Title

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TMOD-17. BRAIN TUMOR PATIENT DERIVED XENOGRAFT FROM LUNG TUMOR METASTASIS: ESTABLISHMENT AND CHARACTERIZATION

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Metastatic cancers to the brain far outnumber those that arise in the brain, yet preclinical models to study such tumors are relatively uncommon. In order to address this deficiency, we are creating a series of novel patient-derived xenografts (PDX) from common metastatic brain cancers. Here, we describe the development, luciferase modification, orthotopic growth, and radio-responsiveness of a metastatic lung adenocarcinoma PDX. The tumor was taken from the brain of a 75-year old woman who had had received carboplatin, taxol, and radiotherapy for her primary lesion, which had been classified as a mucinous micropapillary adenocarcinoma. The tumor was subcutaneously engrafted in nu/nu athymic mice, with the first generation PDX used to establish intracranial PDX in the same mouse strain. Intracranial xenografts showed a remarkable preservation of the original tumor micropapillary architecture, including mucin secretion. Cells from initial subcutaneous growths were also grown in vitro, modified with lentivirus to express luciferase, and re-established as subcutaneous PDX in mice. These luciferase-modified tumors were then established as intracranial PDX, in order to characterize intracranial growth and response to radiation. Serial bioluminescence imaging revealed progressive growth of intracranial tumors in all mice. Four weeks following intracranial implantation, mice were randomized to no treatment vs. 2 Gy whole brain radiation per day, for 5 consecutive days (10 Gy total). Radiotherapy significantly reduced tumor growth rate and extended the survival of engrafted mice ($p < 0.05$: median survival control/untreated mice = 69 days; median survival for mice receiving RT not yet reached). Whole exome sequencing and RNA-Seq of this PDX are underway, including comparison with the original metastatic tumor exome and mRNA profile. We will use the molecular results to test targeted therapeutics, and to compare PDX response to treatment when established intracranially vs. response when established in primary organ site.

TMOD-19. SOMATIC GENOME EDITING WITH THE RCAS-CRISPR/CAS9 SYSTEM FOR PRECISION GLIOMA MODELING

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It has been gradually established that the vast majority of human tumors are extraordinarily heterogeneous at a genetic level. To accurately recapitulate this complexity, it is now evident that *in vivo* animal models of cancers will require to recreate not just a handful of simple genetic alterations, but possibly dozens and increasingly intricate. Here we have combined the RCAS/tv-a system with the CIRSPR/Cas9 genome editing tools to somatically target neural stem cells (NSCs) for precise modeling of human glioma. We show that deletion, both in pups and adult mice, of a variety of known tumor suppressor genes (*Trp53*, *Cdkn2a* and *Pten*), in combination with the expression of an oncogene driver, leads to high-grade glioma formation. Moreover, by simultaneously delivery into NSCs of pairs of gRNAs we show for the first time that the *Bcan-Ntrk1* gene fusions, is able to induce high-grade gliomas. We further established that cells derived from Bcan-Ntrk1 tumors are remarkably sensitive to a Pan-Ntrk inhibitor. Lastly, using homology directed repair (HDR), we generated the Braf V600E mutation into NSCs and we demonstrated that it's sufficient to induce glioma tumor formation. In summary, we have developed an extremely powerful and versatile mouse model for *in vivo* somatic genome editing. Our system will elicit the generation of more accurate glioma models, particularly suitable for preclinical testing.

TMOD-20. EARLY DETECTION OF HDAC INHIBITION IN GLIOBLASTOMA USING ADVANCED HYPERPOLARIZED ^{13}C MRSI

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Today, there is no reliable noninvasive imaging method available to monitor glioblastoma (GBM) response to therapy and predict survival prior to tumor shrinkage. Dissolution Dynamic Nuclear Polarization (DNP) combined with hyperpolarized 13C Magnetic Resonance Spectroscopic Imaging (MRSI) is a novel imaging method that allows probing real-time tumor metabolism. Recent studies using 13C MRSI have shown decreased lactate production from pyruvate in GBM responsive to a dual PI3K/mTOR inhibitor and/or Temozolomide, mediated by lower expression of LDHA or PKM2 enzymes, respectively. SAHA, the histone deacetylase (HDAC) inhibitor, is a novel drug that inhibits cell proliferation by inducing cell cycle arrest followed by apoptosis. The goal of this study was to detect early HDAC inhibition using advanced 13C MRSI. Analysis of dynamic real-time cellular metabolic changes in SAHA-treated U87 live cells in bioreactors demonstrated a significant 37.7% decrease in hyperpolarized [1-13C]-lactate production. SAHA-treated cells also showed a 29.6% decrease of steady-state lactate level in cell extracts. Furthermore, we demonstrated a significant 30.3% decrease in hyperpolarized lactate-to-pyruvate ratio in SAHA-treated U87-bearing mice, which occurred prior to MRI-detectable changes in tumor size that was associated with enhanced animal survival. In order to mechanistically validate our findings, we tested the levels of expression of LDHA, MCT1 and MCT4, the main players in pyruvate-to-lactate interconversion. While expression of LDHA, the enzyme that catalyzes pyruvate-to-lactate conversion was independent of SAHA treatment, expression of both MCT1 and MCT4 transporters, that shuttle pyruvate and lactate in and out of the cell, are increased. We thus propose that increased MCT1/4 led to a decrease in lactate in response to HDAC inhibition and resulted in a reduced pyruvate-to-lactate conversion. Our findings confirm the potential translational value of the hyperpolarized lactate-to-pyruvate ratio as a biomarker for noninvasively assessing the early effects of emerging therapies for patients with GBM.

TMOD-21. CHARACTERIZATION OF PATIENT-DERIVED TUMOR SPHERES AND XENOGRAFTS FOR GLIOBLASTOMA

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Patient-derived tumor spheres and xenografts are essential tools for translational research for malignant gliomas. However, only a subset of glioma samples are established as long-term sphere cultures and/or patient-derived xenografts. We aim to analyze the characteristics of patient-derived tumor spheres and xenografts. We tried primary sphere cultures by serum free medium containing EGF and bFGF from 56 glioma patient-derived samples (48 of grade 4, 4 of grade 3, and 4 of grade 2 tumors) and established long-term sphere cultures. We could establish 14 primary culture cell lines out of 48 glioblastoma samples (22.9% of glioblastoma) as long-term sphere cultures, and no long-term sphere culture was isolated from grade3 and grade 2 tumors. Next we investigated the genetic differences between the cell lines which were successfully established as long-term sphere cultures and those which were not. We found that cell lines with TERT promotor mutations are significantly established as long term-sphere cultures. TP53 mutation, EGFR amplification, and IDH1/2 mutation also might influence the success rate of long-term sphere cultures, but these factors were not statistically significant. We next investigated in vivo characteristics of glioblastoma patient derived xenograft models from these successfully established cell lines. We have injected these cell lines into NOD/Shi-scid IL2Rγ KO mouse and histopathologically analyzed characteristics of xenografts. Each xenograft well recapitulated histological features of original tumors and tumor cells remarkably invade through subventricular zone. These results suggest that long-term sphere culture is possible especially when the tumors are glioblastoma having TERT promotor mutations. Further technical improvement is needed to establish long-term sphere culture at higher percentages especially for glioblastoma samples without TERT promotor mutation. In addition, precise mechanism why tumor cells invade through subventricular zone is unknown, but these patient derived xenograft models are good models to analyze invasion and cancer stem cell properties of glioblastoma.

TMOD-22. WILD-TYPE p53 DRIVES A MESENCHYMAL PHENOTYPE AFTER TREATMENT OF PRIMARY GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and aggressive adult brain malignancy for which conventional surgery, radiation treatment and chemotherapy based on alkylating agent Temozolomide (TMZ) have limited ben-