in GB and the pathways that determine their functions in primary growth versus post-resection recurrence remain largely unknown. In this project, we characterize the immune landscape of GB before and after surgical resection and explore the role of the inflammasome in its dynamics and regulation.
Materials and Methods GL261-GFP-GLuc mesenchymal-type GB cells were orthotopically injected in WT or inflammasome-deficient(*Ice*$^{-/-}$) mice. On day 18 post-implantation, tumors and adjacent parenchyma tissue were collected from the unresected group (group 1). In parallel, tumor resection was performed on a second group of mice (group 2). 10 days later, tumors and adjacent parenchyma tissue were collected from group 2. Following tissue dissociation, immune cells were FACS-sorted from GB tumor-bearing mouse brains. Sorted immune cells were multiplexed using barcoaded lipid indices into 6 different pools and ScRNAseq (10x Genomics) was performed. For the scRNAseq, 30,000 cells/pool, corresponding to 7,500 viable cells/sample were loaded on the 10x chip.
Results Following putative doublet removal and exclusion of stressed or dead cells, we analysed the transcriptomes of ~61,000 single immune cells. Following data integration with Seurat, community detection, non-linear dimension reduction and graph clustering, 23 Louvain clusters were identified, including 15 from the myeloid lineage and 8 from the lymphoid lineage. we observed a significant depletion of microglia (MG)/MG-TAM from the tumor compared to the adjacent non-tumoral parenchyma, which was accompanied by a significant influx of bone-marrow-derived (BM)-TAM and monocytes as already known. Little differences were observed between WT or *Ice*$^{-/-}$mice before resection. However, post-resection remodelling of the GB TME was regulated by the inflammasome. Notably, monocytes, dendritic cells and regulatory T cells (Treg) subsets increased post resection in the adjacent non-tumoral tissue in WT but not *Ice*$^{-/-}$mice. Similarly, the intra-tumoral influx of Treg and the compositional changes of BM-TAMs observed in WT mice were blunted in inflammasome-deficient conditions. These TME differences correlated with faster tumor regrowth and decreased survival rates in WT mice compared to inflammasome-deficient mice.
Conclusion Our data reveal a significant impact of GB resection on TME remodeling and implicate the inflammasome in post-resection recurrence.

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P02.09 INTEGRATED SINGLE-CELL PROFILING DISSECTS CELL-STATE-SPECIFIC ENHANCER LANDSCAPES OF HUMAN TUMOR-INFILTRATING T CELLS

[1]C Schmidl*, [1]D Riegel, [1]E Romero-Fernández, [2]M Simon, [3]A Adenugba, [4]K Singer, [5]R Mayr, [6]F Weber, [7]CD Imbusch, [4]M Kreutz, [7]B Brors, [8]I Ugele, [3]JM Werner, [4]PJ Siska. *[1]Leibniz Institute for Immunotherapy, Regensburg, Germany; [2]Faculty of Biosciences, Heidelberg University, Heidelberg, Germany; [3]Department of Surgery, University Hospital Regensburg, Regensburg, Germany; [4]Department of Internal Medicine III, University Hospital Regensburg, Regensburg, Germany; [5]Department of Urology, Caritas St. Josef Medical Centre, University of Regensburg, Regensburg, Germany; [6]Institute of Pathology, University of Regensburg, Regensburg, Germany; [7]Division of Applied Bioinformatics, German Cancer Research Center (DKFZ), Heidelberg, Germany; [8]Department of Otorhinolaryngology, University Hospital Regensburg, Regensburg, Germany*

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Background Despite extensive studies on the chromatin landscape of exhausted T cells, the transcriptional wiring underlying the heterogeneous functional and dysfunctional states of human tumor-infiltrating lymphocytes (TILs) is incompletely understood.
Materials and Methods We use single-cell chromatin profiling and integrate publicly available single-cell RNA-seq data of TILs from several patients over four cancer entities to study gene-regulation in T cell (dys-)function.
Results We identify gene-regulatory landscapes in a wide breadth of CD8$^+$ TIL functional states. Our analysis predicts enhancer-promoter interactions in human TILs and prioritizes key elements by super-enhancer analysis. We define a human common chromatin trajectory to T cell dysfunction and determine involved key enhancers, transcriptional regulators, and deregulated target genes in this process. Finally, we validate enhancer regulation at immunotherapeutically relevant loci by targeting non-coding regulatory elements with potent CRISPR activators and repressors.
Conclusions Our study provides a framework for understanding and manipulating cell-state-specific gene-regulatory cues from human tumor-infiltrating lymphocytes.

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P03 Vaccine therapy

P03.01 DEVELOPMENT OF A MULTI-TUMOUR ANTIGEN VACCINE FOR HARD-TO-TREAT CANCERS

[1]C Puig Saenz*, [2]D Christensen, [1]SEB McArdle. *[1]Nottingham Trent University, Nottingham, UK; [2]Statens Serum Institut, Copenhaguen, Denmark*

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Background Glioblastoma (GMB), advanced prostate cancer (PCa) and triple negative breast cancer (TNBC) are hard-to-treat cancers with 5-year survival rates of 5%, 12% and 30% respectively in their metastatic stage. With surgery, chemotherapy, and radiotherapy (and hormone therapy for PCa) being the only feasible treatment options, novel therapies are urgently required. Adjuvanted peptide vaccines based on tumour antigens hold promise in prophylactic and therapeutic settings, especially when there is residual disease following treatment. Research suggests that the cancer/testis antigens HAGE and NY-ESO-1 as well as the tumour associated antigen WT1 (antigens of interest) are good candidates for peptide vaccine development, being expressed at various levels in GBM, PCa and TNBC tissues. Additionally, the expression of these antigens can be upregulated by treatment with low-dose DNA methyltransferase inhibitors like decitabine (DAC), offering the possibility to maximise the detection and destruction of residual cancer cells by vaccine-induced T cells.
Materials and Methods A panel of GBM, PCa and TNBC cell lines was treated with 1μM, 5μM and 10μM DAC and tested for antigens of interest using qPCR and western blot, aiming to validate them as immunotherapeutic targets. Peptide sequences derived from these antigens, including a mutated NY-ESO-1-derived sequence, were selected for vaccine development using *in silico* prediction algorithms (SYFPEITHI and IEDB