

cellular invasion, which enables escape from resection and drives inevitable recurrence. Numerous factors have been proposed as the primary driving forces behind GBM's ability to invade adjacent tissues rapidly, including alterations in the tumor's cellular metabolism. Though studies have investigated links between GBM's metabolic profile and its invasive capability, these studies have had two notable limitations. First, while infiltrating GBM cells extending beyond the tumor edge utilize adaptive cellular machinery to overcome stressors in their microenvironment, these cells at the invasive front have not been the ones sampled in invasive studies, which have used cell lines or banked tumor tissue taken from the readily accessible tumor core. Second, studies of invasion have primarily used two-dimensional (2D) culture systems, which fail to capture the dimensionality, mechanics, and heterogeneity of GBM invasion. To address these limitations, our team has developed two parallel approaches: acquisition of site-directed biopsies from patient GBMs to define regional heterogeneity in invasiveness, and engineering of 3D platforms to study invasion *in vitro*. Through utilization of these platforms, and by taking advantage of the system-wide, unbiased screens of metabolite profile and gene expression available, our team looks to identify targetable metabolic factors which drive cellular invasion in GBM. Untargeted metabolomics revealed cystathionine to be selectively enriched in the invasive tumor front of both site directed biopsies (fold change 5.8), and 3D organoid models (fold change 14.2). RNA sequencing revealed 7/30 (23%) metabolic genes upregulated in the invasive tumor front were involved in cysteine or glutathione metabolism. These results highlight a clear role of the transsulfuration pathway in GBM invasion that our team looks to investigate with further targeted assays.

OTME-13. INTEGRATION OF METABOLIC AND TRANSCRIPTIONAL SIGNATURES OF GLIOBLASTOMA INVASION REVEALS EXTRACELLULAR MATRIX REORGANIZATION AND VASCULATURE REMODELING

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BACKGROUND: The invasive behavior of glioblastoma is considered highly relevant for recurrence. However, the invasion zone is difficult to visualize, typically lies outside the resected and irradiated area, and is protected by the blood brain barrier, posing a particular challenge for treatment. We present biological features of invasive growth accompanying tumor progression and invasion based on associated metabolic and transcriptomic changes in patient derived orthotopic xenografts (PDOX) and corresponding patients. **METHODS:** Patients with suspected glioblastoma were enrolled (NCT02904525) and underwent ¹H-MR spectroscopy and imaging (¹H-MRS/I, 7T). Tissue obtained at surgery was transplanted orthotopically into immune-compromised mice. Longitudinal follow-up was performed by ¹H-MRS/I (14.1T) on the injected and the contralateral side. The PDOX, the corresponding contralateral side, and the original human tumors underwent RNA-sequencing. **RESULTS:** The temporal changes of the metabolite profiles characterized the kinetics of invasive growth of PDOX, and were patient specific. Comparison of ¹H-MRS derived metabolite signatures, reflecting temporal changes of tumor development and invasion in PDOX, revealed high similarity to spatial metabolite signatures of combined multivoxel analyses of the patients' tumors. Associations between the metabolite profiles and the combined transcriptome of the xenografts and the host, reflected molecular signatures of invasion, comprising extracellular matrix degradation and reorganization, growth factor binding, and vascular remodeling. **CONCLUSION:** Integrating metabolic profiles and gene expression of highly invasive PDOX allows *in vivo* monitoring of progression in the non-enhancing tumor infiltration zone and provides insights into the remodeling of the extracellular matrix that is essential for cell-cell communication and regulation of cellular processes. The changes of the structural and biochemical properties of the extracellular matrix are of importance for the biological behavior of the tumors and may be subjected to therapeutic targeting.

OTME-14. TGF-BETA SIGNALING IN MICROTUBE FORMATION OF GLIOBLASTOMA

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Microtubes (MTs) are cytoplasmic extensions of glioma cells serving as important cell communication structures while also promoting invasion and treatment resistance through network formation. MTs are abundant in chemoresistant gliomas, in particular glioblastomas, while they are uncommon in chemosensitive IDH mutated and 1p/19q co-deleted oligodendrogliomas. By performing a bioinformatics analysis on data from The Cancer Genome Atlas (TCGA) we identified the TGF- β pathway as being distinctly upregulated in glioblastomas compared to oligodendrogliomas, making this a signaling pathway potentially involved in MT formation. Based on patient-derived GBM stem cell line models we demonstrated that stimulation of TGF- β increased MT formation, while inhibition of TGF- β reduced MT formation. MT formation was verified by expression of GAP43 and nestin, which have previously been shown to be important structural proteins of MTs. Interestingly, we also observed a responder/non-responder relationship between GBM cell lines P3 and GG16/ GG6 regarding MT formation upon TGF- β stimulation. To determine downstream signaling mediators of the TGF- β pathway crucial for MT formation, we subsequently performed RNA sequencing of these cell lines. From the 34 initial candidates common to responders, but absent in non-responders, only 3 genes were left after filtering through TCGA data and *in vivo* RNA sequencing data of a GBM xenograft model derived from P3. Thrombospondin 1 (TSP1) emerged as the most interesting candidate as we have previously shown that transcription of this gene is activated by TGF- β /SMAD signaling and TSP1 also promotes invasiveness of GBM. TSP1 was upregulated by TGF β 1 stimulation in responder cells and promoted MT formation. Transcriptional activation of TSP1 was absent in the non-responder cell line GG6 and could be reversed in the responder cell line P3 by TSP1 shRNAs *in vitro* and *in vivo*. Thus, TSP1 was experimentally verified as an important mediator of microtube formation downstream of TGF- β signaling.

OTME-15. PHYSICAL CONFINEMENT INDUCES DIVERSE TRANSCRIPTOMIC CHANGES AND CHEMORESISTANCE IN MIGRATING GLIOBLASTOMA CELLS

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Glioblastoma multiforme (GBM) cells migrating in physically confined environments are affected by mechanical stress that potentially lead to transcriptomic changes. To simulate those stresses, microfluidic channels were made with micro-patterned polydimethylsiloxane (PDMS) replicating the physical microenvironment of white matter tracts by confining the cells in linear channels similar to the space between axons. We employed a combination of microarray transcriptomic profiling and single cell-sequencing analyses to investigate cells undergoing linear confined space migration (LCSM). GBM cells spontaneously migrate through confined spaces along 5x5 mm (height/width) microfluidic channels, 0.5 to 5 mm in length. Our previous studies demonstrated that cells migrating in LCSM are more resistant to treatment with temozolomide than the same cells growing in standard monolayer culture (SMC). Cells in confined migration evaluated by microarray-based transcriptomic profiling demonstrated that linear confined migration induces increased expression in pathways involving angiogenesis, cell adhesion, cell motility, DNA damage repair, extracellular matrix structure, HIF1 α , and others. Single cell transcriptomic analysis could identify GBM cells in different migratory states (LCSM vs. SMC), and similar pathways were seen upregulated with additional changes in cholesterol biosynthesis pathways and cell cycle regulation pathways. Trajectory Inference aligned single cells according to changes in migration status and demonstrated transcript changes during LCSM were progressive but generally reversible on return to SMC. Pathway analyses showed alterations in the cholesterol biosynthesis pathway and cell cycle regulation in cell clusters of confined migrating cells. Molecular studies confirmed that cholesterol biosynthesis pathway regulatory genes (SQLE, MVD, and HMGCR) are upregulated during LCSM. Expression analysis demonstrated increased G1 phase delay in confined migrating cells (LCSM) confirmed by FUCCI expression analysis. We propose that migration in linear confined spaces like white matter structures produces significant transcriptome changes that produce chemoresistance as a new mechanism for treatment resistance of Glioblastoma.