Abstract

Human immune system consists of a variety of molecules, immune cells, and tissues and has one main task – to protect the host. Regardless if it is an effective infection clearance, tissue repair, or cancer cells elimination, immune system is a vital component of this process. In a homeostasis state, components of innate and adaptive immunity work in tandem, however dysregulation of either leads to pathological conditions such as chronic inflammation or cancer. Human gut is highly exposed to potentially harmful, external factors simultaneously hosting commensal microbiota. Therefore, human gut is rich in immune cells ready to carry out the response if needed, simultaneously tolerating human natural microbiota. It is believed that 70% of the immune system is located in gut-associated lymphoid tissue (GALT). As such, intestinal mucosal tissue contains high numbers of T-cells. T-cells play a vital role in the development of inflammatory bowel disease (IBD) and colorectal cancer (CRC). Particularly, T helper 17 cells (Th17) and T regulatory (Treg), in recent years attracted attention in the context of IBD and CRC. Th17 are a pro-inflammatory subset that exerts inflammation leading to IBD, whilst the main Treg role is to regulate immune response. However, high infiltration of Tregs leads to the immunosuppressive environment and promotes tumor growth. Moreover, IBD is considered as one of the risks factors for the CRC development. Therefore, understanding immune response, especially Th17 and Treg mediated immune response, is vital for the development of effective treatment.

Therefore, the first part of this thesis is focused on the identification of how different housing conditions impacts gut microbiota and the subsequent development of colitis in the T cell adoptive transfer colitis mice models. It was revealed that Helicobacter strains, and *Klebsiella oxytoca* may correlate with increased numbers of IFN-γ $CD4^+$ and IL-17 $CD4^+$ T-cells whilst *Akkermansia muciniphila* had negative correlation with the development of colitis. Further, separate Dextran Sulfate Sodium(DSS)-induced model of colitis was used to investigate the role of USP28 in the development of T-cells. Knockout of USP28 led to the more potent suppressive function of Tregs and participated in IL-22/STAT5 signaling. Collectively, these studies revealed additional aspects of Th17 and Tregs control.

Second part of the thesis involved studies performed on formalin-fixed parafin embedded (FFPE) CRC tissue sections to investigate molecular changes linked to the immune cells infiltration. Spatial transcriptomics analysis of tumor microenvironment (TME) revealed unique upregulation of several genes such as TP53 or CD276 in epithelial tumor clusters, and identified gene expression gradients along the invasive trajectory with identified Tregs interactions with macrophages and epithelial cancer cells in the TME. Furthermore, SIT1, negative regulator of T-cells activation, was identified to be differentially expressed in the tertiary lymphoid structures (TLSs) in the tumor tissue as potential novel indicator of impaired T-cells function. Next, DIA MS-based proteomics of $CD4^+$ enriched CRC FFPE revealed a complex expression patterns of proteins linked to the immune evasion, such as NPM3, and simultaneously expression of pro-inflammatory S100A8 or S100A9 proteins in cancer samples. At the same time, inferred Tregs fractions were found to corelate with IDO1 and ARG1 expression, both associated with immunosuppression in the TME. Furthermore, selective expression of MCEMP1 was identified in CRC samples, comparing to normal tissue, whilst in validation proteomics dataset, $CD4^+$ T-cells isolated from CRC samples, exhibited higher expression of MCEMP1, what may indicate it's potential regulatory role in T-cells in CRC TME. CRC FFPE studies provided new aspects of ongoing regulation of genes and proteins regulating the immune response landscape in TME.

Last part of the thesis comprises of two proteomics studies which investigated changes of the proteins expression in serum of CRC patients as well as healthy controls. Study performed with proximity extension assay (PEA) showed specific upregulation of proteins such as T-cells chemoattractant CXCL9 and CCL23, and IL-6 together with oncogenic SCRN1 with simultaneous downregulation of tumor

suppressor RET protein expression in the serum samples of CRC patients comparing to healthy controls. At the same time, CSF3, IL12RB1, and CD276 were specifically upregulated in the serum samples of patients assigned with inflammation, comparing to patients without such status. Lastly, upregulation of IFN-γ, IL-32,IL-17 and ACP6 was found to correlate with early, and late stages of the disease, respectively. The upregulation of CSF3, IFN-γ, IL6, CXCL9, CCL23, and ACP6 expression levels were validated in a separate cohort. This study provided several new biomarkers candidates for CRC diagnosis. Simultaneously, LC-MS/MS study of CRC patients' serum proteins reported, for the first time, elevated LBP and SAA4 levels associated with CRC tumorigenesis. At the same time, proteins involved in complement cascade namely C5, C1QB, C4A, C8A were upregulated in the CRC conditions, with C4A and C8A upregulated levels being linked to the later stages of the disease. Additionally, C5 expression was validated in a separate cohort indicating its potential role as biomarker.

Collectively, this thesis presents a series of studies aiming at deciphering the heterogeneity of immune responses linked to the T-cells functions, especially Th17 and Tregs, and associated changes in protein and gene expression in the tumor settings and inflammation.