SFB 1389 UNITEGLIOBLASTOMA

FOCUS A – TUMOR INTRINSIC MECHANISMS

FOCUS B – TUMOR MICRO-ENVIRONMENT

FOCUS C – TECHNOLOGICAL INNOVATION

FOCUS D – UNITE CORES

A04 – EVOLUTION OF *IDH* MUTANT GLIOMAS DURING MALIGNANT PROGRESSION

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SUMMARY

Our project goal is to understand epigenetic and genetic mechanisms associated with temozolomide induced resistance in isocitrate dehydrogenase (IDH) mutant lower grade gliomas that exhibit promoter *MGMT* methylation. Our long-term aim is to reveal molecular targets that can be used in combination with alkylating agents to prevent the emergence of acquired resistance.

TASK

VISUAL ABSTRACT

WORKFLOW

Task 1 – Characterize the evolution of molecular alterations during TMZ treatment

Temozolomide (TMZ) treatment and Sequencing

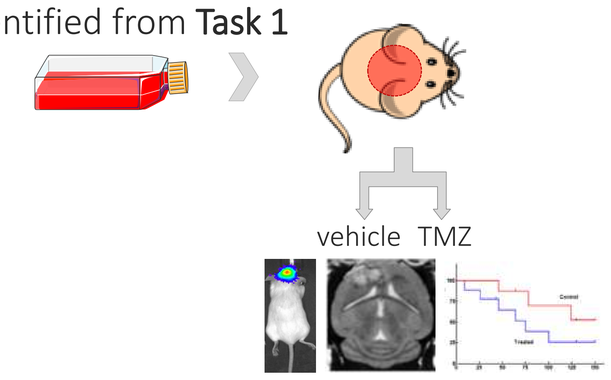
RNA-seq
(detect transcripts, isoforms)
HumanMethylation EPIC arrays
(detect aberrant methylation)
Whole genome sequencing
(variant calling, mutational burden and mutational signatures)

a) Generate TMZ resistant IDH mutant tumorsphere lines
b) Analyze molecular state of cells generated in (a) during start of treatment, stress phase, and acquired resistance using next-gen sequencing (Whole exome, RNA-seq, and bisulfite-seq)
c) Integrate sequencing data from (b) to identify candidate genes for Task 2
d) Direct comparison of findings to N^2M^2 and AMPLIFY-NEOVAC cohort

Task 2 - Determine the functional roles of the candidate drivers of resistance *in vitro* and *in vivo*

Stable cell line generation
Intracranial implantation
Longitudinal tumor phenotype assessment

Create cell lines with candidate genes identified from **Task 1**



a) Generate cell lines with either stable knockout or overexpression of candidates identified in Task 1
b) Generate inducible lines of select candidates to test efficacy in preventing resistance
c) Intracranial implantation of select cell lines in NSG mice for vehicle/TMZ treatment

Task 3 – Investigate the evolution of clonal substructure during TMZ treatment by single cell RNA-seq

Single cell sequencing and Data analysis

Determine pre- and post-treatment transcriptional and structural heterogeneity

a) Single cell copy number and transcriptional analysis of naïve and resistant tumorspheres, and matched primary and recurrent IDH mutated tumors
b) Data analysis to determine clonal heterogeneity due to TMZ treatment

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