Proteomics characterization of immune responses in inflammation and cancer

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The immune system is essential to protect the organism against pathogens, tissue injury and cancer cells. Any disbalance in this complex immune network can cause multiple types of pathologies and persistent inflammatory processes can develop in chronic inflammation. Cancer and chronic inflammatory diseases are increasing their incidence and the most common causes of death. Cancer-associated inflammation has become an important hallmark of cancer, especially in colorectal cancer (CRC) while imbalance between pro-inflammatory and suppressive immune cells and proteins contribute to abovementioned diseases. Despite the great advances in diagnosis and treatment such as immunotherapies in cancer, most of the patients do not have complete responses and develop drug resistance via alternative immunosuppressive mechanisms. Therefore, deeper understanding of the intricate networks of immune responses involved in diseases is urgently needed. Clinical proteomics development allows for high-throughput quantification of proteins. This thesis focuses on the application of proteomics approaches to characterize immune responses in inflammation and cancer contexts, aiming to identify novel immune regulators and discover potential biomarkers.

SARS-CoV-2 infection results in acute inflammation that can develop in exacerbated immune responses, especially in patients with comorbidities such as chronic inflammatory diseases and cancer. In the first part of the thesis, orthogonal proteomics approaches, mass spectrometry and proximity extension assay, were applied to plasma samples from COVID-19 patients with and without pre-existing comorbidities and corresponding controls to determine plasma protein changes related to SARS-CoV-2 infections, the time of infection and specific anti-SARS-CoV-2 responses. Both technologies showed that COVID-19 patients with comorbidities shared a protein signature characterized by alterations in innate immune proteins including complement cascade and acute-phase proteins such as α-2-antiplasmin, that may support post-COVID-19 clotting perturbations. Key immune proteins were detected including CD4 with associated proteins such as CD28 and anti-microbial BST2. Moreover, indicators of tissue remodeling and damage were detected such as MATN2 and COL6A3 as well as extracellular matrix ECM1 and keratin K22E with potential as novel biomarkers for early detection. Several of them were not previously reported including elevated RBP2 and downregulated RNF41 in COVID-19.

CRC diagnosis is mainly based on costly invasive colonoscopy screening programs while CRC prognosis is mainly determined by the tumor stage in the detection time with low survival rates for advanced stages. Therefore, blood-based biomarkers are a promising alternative to improve CRC diagnosis. In the second part of the thesis, previously optimized proteomics approaches were applied to plasma samples from a multi-center CRC cohort and healthy controls to determine protein changes involved in CRC development, progression and cancer associated inflammation. MS-based detected protein changes in CRC patients were associated with cholesterol metabolism including APOC2 associated with CRC progression, several SERPIN family members, and the complement cascade including C5, C1QB as well as C4B and C8A both associated with cancer associated inflammation and CRC progression. Importantly, increased C5 in CRC was validated in an additional cohort. Moreover, increased pro-inflammatory LBP and SAA4 were detected for first time in CRC while acute-phase reactant LRG1 and ceruloplasmin were linked to cancer-associated inflammation. Proximity extension assay revealed plasma protein changes also associated with inflammation such as MDK, proteins associated with activated Th17 and oncogenic signaling pathways at systemic level. Moreover, T-cell attractant CXCL9 and CCL23 were found for first time increased in CRC plasma and were validated in an additional CRC cohort. IFNGγ, IL17C, and IL32 were linked to early CRC stages while ACP6, FLT4, and MANSC1 linked to late stages, being promising prognostic biomarkers.

In the last part, aiming to determine protein changes from immune cells within CRC tumor microenvironment (TME), a deep MS-based proteomics analysis of CRC and normal matched tissue enriched with CD4+ T cells and other immune cells . Protein patterns in CRC tissue reflected the ongoing tumorigenic processes and tissue integrity disruption within CRC TME including cell cycle and other hallmarks of cancer such as angiogenesis, apoptosis dysregulation, cancer stemness, and extracellular remodeling. Importantly, a complex network of increased immune proteins in CRC TME was unveiled with innate pro-inflammatory S100A12, S100A8, and S100A9 as well as immunosuppressive mediators such as CD276, PVR, and NT5E. Moreover, proteins expression indicated high cell immune heterogeneity with co-existence of increased levels of FGF2 producing CAFs together with monocyte/macrophage expressing immune checkpoint ICOSL, both of them linked to CRC progression for first time. Also, higher content of Tregs, activated mast cells, and B cells as well as reduction of IgA plasma cells and CD56 NK cells were predicted within the CRC TME. Interestingly, increased complement cascade within CRC supported findings in CRC plasma analysis which are suggested to have immunosuppressive properties within the TME. Inferred Treg content was correlated with active MHCII presentation with GILT that may mediate tolerogenic responses and immunosuppressive metabolic reprogramming via tryptophan (KYNU, IDO1, AHR), arginine (ARG1), and taurine (SLC6A6) deprivation. Along the novel potential immune regulators within CRC TME, MCEMP1 may play a relevant role in adhesion and migration of myeloid and T cells, especially Tregs.

In conclusion, this thesis contributed to characterize proteins associated with immune responses in inflammation and cancer. Novel plasma proteins associated with SARS-CoV-2 infection under pre-existing chronic inflammatory conditions and in CRC provide novel insights in the disease development. The data generated from this thesis work could facilitate the development of novel clinical biomarkers by further validation studies in larger and more diverse cohorts to evaluate their feasibility for clinical usage. Extensive characterization of CRC TME with high immune infiltration highlighted multiple cell and immune related proteins that may be novel immune regulators. Further functional studies may facilitate to determine underlying molecular mechanisms involved in TME CRC immune responses.