Functional analysis of microRNA in the *ex vivo* animal model of allergic airway inflammation

Allergic airway inflammation is a hallmark of allergic asthma, the most common chronic respiratory disease in paediatric population. Allergic asthma is associated with allergic inflammation causing bronchial airflow obstruction and airway remodelling that increases disease severity and leads to poor clinical outcomes and poor response to treatment in asthmatic patients. Early prevention, in particular in children, could decrease disease severity, but also inhibit irreversible inflammatory and structural changes in the lungs.

Recently *ex vivo* models such as precision cut lung slices (PCLS) have become popular recently as they overcome limitations associated with animal models and *in vitro* studies. They retain the three-dimensional lung structure and native microenvironment, so it is possible to determine the effect of delivered agents on the surrounding tissues. This model was successfully used to model lung fibrosis and develop novel therapies for pulmonary hypertension, but was not previously studied in allergic airway inflammation in the context of modified miRNA expression, despite previous reports showing its suitability to study the mechanisms of allergic inflammatory response. Therefore, the innovative aim of this project it to investigate if modifying the expression of miRNAs altered during airway inflammation in sensitized rats will influence inflammatory responses in the rat and human *ex vivo* model and to identify the potential target proteins of these miRNAs.

Based on our previous studies showing the altered miRNA expression profile in the lungs of rats sensitized to house dust mite extract as compared to non-sensitized rats, we assumed that modifying the expression of these miRNAs in the lung tissue *ex vivo* model using precision-cut lung slices (PCLS) may influence the airways hyperactivity and allergic inflammatory response. We aimed to investigate if transfection of rat-derived organoid model (PCLS) with miRNA mimics/inhibitors will significantly affect allergic response i.e. airway hyperactivity, inflammatory alterations in lung microenvironment and if novel target proteins can be identified in this ex vivo model using rat and human lung tissue.

In the first step, we plan to generate precision-cut lung slices from lung tissue of Brown Norway rats sensitized to house dust mite (n=5) and control rats and compare the airways structure (histological staining) and function (airway hyperactivity test using histamine) between sensitized and non-sensitized lung slices. The next step will include modification of miRNAs expression using transfection of PCLS with fluorescently labelled miRNA inhibitors/mimics to induce downregulation or overexpression. Our preliminary results regarding miRNA expression profile in lungs of sensitized rats showed that 2 miRNAs: rno-miR-223-3p and mir-328a-3p were significantly altered in our previous experiment, so we selected them for analysis. To evaluate the functional effect of miRNA modifiers, we will analyse the airway hyperactivity in transfected PCLS. We will also measure the expression of genes involved in allergic airway inflammation: mucin, cytokines specific for airway epithelial cells, Th2 cells-derived cytokines, chemokines, eosinophils. We will also analyse the inflammatory proteins secreted to culture medium after transfection with miRNA mimic/inhibitors. For the inhibitor or mimic that will show the strongest effect on airway hyperactivity and inflammation, analysis of the expression of possible target proteins will be done to identify the pathways regulated by this miRNAs. In the last step, we aim to analyse if miRNAs mimics/inhibitors showing the strongest anti-inflammatory potential in the rat PCLS have translational potential in human PCLS and will show similar inflammatory response and targets the same proteins.

The results of this project will provide new knowledge about the role of miRNA in allergic airway inflammation. If modifying miRNAs expression involved in inflammation may affect inflammatory cascade in lung tissue, this *ex vivo* model may be further used as a platform for pre-clinical screening of new therapeutics based on miRNA inhibition or overexpression to develop microRNA-targeted therapy focused on allergic airway diseases such as asthma. In addition, identifying novel target proteins of studied miRNAs may be used in future to discover novel pathways involved in allergic inflammation and to develop disease specific therapies.