Abstract

Intercellular communication enables cells to interact with each other and their environment to coordinate their essential activities and functions. Immune cells, dispersed throughout the body, particularly rely on signaling mediator molecules and cognate receptors to exchange information with other immune cells and somatic cells. Accordingly, dysregulation of cell communication can disrupt normal cellular functions, leading to the development of pathological conditions. Extensive research in immunology has identified signaling mediators that are dysregulated or misused in various conditions. Therefore, the main focus of this PhD project is therapeutic targeting of the immune receptors in pathological conditions such as autoimmunity and cancer.

In this project, protein engineering approach was employed to develop small protein binders with potential applications in research, diagnostics, and therapy. These novel protein binders were developed from a highly complex combinatorial library created through mutagenesis of selected residues of albumin binding domain (ABD) or Myomedin protein scaffold. This approach expands the therapeutic protein toolkit with specific high-affinity small protein binders possessing favorable biophysical properties that can be produced in cost-effective bacterial system. The functional activity and efficacy of these binders were evaluated in both *in vitro* and *in vivo* disease models.

For the initial molecular target, interleukin-6 (IL-6) was selected due to its involvement in various autoimmune diseases such as immunoglobulin A nephropathy (IgAN) and inflammatory bowel disease (IBD), as well as significant contribution to cancer progression by affecting tumor cells or tumor microenvironment. To inhibit IL-6 signaling, thus, a collection of ABD-derived small protein binders NEF targeting IL-6 receptor alpha (IL-6Rα) was developed by directed evolution. The resulting NEF variants demonstrated specificity and high binding affinity to the IL-6Rα, as well as inhibitory potential. These ABD binders effectively suppressed B cell maturation, laying foundation for further research about their applicability in IgAN. An emerging body of evidence underscores the importance of IL-6 in IgAN, as discussed in the review article published as part of this PhD study. Also, NEF variants targeting IL-6 signaling efficiently diminished intestinal inflammation markers in chemically-induced colitis murine model. Finally, NEF blockers exhibit significant reduction of the proliferation and migration of cancer cell lines while outperforming the commercial antibody Tocilizumab in some instances. The encouraging experimental outcomes inspired a patent application, which is currently being evaluated by the Industrial Property Office of the Czech Republic.

Another part of the PhD project was focused on targeting IL-22 mediated signaling in IBD. According to the current state of knowledge, IL-22 signaling has dual role in intestinal inflammation depending on the disease context. Using protein engineering, ABR variants were

developed from the ABD combinatorial library, which specifically target IL-22R1 subunit of the IL-22 receptor complex. The specificity and inhibitory potential of IL-22R1 blockers was demonstrated in *in vitro* assays. Additionally, the most promising ABR variants demonstrated anti-inflammatory potential in the DSS-induced acute colitis murine model, highlighting the significant role of IL-22-mediated signaling in intestinal inflammation. Hence, the ABR proteins can provide valuable molecular clues for future IBD drug development.

Furthermore, this PhD thesis describes research efforts on developing therapeutic tools to predict the efficiency of immune checkpoint inhibitor therapy in cancer patients. In this context, MBA ligands targeting PD-1 as molecular target were developed from the Myomedin scaffold, which recognize both the murine and human receptors with high specificity and affinity. The diagnostic potential of MBA ligands was evaluated using tissue sections of human tonsils and patients with non-small cell lung carcinoma (NSCLC) and using Positron Emission Tomography (PET) imaging in mice.

Finally, considering the emerging role of the triggering receptor expressed on myeloid cells 2 (TREM-2)—a member of immunoglobulin superfamily, in inflammatory disorders and cancer, the cellular and molecular biology of the TREM-2 was reviewed. Additionally, the clinical relevance of the TREM-2 in cancer progression and a therapeutic potential of TREM2-targeting molecules in cancer therapy alone or in combination with immune checkpoint inhibitors was emphasized.