Simultaneous transcriptomic and immune phenotyping of glioma-infiltrating microglia and macrophages at the single-cell level

Glioblastoma is the most aggressive brain tumor, characterized by poor patient prognosis and median survival of 15 months post diagnosis. Glioblastomas are highly infiltrated by microglia and macrophages -the immune system cells that are supposed to create a first defense line. However, under the influence of glioma-derived signals they are transformed into **Glioma Associated Macrophages (GAMs)** that have suppressed anti-tumor activity. GAMs promote glioma invasion and progression, and support formation of new blood vessels that vascularize the tumor.
Although the role of microglia and macrophages in tumorigenesis has been extensively studied, it remains disputable whether brain-resident microglia and peripheral, infiltrating macrophages have similar or distinct role in glioma pathogenesis. The discrepancies in the field emerge mainly from the fact that up to date markers allowing to reliably distinguish microglia and macrophages in the glioma microenvironment has not been identified.

In the proposed project, we first aim to determine specific marker candidates for specific isolation of glioma infiltrating microglia and macrophages. Secondly, we plan to perform detailed bioinformatical analysis of molecular pathways upregulated by GAMs to explore specific functions of immune subpopulations in glioma progression.

We will employ state-of-the-art single-cell RNA sequencing (scRNA-seq) technology combined with simultaneous assaying of cell-surface markers at the protein level. scRNA-seq provides the gene expression profile of each single cell in a cell mixture. Thanks to that we will be able to identify sub-groups of similar cells within the mixture of GAM population and characterize them. This technique outperforms standard RNA-seq that averages the gene expression over entire input population and loses the information about inter-population differences.
Due to large amount of generated data, bioinformatic analysis will be a crucial step in the proposed project. Data analysis has become a laborious step, which most frequently is out of scope of a scientist with biological background. Simultaneously, biological knowledge is indispensable in narrowing down the high-throughput data to specific biological processes that can be further validated with functional biochemical assays. We will engage close cooperation of a bioinformatics professional with a biologist of intermediate programming proficiency. By providing a common communication platform we intent to create a feedback loop between the two specialists and foster obtaining biologically meaningful results.

Our studies will help to better understand role of immune system cell in glioma progression. Currently, there are no studies on glioma immunopathology employing the scRNA-seq technology. Detailed insight into the single-cell resolution transcriptomics and finding specific markers for microglia and macrophages may promote further on glioma immunopathology. Deciphering specific role of microglia and macrophages will help to better understand the function of those cells in glioma-genesis, which could be translated into new immunotherapy targets in the future.