Exploring the Influence of Cigarette Smoke on TROP2 Expression in Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a major public health problem, with a trend of increasing estimated deaths in the coming years (1). Cigarette smoke, a major risk factor for COPD, promotes inflammation, senescence (2, 3), and repeated cycles of injury and repair that can compromise the integrity of lung tissue, thereby depleting the repair potential of stem cells (4). The repair processes often recapitulate or reactivate events previously active during embryogenesis. Cigarette smoke exposure in a bronchial epithelial cell monolayer affects Notch-1 signaling, a pathway that regulates stem cell survival and differentiation and tissue repair mechanisms (5).

TROP2 (trophoblast antigen 2) exerts a relevant role in lung development and is recognized as a major tumorigenic factor and as a molecule involved in stem cell maintenance (6). TROP2 is a transmembrane glycoprotein that undergoes intramembrane proteolysis at two cleavage sites mediated by the γ-secretase complex that yields a large extracellular fragment and a short intracellular fragment. TROP2 can promote cell proliferation via direct and indirect pathways. The intracellular fragment can enter the nucleus where it binds to the β-catenin transcription factor, thereby increasing the expression of cyclin D1 and c-myc (7), promoting cell proliferation and self-renewal. In addition, TROP2 by ERK (extracellular signal-regulated kinases) activation *1*) induces the activity of the AP-1 (activator protein 1) transcription factor, thus increasing the expression of cyclin D1, cyclin E, and CDKs (cyclin-dependent kinases), and *2*) decreases the cell cycle inhibitor $p27^{KIP1}$, which inhibits the activity of the cyclin E/CDK2 complex (8).

With regard to cell adhesion, TROP2 decreases fibronectin binding and brings the integrin α5β1/talin complex to the leading edge of the cell, decreasing cell adhesion and increasing cell motility (9), as TROP2 activation increases intracellular calcium concentration (10).

The aberrant and continuous injury that cigarette smoke exposure causes in the airways could lead to uncontrolled aberrant activation of TROP2 that could facilitate both cancer initiation and progression. TROP2 correlates with mutant p53 and is involved in adenocarcinoma cell growth, invasion, and neovascularization (11). A previous study demonstrated that TROP2 is increased in adenocarcinoma and correlates with poor clinical outcome and with shorter overall survival (11).

In this issue of the *Journal*, Li and colleagues (pp. 747–759) provide *ex vivo* and *in vitro* evidence that human small and large airways from smokers have increased cytoplasmic TROP2 expression and that TROP2 expression correlates with the smoke index (12). Further experiments using primary basal cells (BCs) isolated from bronchial brushing of healthy donors provide evidence that cigarette smoke extract (CSE) is able to upregulate TROP2 gene and protein expression in a time- and dose-dependent manner. Protein and phosphorylation levels of both p38 MAPK (mitogen-activated protein kinases) and NF-κB are increased by exposure to CSE. Inhibitors of p38 MAPK (PD169316 or SB203580) or of NF-κB (BAY11-7082) blunt the increase of TROP2 caused by CSE exposure, supporting the concept that CSE increases TROP2 expression in airway BCs through p38 MAPK and NF-κB pathway cooperation. Therefore, the effects of p38 MAPK and NF-κB in TROP2 overexpression could add to the recognized role of other factors, including p63 or WT1 (Wilms' tumor-1) inactivation (13).

Li and colleagues also provide new evidence that Ginsenoside Rb3 inhibits CSE activation of p38 MAPK and of NF-κB, thereby alleviating CSE-induced TROP2 expression. It is conceivable that Ginsenoside Rb3 mediates these effects by acting on key mechanisms that lead to the reduction of oxidative stress (14). Experimental evidence demonstrates that CSE increases oxidative stress in airway epithelial cells both by increasing reactive oxygen species generation and by impairing antioxidant mechanisms (15). Ginsenoside Rb3 is effective in mitigating CSE effects in BC monolayers and in air–liquid interface (ALI). The use of primary cells directly isolated from human lung tissues addresses the need to replace animal models with *in vitro* models that reproduce *in vivo* characteristics (16). The ALI culture method yields a layer of polarized mucociliary differentiated epithelial cells that morphologically resemble the organization and stratification of the *in situ* airway epithelium. For this reason, the results obtained in this study can be considered closer to what actually happens *in vivo*.

Although the study by Li and colleagues has the potential to identify, for the first time, the molecular mechanisms by which cigarette smoke upregulates TROP2 expression, it has some limitations. First, TROP2 can be expressed in the nucleus or in the cytoplasm under different contexts, and the authors do not specify whether the increase due to smoking occurs in both or only one of the two compartments. The experimental model chosen by the authors also has some limitations and does not fully exploit the potential of an ALI culture. Notably, the ALI model allows delivery of toxic compounds using the air exposure route, which resembles the *in vivo* inhalation route (16). Instead, the authors chose to stimulate the cells by adding a CSE solution only to the basal medium. This could conceivably affect the results, because the cells that are exposed to the cigarette smoke *in vivo* are the most apical ones of the airway mucosa, rather than the basal cells. Furthermore, the cells are stimulated before differentiating into a multilayer comprising multiple cell types, characteristic of the lung tissue. In this way, the CSE could modify the physiological balance of the different cellular types during the differentiation process, driving the formation of one cell type over another.

Finally, the effects of Ginsenoside Rb3, here demonstrated only *in vitro*, warrant future *in vivo* studies on smokers and subjects with COPD.

Originally Published in Press as DOI: 10.1165/rcmb.2021-0107ED on March 30, 2021

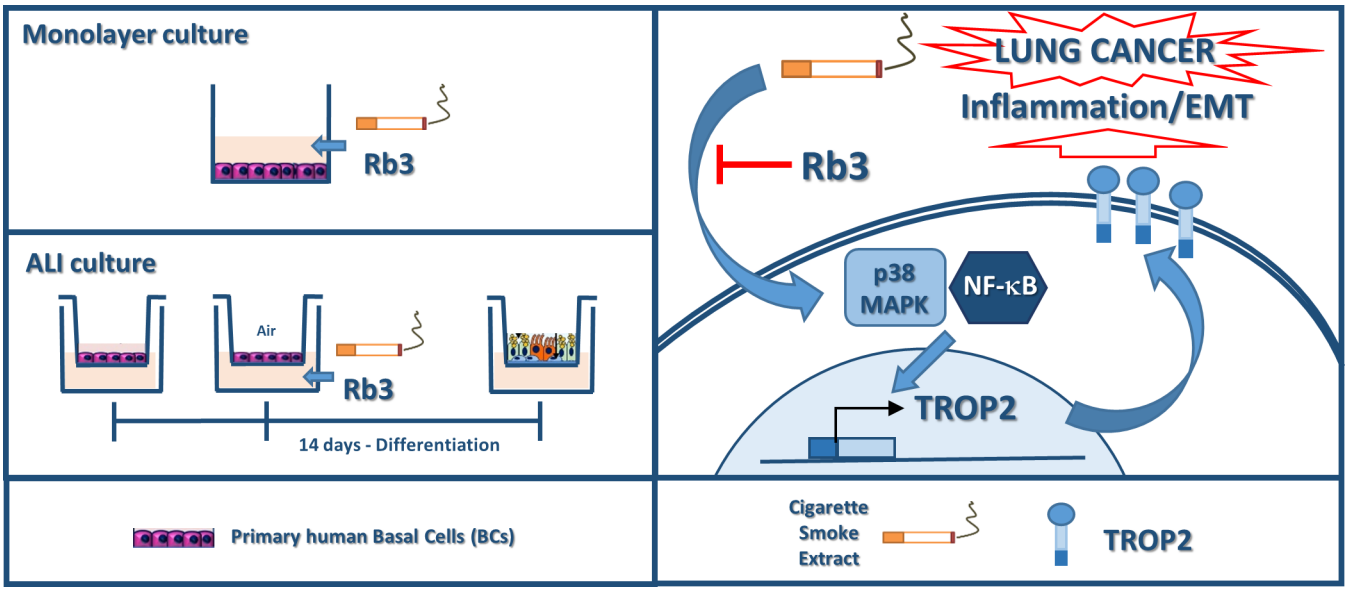

Figure 1. Schematic representation of the effects of cigarette smoke extract and Ginsenoside Rb3 on TROP2 expression in monolayer and ALI models. ALI = air–liquid interface; EMT = epithelial–mesenchymal transition; p38 MAPK = p38 mitogen-activated protein kinase; TROP2 = trophoblast antigen 2.

In conclusion, TROP2 appears to be involved in various mechanisms necessary to maintain airway tissue homeostasis. Smoking, by altering TROP2 expression and related homeostatic mechanisms, could induce epithelial–mesenchymal transition and inflammation. Based on the findings of the study, Ginsenoside Rb3 counteracts the effect of cigarette smoke and could potentially be used as add-on therapy in patients with cancer (*see* Figure 1). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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