

1 Research Program

1.1 Project Title: Endolysosomal cation channels and lung disease

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1.1.1 Current State of Relevant Research

The most prevalent chronic lung diseases today are lung fibrosis and COPD (chronic obstructive pulmonary disease), the latter one affecting nearly 300 million people worldwide, resulting in the death of 3 million individuals each year. Currently, there are no drugs available that can cure lung fibrosis, emphysema formation or COPD. Hence, there is an urgent need for novel and innovative strategies and targets. In COPD and emphysema patients, the lung is chronically inflamed. In response to cigarette smoke or inhalation of environmental and occupational pollutants such as metals in asbestosis or silicosis, high levels of dust in coal mining and certain gases, cells such as neutrophils, T-lymphocytes, B cells and macrophages accumulate. When activated, these cells initiate an inflammatory cascade that triggers the release of inflammatory mediators such as tumour necrosis factor alpha (TNF- α), interferon gamma (IFN- γ), matrix-metalloproteinases (MMP-6, MMP-9), cathepsins, C-reactive protein (CRP), interleukins (IL-1, IL-6, IL-8) or fibrinogen (Barnes, 2004; Vlahos and Bozinovski, 2014). These inflammatory mediators sustain the inflammatory process and lead to tissue damage as well as a range of systemic effects. In the setting of COPD, respiratory tract infections, both acute and chronic, also occur with increased frequency (Sethi et al., 2010). These infections contribute considerably to the clinical course of COPD patients and constitute a significant comorbidity in COPD.

Endolysosomal cation channels, in particular TRPML channels (TRPML1, TRPML2, TRPML3) and two-pore channels (TPC1, TPC2) have been shown recently to be highly expressed in macrophages (Samie et al., 2010; Cang et al., 2013), but also in alveolar epithelial cells, and have been postulated to control intracellular vesicle trafficking and endolysosomal exocytosis, and to play critical roles in endocytosis and phagocytosis.

1.1.2 Preliminary Work Directly Relating to the Research Program

We have already preliminary data obtained from the analyses of TRPML and TPC knockout mice indicating significant changes in lung function parameters. A particular focus of this proposal shall be on TPCs, i.e. TPC1^{-/-} and TPC2^{-/-} mice where we identified robust changes in lung function parameters.

1.1.3 Hypothesis and Aims

We hypothesize that in the lung TRPML channels and TPCs may be key regulators of the secretion of proteolytic enzymes and inflammatory mediators on the one hand, and, on the other hand may regulate phagocytosis and intracellular trafficking of lung pathogens. Malfunction of these channel proteins is hence postulated to impact development and progression of emphysema formation, COPD, chronic lung inflammation as well as lung fibrosis and are thus exciting novel targets for the treatment of lung diseases.

1.1.4 Work Program and Methods

The following work packages (WP) and research tasks shall be addressed:

- **WP1: Use qPCR and endolysosomal patch-clamp techniques to evaluate functional expression of TPC1 and TPC2 in lung primary cells.** One important first step in determining

the role of TPCs in lung function is the exact cellular and also subcellular (early endosomal?, late endosomal? lysosomal?) localization of these channels and the characterization of the respective endogenous currents. To address these fundamental questions we will make use of the endolysosomal patch-clamp technique as described previously (Grimm et al., 2014; Chen et al., 2014) and specific small molecule agonists of these channels. Furthermore, for TPC2 a newly generated TPC2 GFP reporter mouse model is available for tissue preparation and visualization of green fluorescent cells in the lung, i.e. TPC2 expressing cells using laser scanning microscopy.

- **WP2: Assess lung function in wild-type and TPC/TRPML knockout mouse models.** In parallel to WP1, in WP2 lung function measurements shall be repeated for TPC1 and TPC2. In addition to basal lung function measurements, we will also assess lung function of WT and knockout animals under chronic noxious or stress conditions such as e.g. exposure to tobacco smoke (cooperation with Dr. A.Ö. Yildirim, Helmholtz Institute Munich).
- **WP3: Assess the molecular mechanism(s) causing lung phenotypes in TPC/TRPML knockout mouse models.** In WP3 we will investigate the molecular mechanisms leading to the observed phenotypes in TPC1^{-/-} and TPC2^{-/-} mice (cooperation with Prof. Dr. Manfred Frick, University of Ulm).