Commentary

Control of Lung Epithelial Growth by a Nicotinic Acetylcholine Receptor

The Other Side of the Coin

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Nicotine, a primary active component of tobacco, is a powerful stimulator of cell proliferation. The search for nicotine receptors linked to aberrant lung growth led to the discovery that nicotinic acetylcholine receptors (nAChRs) are significantly expressed in the non-neuronal tissues of the lung.¹ There are more than a dozen different nAChR subunit proteins, subdivided into α and β subfamilies, which form pentameric ion channels consisting of either a single type of α subunit (homopentamers) or a combination of α and β subunits (heteropentamers).

As ligand-gated ion channels, nAChRs undergo complex allosteric changes in response to binding either the endogenous ligand acetylcholine or exogenous ligands, including nicotine. Although nAChRs are classically linked to the plasma membrane depolarization required for neurotransmission, non-neuronal nAChRs in the lung act most frequently as calcium channels and have been linked to regulatory proteins such as *src* and phosphatidylinositol 3-kinase, which can control cell proliferation.² Moreover, although nAChR activation often leads to a positive feedback loop that induces receptor expression, chronic stimulation of nAChRs can lead to channel desensitization and decreased activity. Thus, elucidating functional roles for nAChRs is particularly complex and requires consideration of subunit composition, dose response, and duration of ligand stimulation.

Although the majority of studies of nAChR function in the lung are related to the effects of nicotine on these receptors, little is known about the physiological functions of these receptors in regulating lung growth and repair. Emerging data show that airway epithelia secrete, process, reabsorb, and synthesize acetylcholine, underscoring a physiological role for nAChRs in normal lung function.³ In this issue of *The American Journal of Pathol-*

ogy, Maouche et al⁴ used a multipronged approach to investigate roles for $\alpha 7$ nAChR, a widely expressed homopentamer, in regulating airway epithelial growth and differentiation.

As a pseudostratified epithelium, the airway is composed of several phenotypically distinct cell types including ciliated cells, secretory cells, and basal cells, all in close proximity. By immunohistochemistry, Maouche et al found that basal cells, normally localized at the basement membrane, were enriched for $\alpha 7$ nAChR expression. Importantly, expression of $\alpha 7$ nAChRs was pronounced in proliferating cells in the developing lung. Given this and the more typical role for non-neuronal nAChRs in stimulating cell proliferation, it was interesting that functional experiments revealed a role for $\alpha 7$ nAChRs in limiting, not stimulating, basal cell proliferation. Specifically, the authors found that the injury response in airways of $\alpha 7$ nAChR-deficient mice was characterized by significant basal cell hyperplasia compared with wild-type mice.⁴ Treatment of cultured human airway cells or *ex vivo* human lung explants with α -bungarotoxin, a neurotoxin capable of blocking $\alpha 7$ nAChR function, had a similar effect on the injury response. As a direct demonstration that $\alpha 7$ nAChR expression controls proliferation in cultured cells, a human lung basal epithelial cell line transfected with either small interfering RNA or cDNA (to deplete or enhance $\alpha 7$ nAChR expression) showed an inverse correlation between the level of $\alpha 7$ nAChR expression and the

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rate of cell division. Consistent with these studies on lung epithelia, keratinocytes from $\alpha 7$ nAChR-deficient mice favored progression through the cell cycle over terminal differentiation.⁵ These data suggest an intriguing parallel role for $\alpha 7$ nAChRs in the airway to promote basal cell differentiation by restricting cell growth.

In addition, Maouche et al showed that the effect of inhibiting $\alpha 7$ nAChR was most prominent in altering the injury response. However, the airways of uninjured $\alpha 7$ nAChR-deficient mice also had significant areas with disrupted morphology and basal cell hyperplasia.⁴ Nevertheless, the data presented are consistent with the work of others showing that $\alpha 7$ nAChR-deficient mice are sensitive to acute lung injury and have deficiencies in fluid clearance mediated by alveolar epithelium.⁶ In this study, acute nicotine treatment facilitated recovery from acute lung injury; however, whether chronic low-dose nicotine administration would have a deleterious effect was not tested.

It is notable that Maouche et al did not directly test the effects of nicotine in their *in vitro* models. Therefore, it would be of interest to identify the effects of distinct nicotine treatment protocols that either desensitize or stimulate basal cell $\alpha 7$ nAChRs. These experiments could unveil other possible modes of action for $\alpha 7$ in regulating cell proliferation. For instance, one possibility is that $\alpha 7$ nAChRs deliver both mitogenic and antiproliferative signals, with the latter being predominant at baseline, but overcome in the presence of exogenous stimulation with nicotine. Another possibility is that $\alpha 7$ nAChRs deliver only antiproliferative signals that, when inhibited by α -bungarotoxin, enable mitogenic signals elicited by nicotine stimulation of other nAChRs receptors.

The use of α -bungarotoxin in several of the experiments presented by Maouche et al should be interpreted cautiously because this agent can also inhibit other classes of nAChRs, such as $\alpha 3$ nAChRs. The same is true when one is evaluating the effects of nicotine, which can up-regulate several nAChR proteins beyond $\alpha 7$ nAChRs.⁷ Importantly, tobacco smoke contains other active components besides nicotine that may also affect the expression of several nAChRs.⁸ This raises the possibility that any effect of nicotine (or tobacco) that desensitizes $\alpha 7$ nAChR may have a stimulatory effect on other classes of nAChRs. New pharmacological agents that recognize different nAChRs to varying degrees of specificity are continually being developed and are expected to help define the roles for individual nAChR proteins in the future.⁹

A similar complexity is encountered with the use of $\alpha 7$ nAChR-deficient animals. Silencing of this receptor has been associated with increased compensatory expression of other nAChR subunits (including $\alpha 3$, $\alpha 9$, and $\alpha 10$).⁵ Moreover, nAChR heterogeneity makes defining roles for specific subunits difficult, particularly because cells expressing multiple subunit genes have the potential to produce several different combinations of functionally distinct nAChRs.¹⁰ Nonetheless, the combination of molecular approaches and the use of multiple systems by Maouche et al was a strength of the study.

The study by Maouche et al has important clinical implications, because the pathology of injured $\alpha 7$

nAChR-deficient mice closely resembles changes noted in the airways of smokers with chronic obstructive pulmonary disease. Intriguingly, the airways of lungs isolated from heavy smokers showed areas with significant basal cell hyperplasia, which were morphologically similar to the injury models in which $\alpha 7$ nAChR expression or function was inhibited.⁴ Because nicotine exposure would normally be expected to activate $\alpha 7$ nAChRs, one way to reconcile these apparently discordant results is to consider that smokers' lungs are exposed to a long-term low dose of nicotine, which could desensitize $\alpha 7$ activity,^{11,12} and thus physiologically mimic $\alpha 7$ nAChR-deficient mice.

Another important implication of the findings by Maouche et al relates to the fact that the antimitogenic effects exerted by $\alpha 7$ nAChR activity in airway epithelia contrast with the mitogenic effects observed in cultured lung cancer cells.¹³ nAChRs in general and $\alpha 7$ nAChRs in particular have been linked to nicotine-stimulated proliferation of lung carcinoma cells. These observations suggest that, although $\alpha 7$ nAChR antagonists might promote epithelial cell hyperplasia, they might also serve to restrict lung cancer cell growth as has been shown *in vitro*.² In fact, the $\alpha 7$ nAChR antagonist α -cobratoxin was recently found to prolong survival in an animal model of lung cancer.¹⁴ The relevance of this work in relation to the development of new and effective anticancer therapeutic strategies is obvious.

Three recent, large-scale epidemiological studies have identified a gene locus, 15q25, which is linked to smoking dependence, chronic obstructive pulmonary disease, and lung cancer, that contains a cluster of nAChR genes, including $\alpha 3$, $\alpha 5$, and $\beta 4$.^{15–17} This locus does not contain the $\alpha 7$ nAChR gene, which instead is located nearby in locus 15q14 on the same chromosome. It is tempting to speculate that $\alpha 3$, $\alpha 5$, and/or $\beta 4$ nAChRs are linked to lung disease through a misregulated interaction with $\alpha 7$ nAChR. Whether this misregulation is due to a direct interaction, through formation of heteromeric nAChRs or by interplay between parallel intracellular signaling pathways remains an open question.

It is notable that the data presented by Maouche et al are more supportive of a role for $\alpha 7$ nAChRs in restricting cell proliferation than in promoting cell differentiation; the latter is mainly inferred from histological analysis of tissue phenotype. Defining signaling pathways controlled by $\alpha 7$ nAChR that are linked to differentiation would strengthen this case, but these studies await further exploration. Potential $\alpha 7$ nAChR regulatory candidates include the Foxj1,⁴ the wnt/ β -catenin axis,^{18,19} and the peroxisome proliferator-activated receptors.²⁰

Furthermore, it will be interesting to examine whether the effects on epithelial cells noted by Maouche et al are shared by other cells, because $\alpha 7$ nAChRs are expressed in a wide array of lung cells including endothelial cells, fibroblasts, alveolar epithelial cells, and macrophages. These data will be important when the findings of this study are considered in the context of emerging data linking $\alpha 7$ nAChR to normal lung development.^{21,22}

In summary, the work by Maouche et al further extends our understanding of the role of nAChRs, particularly $\alpha 7$ nAChRs, in regulation of cell proliferation in lung epithe-

lia. Further work will be needed to define the mechanistic basis for this and other nAChRs in regulating airway epithelial cell differentiation and regeneration. However, the data available to date implicate $\alpha 7$ nAChRs in fundamental cellular processes relevant to lung development, injury and repair, and carcinogenesis. Generation of new and more specific nAChR agonists and antagonists is expected to enable the functions of $\alpha 7$ and related nAChRs to be more finely dissected, thereby opening new avenues for therapeutic intervention.

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