

Interrogation of the antigen presentation dynamics via MHCII in tumor immune evasion through multimodal single-cell sequencing

The intricate interplay between the immune system and tumors plays a pivotal role in cancer development. While immune cells possess the inherent ability to identify and eliminate cells undergoing malignant transformation, cancer cells evolve mechanisms to evade immune detection, posing significant challenges in treatment. Immunotherapies like Immune Checkpoint Blockade (ICB) that counteract immune evasion revolutionized cancer treatment and improved patient survival in multiple types of cancer. Still, response to ICB treatment varies largely between individuals and around 50% of patients show no benefit. Thus, highlighting the need for further investigation of the immune evasion mechanisms and the development of improved immunotherapies.

Effective activation of anti-tumor response hinges on the recognition of malignant cells by T lymphocytes. T cells detect invaders and abnormal cells by molecular tags called antigens, which are tiny protein fragments made in all cells. While antigens of healthy cells do not typically trigger T cell response, cancer cells might display aberrant antigens coming from e.g. mutated protein, which signals T cells to attack and destroy the cancerous cell. All cells display self-antigens via Major Histocompatibility Complex (MHC) class I. However, specialized immune cells called Antigen Presenting Cells (APCs) go further by showcasing antigens from external proteins that they have ingested, helping to identify and track down abnormal cells. APCs present these non-self antigens via MHC class II to T helper lymphocytes, which is crucial for sustaining a long-lasting immune response against tumors. However, we still do not fully understand this process.

We aim to elucidate the mechanisms underlying antigen presentation via Major Histocompatibility Complex class II (MHC-II) in tumor-activated antigen-presenting cells (APCs) and their contribution to immune evasion. Leveraging innovative molecular biology techniques we seek to comprehensively understand the dynamics of immune responses in the tumor microenvironment. We will employ CRISPR-Cas technology known as ‘molecular scissors’ to switch off individual genes involved in the process of MHC-II antigen presentation. By directed shutdown of a gene of interest, we can observe how its absence or modification affects the cell performance and assess its function. With this approach, we aim to identify genes essential for MHC-II antigen presentation and obtain insights into their role in the induction of anti-tumor response. We will also utilize multiomic single-cell sequencing, to evaluate the consequences of the “gene shutdown” within individual cells at the gene expression and chromatin accessibility levels simultaneously.

This innovative approach offers a comprehensive view of the molecular landscape within individual cells, shedding light on the regulation of MHC-II antigen presentation machinery in the tumor microenvironment. While this project will be predominantly conducted *in vitro*, leveraging CRISPR-Cas and single-cell sequencing technologies will provide a foundation for potential continuation in more complex systems, including *in vivo* models and clinical samples. Elucidating the mechanisms underlying MHC-II antigen presentation in tumor-activated APCs might provide valuable insights into cancer immune evasion mechanisms and contribute to the development of more effective immunotherapeutic strategies.