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# COVID-19 and smoking: is nicotine the hidden link?

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**Nicotine *via* alpha7-nicotinic receptor induces ACE-2 overexpression in human bronchial epithelial cells (HBEpC)** https://bit.ly/3eJ5b35

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*To the Editor*:

Leung *et al.* [1] have recently published, in the *European Respiratory Journal*, a paper on the expression of angiotensin-converting enzyme II (ACE-2) in the small airway epithelia of smokers and COPD patients, discussing its effects on the risk of severe coronavirus disease 2019 (COVID-19). The authors found an increased expression of the ACE-2 gene in the airways of subjects with COPD and in current smokers. Indeed, a recent systematic review reporting data on the smoking habits of patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), concluded that smoking may be associated with a negative progression of the disease and with the adverse outcome [2]. These conclusions were challenged in a correspondence by Cai [3] on the basis that a reliable mechanism explaining this association was missing. The need for these results to be supported by additional studies is quite clear, but we believe that a robust mechanistic explanation exists. Nicotine has a known influence on the homeostasis of the renin–angiotensin system (RAS) up-regulating the angiotensin-converting enzyme (ACE)/angiotensin (ANG)-II/ANG II type 1 receptor axis, and down-regulating the compensatory ACE-2/ANG-(1–7)/Mas receptor axis, contributing in turn to the development of cardiovascular and pulmonary diseases [4]. Different airway cells, such as bronchial epithelial cells, type II alveolar epithelial cells and interstitial lung fibroblasts, express nicotinic acetylcholine receptors (nAChR), specifically the α7subtype [5]. All these cells express components of the RAS [4]. In addition, nicotine increases the expression and/or activity of ACE in the lung [4], an increase which has been found also in the serum of smokers, and that required at least 20 min to return to control level [4]. ACE-2 serves as a physiologically relevant cellular entry receptor for SARS-CoV, for the human respiratory coronavirus NL63, and probably for SARS-CoV-2 [6]. ACE binds the SARS-CoV-2 S protein, and through its tissutal expression mediates the localisation and the efficiency of the infection [6]. Moreover, nicotine induces the epithelial–mesenchymal transition (EMT) [5, 7], a mechanism sufficient to allow "normal" differentiated cells to acquire the stem cell-like characteristics and properties. We planned experiments on human bronchial epithelial cells (HBEpC), obtained from Cell Applications Inc. (www.cellapplications.com/product number 502K-05a). Cells were maintained as

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adherent monolayer in complete bronchial/tracheal epithelial cell growth medium (www.cellapplications.com/product) at 37°C in 95% air/5% $CO_2$, seeded at an initial density of $7.5\times10^4$ cells·cm$^{-2}$, and sub-cultured with a 0.25% trypsin–1 mM EDTA solution (Sigma-Aldrich, Milan, Italy) when cultures reached 80% confluence. HBEpC are derived from the surface epithelium of normal human bronchi non-diseased (*i.e.* asthma, COPD or type 2 diabetes). The morphology is consistent with epithelial origin, and is positive for epithelial cell marker cytokeratin 18. Semi-confluent HBEpC at fourth passage ($7.5\times10^4$ cells·cm$^{-2}$) were treated: 1) for 1 h with zero or $1.0\times10^{-7}$ M nicotine (Sigma-Aldrich, Milan, Italy) dissolved in saline in complete medium; 2) with $1.0\times10^{-6}$ M α-Bungarotoxin (α-BTX; Sigma-Aldrich, Milan, Italy) dissolved in saline, in the continued presence of nicotine at zero or $1.0\times10^{-7}$ M for 1 h; 3) treated continuously with nicotine for additional passages, 1 passage every 48 h for a total of 16 passages. We showed, for the first time, that nicotine at $1\times10^{-7}$ M (the concentration present on the alveolar lining fluids after one cigarette is in the range $6\times10^{-6}$ to $6\times10^{-5}$ M [5]) is able to increase ACE-2 (figure 1a) in HBEpC. Treatment with nicotine induces phospho-S6 ribosomal protein (Ser235/236), Akt1, phospho-Akt (Ser473), phospho-Akt (Thr308) and phospho-p44/42 MAPK (Thr202/Tyr204) (figure 1b). To verify the hypothesis that ACE-2 is induced by nicotine through α7-nAChR, HBEpC, at fourth passage, in the exponential growth phase, plated at a density of $1\times10^6$ cells·mL$^{-1}$, were incubated with α7-nAChR siRNA (0.1 µg) diluted in 100 µL of siRNA transfection medium. Transfection was performed as described by Li *et al.* [8], who transfected, successfully, HBE16 human airway epithelial cell line (unaffected cells). A clone of transfected HBEpC that did not express α7-nAChR proteins, also after treatment with nicotine (figure 1c), and is not able to induce phospho-S6 ribosomal protein (Ser235/236), Akt1, phospho-Akt (Ser473), phospho-Akt (Thr308) and phospho-p44/42 MAPK (Thr202/Tyr204) after nicotine treatment (figure 1d), was selected for further experiments. Nicotine did not induce ACE-2 in this clone (si-mRNA-α7-HBEpC) (figure 1a). This observation supports the hypothesis that ACE-2 increase is specifically mediated by α7-nAChR. Moreover, when HBEpC were incubated simultaneously with nicotine and α-BTX, an α7 nicotine antagonist [9], no induction of ACE-2 was observed (figure 1d). Importantly, treatment with nicotine, α-BTX or with the combination is not cytotoxic (data not shown). On these bases, we suggest that smoking may promote cellular uptake mechanisms of SARS-CoV-2 through α7-nAChR signalling. A possible α7-nAChR down-stream mechanism may be the induction of phospho-Akt and phospho-p44/42 MAPK. This mechanism was hypothesised, partially, by Olds and Kabbani [10] on their schematic model explaining how nicotine exposure increases the risk of SARS-CoV-2 entry into lung cells. α7-nAChR is present both in neuronal and non-neuronal cells (*i.e.* lung, endothelial, lymphocyte); consequently, smoking may impact COVID-19 pathophysiology and clinical outcome in several organ systems, including the brain.