol Physiol* 2005;289: L667–L676.
9. Krebs M, Sakurai R, Torday JS, Rehan VK. Evidence for *in vivo* nicotine-induced alveolar interstitial fibroblast-to-myofibroblast transdifferentiation. *Exp Lung Res* 2010;36:390–398.
10. Wang Q, Sundar IK, Blum JL, Ratner JR, Lucas JH, Chuang TD, *et al*. Prenatal exposure to electronic-cigarette aerosols leads to sex-dependent pulmonary extracellular-matrix remodeling and myogenesis in offspring mice. *Am J Respir Cell Mol Biol* 2020;63:794–805.
11. Vicary GW, Ritzenthaler JD, Panchabhai TS, Torres-González E, Roman J. Nicotine stimulates collagen type I expression in lung via α7 nicotinic acetylcholine receptors. *Respir Res* 2017;18:115.
12. Vang A, Clements RT, Chichger H, Kue N, Allawzi A, O'Connell K, *et al*. Effect of α7 nicotinic acetylcholine receptor activation on cardiac fibroblasts: a mechanism underlying RV fibrosis associated with cigarette smoke exposure. *Am J Physiol Lung Cell Mol Physiol* 2017; 312:L748–L759.
13. Jensen K, Nizamutdinov D, Guerrier M, Afroze S, Dostal D, Glaser S. General mechanisms of nicotine-induced fibrogenesis. *FASEB J* 2012;26:4778–4787.
14. Huang LT, Chou HC, Lin CM, Yeh TF, Chen CM. Maternal nicotine exposure exacerbates neonatal hyperoxia-induced lung fibrosis in rats. *Neonatology* 2014;106:94–101.
15. Dasgupta C, Xiao D, Xu Z, Yang S, Zhang L. Developmental nicotine exposure results in programming of alveolar simplification and interstitial pulmonary fibrosis in adult male rats. *Reprod Toxicol* 2012; 34:370–377.
16. Wang Q, Khan NA, Muthumalage T, Lawyer GR, McDonough SR, Chuang TD, *et al*. Dysregulated repair and inflammatory responses by e-cigarette-derived inhaled nicotine and humectant propylene glycol in a sex-dependent manner in mouse lung. *FASEB Bioadv* 2019;1:609–623.
17. Maritz GS. Perinatal exposure to nicotine and implications for subsequent obstructive lung disease. *Paediatr Respir Rev* 2013;14: 3–8.
18. Maritz GS, Rayise SS. Effect of maternal nicotine exposure on neonatal rat lung development: protective effect of maternal ascorbic acid supplementation. *Exp Lung Res* 2011;37:57–65.
19. Wongtrakool C, Wang N, Hyde DM, Roman J, Spindel ER. Prenatal nicotine exposure alters lung function and airway geometry through α7 nicotinic receptors. *Am J Respir Cell Mol Biol* 2012;46:695–702.