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Generation of a novel mouse model for conditional expression of epithelial Na^+ channel in the lung

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The complex pathogenesis of cystic fibrosis (CF) lung disease remains incompletely understood. βENaC-transgenic mice with airway-specific overexpression of ENaC mimick increased airway Na^+ absorption in CF patients and develop spontaneous CF-like lung disease. However, the high and early pulmonary mortality and the very early onset of the disease limits the use of this model for studies that require long-term observation including the pathogenesis of chronic infections, effects of inhaled toxicants and particulates, and long-term effects of pharmacotherapy. Conditional overexpression of ENaC in mice by combinatorial use of lung-specific promoter elements and the tetracycline-regulated system provides a powerful tool to elucidate its role in airway ion transport, inflammation, host defense and tissue remodeling in the *in vivo* pathogenesis of CF and in a development-independent fashion. However, the original version of the reverse tetracycline-dependent transactivator (rtTA) exhibited limited doxycycline sensitivity and residual affinity to its promoter (P_{tet}) producing leaky transgene expression in the absence of doxycycline impeding the use of this system for such mechanistic studies. We, therefore, used a new generation rtTA, designated rtTA2^S-M2, with no basal activity and increased doxycycline sensitivity, and the rat Clara cell secretory protein (CCSP) promoter to target its expression to pulmonary epithelia in mice. To develop a model with conditional expression of ENaC, we first generated novel CCSP-rtTA2^S-M2 founder lines by pronuclear injection. For functional characterization, activator founder lines were crossed with bi-transgenic reporter mice expressing luciferase and Cre recombinase (LC-1). Background activity, doxycycline sensitivity, tissue and cell-type specificity, inducibility, and reversibility of doxycycline-dependent gene expression were determined by luciferase activity, immunohistochemistry, morphometry and

bioluminescence measurements in neonatal and adult lungs. These analyses demonstrated that we generated two distinct novel CCSP-rtTA2^{S}-M2 activator mouse lines that confer tight and doxycycline dose-dependent regulation of transgene expression with high inducibility, complete reversibility and no background activity, in airway or alveolar epithelia. Second, we generated responder lines for conditional expression of i) βENaC alone and ii) β- and γENaC. We used CCSP-rtTA2^{S}-M2 activator lines to mediate doxycycline controlled ENaC expression in selected βENaC responder lines (βENaC-TRE-Luc), determined luciferase activity and performed quantitative real-time PCR and showed high induction of luciferase and βENaC in double transgenic CCSP-rtTA2^{S}-M2/βENaC-TRE-Luc mice. The externally induced ENaC expression resulted in increased Na^{+} absorption of tracheal tissues measured in transepithelial Ussing chamber recordings. We demonstrate that rtTA2^{S}-M2 enables quantitative control of conditional expression of reporter genes (luciferase and Cre) as well as βENaC in different compartments (conducting airways and alveoli) of the murine lung. Our results predict that the new conditional ENaC lines, i.e. activator and responder lines will be useful to study the pathogenesis CF lung disease in a development independent fashion and to determine the specific role of ENaC subunits in the context of disease in future studies. Additionally, it will allow studying lung repair of the diseased lung. Moreover, the developed CCSP-rtTA2^{S}-M2 activator mice will be useful tools to study the role of other genes of interest in lung biology or disease pathogenesis of CF and other common lung diseases including Asthma or chronic obstructive pulmonary disease (COPD) and might ultimately lead to the development of novel therapies for these common diseases.