Airway epithelium provides the first line of defense against environmental risk factors and plays a crucial role in the integrating of innate and adaptive immune responses. Increased permeability for environmental allergens, pathogens and toxic substances caused by the loss of barrier function upon injury requires the wounded airway epithelium to restore the cellular barrier. Chronic airway inflammation, the underlying cause of lung diseases such as asthma and chronic obstructive pulmonary disease, induced by aberrant repair of the airways, leads to structural and functional changes of the airway walls, referred to as remodeling. Main steps of the wound healing include cell migration, dedifferentiation, spreading of the cells to close the wound, proliferation and differentiation. These processes require the phased expression of networks of genes. One of the plausible biological mechanism of regulating gene expression are small, noncoding RNAs called microRNAs (miRNAs). The exact involvement of miRNAs in the repair of injured airway epithelium is still not well known, and only a few studies exist on this subject. Our previous research has shown altered expression of numerous miRNAs during wounding assays of human airway epithelium *in vitro* model at different stages of wound repair, implying that they play an important role in this process. One of studied miRNAs, miR-328, has shown over 10-fold changes in expression levels at different stages of wound repair. The inhibition of miR-328 has demonstrated a significantly longer wound closure in comparison to the negative control cells. Therefore, the aim of this study is to identify the target genes (at mRNAs and protein level) which expression may be regulated by miR-328 and that may be crucial to the process of wound repair in airway epithelium.

We plan to conduct the experiments on human airway epithelium cell line 16HBE14o-. Cells will be cultured on plates until confluence and then wounding assays will be performed. RNA and protein will be isolated at specific time points following injury established in previous experiments (at baseline, 4, 8 and 16 hours post-wounding). Expression analysis of genes selected from in silico analysis and potentially targeted by miR-328 will be performed using Next-Generation Sequencing. Luciferase assay will be used to verify the interaction between studied mRNA and miR-328. Expression of proteins, encoded by confirmed mRNAs, will be detected with Western Blot method. The proteins showing significantly altered expression during repair will be analyzed by immunocytochemistry to identify their localization and possible function at different time points of wound closure.

Chronic inflammation of the airways underlies aberrant repair of the airway epithelium, which subsequently leads to structural and functional changes in the airway wall, the underlying cause of lung diseases such as asthma or chronic obstructive pulmonary disease. Identification of target genes, which expression is regulated by miR-328 (significant in the process of epithelial wound repair) offers possibility to further develop novel therapeutic strategies such as targeted therapy with the use of inhibitors or synthetic miRNA, which might be a breakthrough to the treatment of these diseases. Results obtained in the course of this project will be the starting point to our future research on the biology of wound repair in the airway epithelium, including the *in vivo* animal model and clinical studies. This is, however, beyond the budget of this project.