ABSTRACT

Background. RNA-binding proteins (RBPs) play a key role in post-transcriptional gene regulation (PTGR) of genes involved in numerous biological processes. These proteins act through the binding to specific *cis*-elements present in their RNA targets and by forming, with other regulatory factors, dynamic ribonucleoprotein complexes (RNPs) that ultimately determine the fate of different type of RNAs. There are several families of RBPs classified according to the type of RNA target that they bind and this thesis focused on the study of RBPs that regulate the turnover and translation of messenger RNA (mRBPs). Multiple studies in the last decade have established that aberrant expression and function of RBPs participate to cancer pathogenesis by altering the stability and translation of genes involved in many mechanism of neoplastic transformation. Many of the cancer-related pathways for which PTGR mediated by RBPs has been established are also critically involved in chronic inflammation; to this end, many important basic and preclinical studies and gene ablation animal models indicate that RBPs are critically involved also in inflammatory responses and immunity. In contrast to the growing number of studies on the role pf RBPs in human cancer, translational studies based on chronic inflammatory disease are still scarce.

Aims. In this thesis we aimed at studying the expression profile of mRBPs in two major human chronic lung inflammatory diseases: chronic obstructive pulmonary disease (COPD) and bronchial asthma and to proceed with the characterization of their functional role in disease pathogenesis. On these studies, our long-term aim is to explore mRBP therapeutic targeting, for which we plan to develop dedicated experimental models.

Results. In our first study we focused on three RBPs, Tristetraprolin (TTP), AU-binding factor 1 (AUF-1) and Human Antigen R (HuR). These proteins recognize core sequences mostly present in the 3'-Untraslated Region (UTR) of numerous mRNAs involved in inflammatory process. HuR acts mainly as a positive regulator of mRNA stability and translation, while TTP and AUF-1 appear to limit these functions. RBPs expression was evaluated by immunohistochemistry in bronchial and peripheral lung samples from mild-to-moderate stable COPD patients and age/smoking history-matched smoker with normal lung function as controls (n=12 both cohorts)*. For the first time, selective downregulation of AUF-1 was identified in airway epithelium of COPD patients vs. controls. Results were confirmed in a publicly available microarray database of primary epithelial cells obtained from bronchial brushing of COPD patients and non-smoking and smoking subjects as controls (n=6/12/12 each). Results were also validated in *in vitro* studies, using the normal human airway epithelial BEAS-2B cell line stimulated with hydrogen peroxide, cytokine combination (Cytomix), cigarette smoke extract (CSE) - which are well-established models of chronic inflammation and oxidative stress occurring in COPD - also following siRNA-mediated silencing. The chosen i*n vitro* stimulations recapitulate the selective decrease of AUF-1. We also observed, in the public transcriptomic database previously probed, that decrease in AUF-1 in epithelial cells of COPD patients paralleled significant alteration of a curated list of AUF-1-regulated inflammatory transcripts.

Based on these results, the investigation continued in two directions: in one study, the analysis of RBP profile was broadened, by performing an *in silico* analysis of the expression profile of ~ 600 mRBPs, derived from a published RBP census, in stable COPD and severe bronchial asthma. The mRBP list was searched in two selected transcriptomic datasets of primary airway epithelium, isolated by bronchial brushing in patients vs control cohorts. *In silico* analysis revealed a peculiar mRBPs modulation in COPD patients compared with non-smoking and smoking controls, with predominant RBP downregulation. Cluster analysis identified a group of disease-related coexpressed RBPs. Genome ontology identified in the RBP profile the involvement in important COPD pathogenic processes. Strikingly, no significant modulation of RBPs was found in the dataset of patients with severe asthma versus controls.

As for the second direction, we focused on further characterization of the role of AUF-I in airway epithelium. Using an RNA immunoprecipitation and sequencing (RIP-Seq) approach, we identified AUF-1 targets in the airway epithelial cell line BEAS-2B and identified specific interacting sequences in targeted transcripts. The list of AUF-1 targeted transcripts was then searched within the public transcriptomic database of COPD patients and controls previously probed. Dysregulation of AUF1-trascripts in COPD patients compared to no-smoker and smoker controls was therefore identified and used to guide the selection of the transcripts to perform validation studies (ongoing).

Conclusions. The studies performed for this thesis have identified novel expression profiles of RBPs in COPD and severe asthma. In particular, significant changes in RBPs expression characterize the bronchial epithelial response elicited by the COPD pathophysiology, while surprisingly lacking in epithelial transcriptomics of severe asthma patients compared to healthy controls. Among the RBPs, AUF-1 may play a pathogenic role in COPD by altering post-transcriptional control of epithelial gene expression, thus contributing to increased airway inflammation. Identification of these changes can be used to infer putative pathogenetic roles of mRBPs and identify novel disease-related regulatory networks.

** Complete list of study subjects' clinical characteristics are in Study #1 (page 83, Table 1)*