OPTC-5. MOLECULAR SIGNATURES OF PODOPLANIN EXPRESSING GLIOBLASTOMA CELL SUBSETS WITH PUTATIVE ROLE IN CANCER ASSOCIATED THROMBOSIS AND MICROTHROMBOSIS

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Vascular anomalies, including thrombosis, are a hallmark of glioblastoma (GBM) and an aftermath of dysregulated cancer cell genome and epigenome. Upregulation of podoplanin (PDPN) by cancer cells has recently been linked to an increased risk of venous thromboembolism in glioblastoma patients. Thus, regulation of this platelet activating transmembrane protein by transforming events and release from cancer cells into the circulation are of considerable interest. We took advantage of single-cell and bulk GBM transcriptome dataset mining and investigated the pattern of PDPN expression across several databases. Our analysis indicated that PDPN is expressed by distinct (mesenchymal) glioblastoma cell subpopulations and is downregulated by oncogenic mutations of EGFR and IDH1 genes, via changes in chromatin modifications (EZH2) and DNA methylation, respectively. Additionally, we utilized isogenic and stem GBM cell lines, xenograft models in mice, ELISA assays for PDPN, tissue factor (TF), platelet factor 4 (PF4) and clotting activation markers (D-dimer), and multicolor nano-flow cytometry to show that GBM cells exteriorize PDPN and/or TF as cargo of exosome-like coagulant extracellular vesicles EVs. We also documented an increase of platelet activation (PF4) or coagulation markers (D-dimer) in mice harboring the corresponding PDPN- or TF-expressing glioma xenografts, respectively. While PDPN was a dominant regulator of systemic platelet activation, co-expression of PDPN and TF impacted local microthrombosis. Our work suggests that distinct cellular subsets drive multiple facets of GBM-associated thrombosis and may represent targets for diagnosis and intervention.

FINAL CATEGORY: OMICS OF RESPONSE TO THERAPY

OMRT-1. CANNABIDIOL CONVERTS NFKB INTO A TUMOR-SUPPRESSOR IN GLIOBLASTOMA WITH DEFINED ANTIOXIDATIVE PROPERTIES

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BACKGROUND: The transcription factor NFKB drives neoplastic progression of many cancers including primary brain tumors (glioblastoma; GBM). Precise therapeutic modulation of NFKB-activity can suppress central oncogenic signalling pathways in GBM, but clinically applicable compounds to achieve this goal have remained elusive. METHODS: In a pharmacogenomics study with a panel of transgenic glioma cells we observed that NFKB can be converted into a tumor-suppressor by the non-psychotropic cannabinoid Cannabidiol (CBD). Subsequently, we investigated the anti-tumor effects of CBD, which is used as an anticonvulsive drug (Epidiolex) in pediatric neurology, in a larger set of human primary GBM stem-like cells (hGSC). For this study we performed pharmacological assays, gene-expression profiling, biochemical and cell-biological experiments. We validated our findings using orthotopic in vivo models and bioinformatics-analysis of human GBM-datasets. RESULTS: We found that CBD promotes DNA-binding of the NFKB-subunit RELA and simultaneously prevents RELA-phosphorylation on serine-311, a key residue which permits genetic transactivation. Strikingly, sustained DNA-binding by RELA lacking phospho-serine 311 was found to mediate hGSC-cytotoxicity. Widespread sensitivity to CBD was observed in a cohort of hGSC defined by low levels of reactive oxygen-species (ROS), while high ROS-content in other tumors blocked CBD induced hGSC-death. Consequently, ROS-levels served as predictive biomarker for CBD-sensitive tumors. CONCLUSIONS: This evidence demonstrates how a clinically approved drug can convert NFKB into a tumor-suppressor and suggests a promising repurposing option for GBM-therapy.

OMRT-2. LIQUID BIOPSY FOR PATIENT STRATIFICATION AND MONITORING OF DACOMITINIB CLINICAL TRIAL IN PATIENTS WITH EGFR AMPLIFIED RECURRENT GLIOBLASTOMA

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INTRODUCTION: Liquid biopsy for the detection and monitoring of brain tumors is of significant clinical interest. The ability to non-invasively profile tumors can avoid a risky biopsy and opens avenues for testing novel therapies by accurately stratifying patients to receive the right therapy. Here, we provide evidence of EV RNA-based diagnosis, patient stratification, and assessment of response to therapy in the setting of a clinical trial evaluating the efficacy of dacomitinib, an EGFR tyrosine kinase inhibitor in patients with recurrent, EGFR amplified GBM(NCT01112527). METHODS: We performed RNASeq on long RNA extracted from the serum samples, pre-treatment and 1-month post-treatment. RESULTS: Firstly, longRNASeq allowed the detection of thousands of mRNA, lincRNAs and antisense RNAs enabling the study of a wider repertoire of potential RNA based biomarkers. Secondly, we observed a differential expression profile in serum EV RNA of GBM patients and healthy controls. Combining our findings with TCGA data and literature screening, we generated a 25 gene signature representative of critical pathways in several hallmarks of cancer. Thirdly, we observed a differential expression profile in serum EV RNA of responders to dacomitinib compared to non-responders in pre-treatment serum. Specifically, the EV mRNAs ZNF35 and LAMTOR2 distinguish responders from non-responders (p-adjusted = 2.6E-8 and 2.4E-6, respectively) allowing potential patient stratification. Finally, we observed a differential expression profile in serum EV RNA of responders to dacomitinib compared to non-responders in post-treatment serum. EV mRNA DNMT3A is significantly enriched (p-adjusted = 1.8E-4) in post-treatment serum of responders compared to non-responders to dacomitinib allowing potential monitoring of response to therapy. CONCLUSION: This study represents the first longitudinal profiling of the EV transcriptome in a cohort of genomically selected GBM patients. These findings are a tantalizing step toward liquid biopsy-based biomarkers for the detection of GBM, as well as patient stratification and monitoring.

OMRT-3. LONGITUDINAL ANALYSIS OF DIFFUSE GLIOMA REVEALS CELL STATE DYNAMICS AT RECURRENCE ASSOCIATED WITH CHANGES IN GENETICS AND THE MICROENVIRONMENT

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Diffuse glioma is an aggressive brain cancer that is characterized by a poor prognosis and a universal resistance to therapy. The evolutionary processes behind this resistance remain unclear. Previous studies by the Glioma Longitudinal Analysis (GLASS) Consortium have indicated that therapy-induced selective pressures shape the genetic evolution of glioma in a stochastic manner. However, single-cell studies have revealed that malignant glioma cells are highly plastic and transition their cell state in response to diverse challenges, including changes in the microenvironment and the administration of standard-of-care therapy. Interactions between these factors remain poorly understood, making it difficult to predict how a patient's tumor will evolve from diagnosis to recurrence. To interrogate the factors driving therapy resistance in diffuse glioma, we collected and analyzed RNA- and/or DNA-sequencing data from temporally separated tumor pairs of 292 adult patients with IDH-wild-type or IDH-mutant glioma. Recurrent tumors exhibited diverse changes that were attributable to changes in anatomic composition, somatic alterations, and microenvironment interactions. Hypermutation and acquired *CDKN2A* homozygous deletions associated with an increase in proliferating stem-like malignant cells at recurrence in both glioma subtypes, reflecting active tumor expansion. IDH-wild-type tumors were more invasive at recurrence, and their malignant cells exhibited increased expression of neuronal signaling programs that reflected a possible role for neuronal interactions in promoting glioma progression. Mesenchymal transition was associated with the presence of a specific myeloid cell state defined by unique ligand-receptor interactions with malignant cells, providing opportunities to target this transition through therapy. Collectively, our results uncover

recurrence-associated changes in genetics and the microenvironment that can be targeted to shape disease progression following initial diagnosis.

OMRT-5. THERAPY-INDUCED REPROGRAMMING DRIVES GLIOMA VASCULAR TRANSDIFFERENTIATION AND RECURRENCE

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Therapy-resistant glioma cells elicit remarkable phenotypic plasticity leading to aggressive tumor recurrence. Here, we used single-cell and whole transcriptomic sequencing to uncover that radiation treatment induces a dynamic shift in functional states of glioma cells allowing for acquisition of either stem-like, mesenchymal-like or vascular-like phenotypes. The predominant phenotype switch induced by radiation in surviving tumor cells is the vascular-like cell state, resulting in transdifferentiation to endothelial-like and pericyte-like cells in distinct cell clusters. The transdifferentiated endothelial-like and pericyte-like cells secrete trophic factors to support proliferation of tumor cells, and their selective ablation results in reduced tumor growth and recurrence post-treatment. Mechanistically, the acquisition of vascular-like phenotype is driven by increased acetylation and chromatin accessibility in vascular genes and in regions for binding of vascular specification transcription factors. Blocking histone acetylation using a small molecule inhibitor targeting P300 histone acetyltransferase activity prior to radiation treatment inhibits the vascular-like transdifferentiation of glioma cells and tumor growth. Our findings indicate that radiation therapy-induces rewiring of glioma cells that promotes vascular cell-like transdifferentiation, tumor growth and recurrence.

OMRT-6. OPTIMIZING MDM2 INHIBITION FOR THE TREATMENT OF HIGH-GRADE GLIOMA

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Over 80% of high-grade gliomas have alterations in members of the p53 pathway, a central regulator of cell cycle progression and apoptosis that becomes activated in response to cellular stress and DNA damage. For tumors that retain wild-type p53, pathway deregulation frequently occurs through the amplification of negative regulators of p53, including the E3 ubiquitin ligase MDM2. The p53/MDM2 interaction axis has served as basis for the development of several classes of MDM2 inhibitors, with AMG232 being the most potent molecule currently undergoing clinical evaluation. As the effects of MDM2 inhibition (MDM2i) remain poorly understood in high-grade glioma, we performed genomic and transcriptomic analyses in patient-derived models to better characterize sensitive tumors and identify putative biomarkers of drug response. Treatment with AMG232 impaired the growth of cell lines with wild-type p53 status, particularly in tumors with additional amplification of MDM4 or PPM1D activating mutations. Treatment with AMG232 upregulated both cell cycle arrest and apoptotic cellular responses, as measured by annexin V/PI staining and immunoblotting. Interestingly, the dynamics of these two downstream p53 signaling axis were dependent on treatment duration across models. In addition to p53 pathway activation and apoptotic induction, RNA-sequencing revealed MDM2i to be associated with the activation of oncogenic MAPK and KRAS signaling as well as epithelial to mesenchymal transition markers. In most solid tumors, resistance to MDM2i is mainly mediated by acquisition of p53 inactivating mutations. We hypothesized that resistance mechanisms in glioma may be partially driven by transcriptional changes, as these tumors consist of subpopulations with diverse cell differentiation states. By chronic AMG232 treatment, we have developed in vitro and in vivo models of acquired MDM2i resistance that are not mediated by p53 inactivation. Ongoing work is focused on characterizing the transcriptional profile of these cells to identify transcriptional changes leading to decreased drug response.

OMRT-7. ANGIOGENESIS INHIBITORS STRONGLY SYNERGIZE WITH THERAPEUTICS TARGETING TUMOR METABOLISM

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Angiogenesis inhibition has become a mainstay of oncology despite having fallen short of its early promise. As originally envisioned, angiogenesis inhibition would cut off the blood supply, deprive tumor cells of key nutrients, leading to their death. In practice, while there is evidence that tumors under angiogenesis treatment do in fact exhibit some degree of metabolic stress, this is stress is not sufficient to induce significant cancer cell death. We posit that the full potential of angiogenesis inhibition can be realized by the combination of angiogenesis inhibition with emerging tumor metabolism targeting therapies. Because tumors under angiogenesis inhibition are already in a state of nutrient stress, the effects of metabolically targeted therapies such as amino acid depletion (e.g. asparginase, methionine restriction), inhibitors of stress adaption (AMPK and GCN2 inhibitors) or energy metabolism (e.g. IACS-010759, Metformin, POMHEX) stand to dramatically increase in potency whilst remaining selective for (angiogenic) tumor versus (non-angiogenic) normal tissue. Here, we provide proof-of-principal for this thesis. First, we performed metabolomic profiling of angiogenesis-inhibited tumors, which corroborates a state of nutrient stress in angiogenesis-inhibited tumors. Second, we demonstrate dramatic anti-neoplastic synergy (effectively curing of xenografted tumor-bearing mice, irrespective of initial tumor size), without enhanced adverse toxicities, between the OxPhos inhibitor IACS-010759 and the angiogenesis tyrosine kinase inhibitor, Tivozanib. The same results were recapitulated with the anti-VEGFA antibody, Avastin, and the OxPhos inhibitor could be substituted with the Enolase inhibitor HEX, with similar effects. The synergy was observed in a broad range of tumor types, even those without clear genetic susceptibilities. Together, these results suggest that angiogenesis inhibitors synergize broadly with cancer therapies targeting metabolism, allowing the realization of the full potential of these previously disappointing drugs. Our results warrant systematic combination clinical trials between angiogenesis inhibitors and established, as well as emerging anti-metabolic cancer therapies.

OMRT-8. PRECISION TARGETING OF CELLULAR PATHWAYS WITH COMPLEMENTARY DIAGNOSTICS

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Precision medicine tailors treatment for each patient by identifying the molecular drivers of their disease. This can allow more effective tumour targeting, avoid harmful standard chemotherapeutic side-effects, and offer savings to the healthcare system through not treating patients who are unlikely to respond to a specific agent. Treatment regimes are usually designed by identifying DNA-level alterations and selecting drugs tailored to that mutation. However, cancer is not a one-pathway disease and not all patients with particular mutations will respond to treatment, while patients without canonical pathway-activating mutations are excluded from potentially life-saving treatment. To address this, we have developed a NanoString assay combining proteomic and transcriptomic profiles of 4 key actionable, cancer-related pathways (MAPK, PI3K, NFκB and JAK/STAT). We used RNA-Seq data from gold standard cell lines with defined pathway changes to identify minimal gene sets indicative of pathway activation, and integrated them with phospho protein measurements to generate a pathway activation score. The combined panel was run on isogenic cell lines as well as glioma samples with both known and unknown driving alterations. We found pathway activation to be more variable than expected based on DNA alterations alone, implying that consideration of proteomic and/or transcriptomic-level information is important for future therapeutic decision-making.

OMRT-9. EFFECT OF PRE-OPERATIVE STEREOTACTIC RADIOSURGERY ON BRAIN METASTASIS: ANALYSIS OF DNA AND RNA GENOMIC PROFILES FROM PHASE-II CLINICAL TRIAL NCT03398694

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BACKGROUND: With improved systemic therapy that has limited impact on the intracranial compartment, the incidence of brain metastasis (BM) from solid cancers is rising and negatively impacting patient's overall survival (OS). Treatment varies based on presentation, however, for patients with <4 symptomatic BMs current clinical practice involves surgical resection followed by stereotactic radiosurgery (SRS) to the resection cavity. Post-operative SRS is associated with increased risk of leptomeningeal disease (LMD) and local recurrence in the follow-up period. We hypothesize that pre-operative SRS will decrease the incidence of LMD as well as local recurrence and increase patient's OS by delivering a lethal dose of radiation to tumor cells before they are disturbed by surgical resection. In a Phase II clinical trial (NCT03398694) we are treating patients with 1–4 symptomatic BMs with pre-operative SRS while collecting DNA and RNA sequencing data from core and peripheral edges of the resected tumor to examine the genomic effects of SRS on tumor. METHODS: Post-SRS resected tumor specimens were divided into two groups: 'center' and