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# Layman Summary of Progress (2021-2022)

Nicholas Mikolajewicz

***Background***. Glioblastoma (GBM) tumors are capable of evading immunotherapy and result in aggressive recurrent tumors with poor clinical outcomes. This project aims to combine bioinformatic and genetic tools to establish the usefulness of GL261 and CT2A as mouse models of human GBM, and to use these mouse models to identify gene expression regulators in GBM cells (i.e., transcription factors) that enable the reprogramming of heterogenous GBM tumors into uniform states that are responsive to immunotherapy.

***Progress***. Two distinct mouse models of glioblastoma, CT2A and GL261, were engrafted into mouse brains and subsequently profiled using single-cell transcriptomics to obtain high-resolution data characterizing the tumor and surrounding microenvironment. Using this approach, we found that the transcriptomic profile of tumors engrafted in mice resembled human grade IV glioblastomas, and that the mouse tumors recapitulated similar degrees of diversity as seen in patient tumors, thereby supporting the validity of these mouse models. We also performed pooled loss-of-function genetic screens in CT2A and GL261 cells and found that these mouse models recapitulate many of the genetic dependencies that are specifically seen in human GBM, suggesting that these mouse models have similar therapeutic vulnerabilities as human GBMs. Then using a collection of public and in-house GBM datasets profiled using single-cell transcriptomics, we applied machine-learning methods to identify candidate transcription factors that regulate GBM subtypes. Using a complementary genome-wide CRISPR screening approach, we also found genes that enable glioma cells to evade killing mediated by cytotoxic T lymphocytes (CTLs). While no additional transcription factors were identified using this alternative strategy, we found that the autophagy pathway is a critical regulator of glioma-intrinsic evasion of killing by CTLs. From the transcription factors that were identified through our bioinformatic approach, four candidate transcription factors were selected for experimental validation and CRISPR-Cas9-mediated knockout cell lines were generated. Experiments characterizing these genetically-engineered cell lines are in progress, and we expect that they will provide insights into how the GBM phenotype is affected following perturbation of key transcription factors.