EXTH-08. REPLACEMENT OF MICROGLIA BY BRAIN-ENGRAFTED MACROPHAGES PREVENTS MEMORY DEFICITS AFTER THERAPEUTIC WHOLE-BRAIN IRRADIATION

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EXTH-04. BLOCKADE OF NRF2/GLUTATHIONE METABOLISM AS A SYNTHETIC LETHALITY APPROACH FOR IDH1-MUTATED GLIOMA

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BACKGROUND: Mutations in isocitrate dehydrogenase (IDH1/2) are frequent genetic abnormalities in human malignancies. IDH1/2-mutated cancers are a recently defined disease entity with distinctive patterns of tumor cell biology, metabolism and resistance to therapy. Molecular targeting approaches against this disease cluster remain limited. METHODOLOGY: We investigated the redox homeostasis in IDH1 mutant-transduced cells and patient-derived brain tumor initiating cells. The importance of antioxidant genes was confirmed through COX regression analysis on a large cohort of lower grade glioma. We investigated the biologic impact of Nuclear factor erythroid 2-related factor 2 (Nrf2) on the glutathione de novo synthesis in IDH1-mutated cells. Finally, we evaluated the value of targeting NRF2/glutathione metabolic pathway as a potential synthetic lethality approach for IDH1-mutated cell in vitro and in vivo. RESULTS: We discovered that acquisition of cancer-associated IDH1 mutants results in constitutive activation of NRF2-protected cytoprotective pathways through decoupling of NRF2 from its E3 ligase Kelch-like ECH-associated protein 1. NRF2 mediated the transcriptional activation of GCLC, GCLM and SLC7A11, which stimulate the biosynthesis of glutathione de novo synthesis, and relieves the metabolic stress imposed by the IDH1 mutant cancers. In conclusion: Our findings suggest that blockade of the NRF2/glutathione synthetic pathway is a novel targeting strategy for IDH1-mutated malignancies.

EXTH-05. THERAPEUTIC IMPLICATIONS OF TTFIELDS INDUCED DNA DAMAGE AND REPLICATION STRESS IN NOVEL CELL LINES FOR CANCER TREATMENT

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TTFields are low-intensity, intermediate frequency, alternating electric fields which are applied to tumor regions using non-invasive arrays. TTFields is approved for the treatment of glioblastoma and mesothelioma with clinical trials ongoing in other cancer types. The mechanism of action for TTFields includes interference with mitotic, reduced DNA double strand break (DSB) repair capacity and the frank induction of DNA DSBs. The mechanism by which TTFields induces DNA DSBs appears to be through the enhancement of DNA replication stress with continued TTFields exposure. The induction of DNA DSBs appears to be as a result of the upregulated expression of mitotic checkpoint genes MCM6 and MCM10 as well as the Fanconi’s Anemia (FA) pathway genes. TTFields treatment increases the number of RPA foci, decreases nascent DNA length and increases R-loop formation which are markers of DNA replication stress. These results suggest that TTFields-induced replication stress is the underlying mechanism and cellular endogenous source of DNA DSB generation via replication fork collapse. The current study suggests that TTFields exposure causes a conditional vulnerability environment that renders cells more susceptible to chemotherapeutic agents that induce DNA damage and/or cause replication stress. Supporting this is the synergetic cell killing seen with TTFields exposure concomitant with cisplatin, TTFields plus concomitant PARP inhibition with or without subsequent radiation, or radiation given at the completion of a TTFields double exposure. Finally, TTFields-induced mitotic aberrations and DNA damage/replication stress events, although intimately linked to one another as one can expose the other, are likely initiated independently of one another as suggested by the gene expression analysis of 47 key mitosis regulator genes. These results establish that enhanced replication stress and reduced DNA repair capacity are major mechanisms of TTFields effects, effects for which there are therapeutic implications.

EXTH-06. DOWN-REGULATION OF PD-L1 VIA FKBP5 LOWERED BY A CYCLOXYGENASE-2 INHIBITOR IN GSCS AND GBM CELLS MAY BE AN INHERENT THERAPY TO ENHANCE ANTITUMOR EFFECTS OF IMMUNOTHERAPY

Jumi Yamaguchi, Kohei Nakajima, Kenji Shono, Yoshihumi Mizobuchi, Toshitaka Yamaguchi, Keiko Kitazato, and Yasushi Takagi; Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan

BACKGROUND: Antitumor therapies targeting programmed cell death-1 (PD-1)/its ligand-1 (PD-L1) are plentiful at present stage. However, in glioblastoma (GBM), the expression of PD-L1 is variable and the role of anti-PD-1 antibody therapy is still unclear. The high expression of PD-L1 affects cell proliferation and invasion in GBM cells. As COX-2 modulates PD-L1 expression in cancer cells, we tested our hypothesis that a COX-2 inhibitor, celecoxib may play a role on anti-PD-1 antibody treatment for glioma. METHODOLOGY: We examined mRNA expression of the 143 GBM and 16 GSCs were randomly divided into four treatment groups; vehicle (VC), celecoxib, anti PD-1 antibody or the combination of celecoxib and an antibody against PD-1. RESULTS: Down-regulation of PD-L1 was associated with post-transcriptional regulation of co-chaperone FK506-binding protein 5 (FKBP5) by celecoxib. The combination therapy of anti-PD-1 antibody with celecoxib could be a promising therapeutic strategy targeting PD-L1 in GSCs and GBM. CONCLUSIONS: Down-regulation of PD-L1 via FKBP5 by celecoxib may play a role on the antitumor effects under the overwhelmed expression of PD-L1.

EXTH-07. OPTIMIZATION OF TARGETING ELTD1 IN GlioBLASTOMA USING A MOLECULAR TARGETING APPROACH

Narthi Smith, Derb Soudi, Dhaval Trivedi, and Michelle Zalles; Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

The standard of care for glioblastoma multiform (GBM), an aggressive form of brain cancer, has significantly decreased the overall survival rate for patients. New targeted therapies are urgently needed to improve patient outcomes. Targeting ELTD1 (epidermal growth factor, latrophilin, and 7 transmembrane domain 1) in GBM is a potential cancer therapy in a G53 xenograft mouse model. While our studies have demonstrated that the blood brain barrier (BBB) was leaky around the tumor region, other studies have shown that the BBB is not equally disrupted in GBM patients, therefore suggesting that the mAb may have difficulty crossing the BBB and infiltrating the tumor due to its size. To overcome these limitations, this study focused on the optimization of targeting ELTD1 by using an optimized sFc antibody fragment derived from our mAb targeting ELTD1. Immunocompromised mice were intracerebrally injected with human-G55 cells. Morphological MRI was used to monitor and calibrate tumor volumes. Treatments using IgG, anti-ELTD1 mAb or fragment upon tumor detection. Vascular perfusion images were obtained to examine vascular alterations. Molecular targeting imaging (mMRI) was used to assess the binding specificity of our antibodies against the tumor region. Targeting ELTD1 with varying antibodies (anti-ELTD1 mAb and sFc fragment) resulted in increased survival and decreased tumor volumes in a G53 xenograft GBM mouse model. Additionally, through the use of mMRI, we determined altered levels of VEGF and increased expression of the complex genes MCM6 and MCM10 as well as the Fanconi’s Anemia (FA) pathway genes. Our data suggest that the optimization of an anti-ELTD1 therapy could be used to better target angiogenesis in glioblastomas.

EXTH-08. REPLACEMENT OF MICROGLIA BY BRAIN-ENGRAFTED MACROPHAGES PREVENTS MEMORY DEFICITS AFTER THERAPEUTIC WHOLE-BRAIN IRRADIATION

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Microglia have a distinct origin compared to blood circulating myeloid cells. Under normal physiological conditions, microglia are maintained by self-renewal, independent of hematopoietic progenitors. Following genetic or pharmacologic depletion, newborn microglia derive from the local resident pool and quickly repopulate the entire brain. The depletion of brain resident microglia during therapeutic whole-brain irradiation fully prevents irradiation-induced synaptic loss and recognition memory deficits but the mechanisms driving these protective effects are unknown. Here, we demonstrate that after CSF-1R-inhibiting microglia depletions and therapeutic whole-brain irradiation, circulating monocytes invade the brain and replace the microglia pool. These monocyte-derived brain-engrafted macrophages have reduced phagocytic activity compared to microglia from irradiated brains, but similar to locally repopulated microglia without brain irradiation. Transcriptional comparisons reveal that brain-engrafted macrophage
phages have both monocyte and embryonic microglia signatures. These results suggest that monocytederived brain-engrafted macrophages represent a novel therapeutic avenue for the treatment of brain radiotherapy-induced cognitive deficits.

EXTH-09. FIRST-IN-HUMAN DOSE CONSIDERATIONS OF A BISPECIFIC ANTIBODY FOR TREATING GliOBLASTOMA Teilo Schaller, Matthew Foster, Ivan Spasojevic, Patrick Gedeon, Luis Sanchez-Perez, and John Sampson; Duke University Medical Center, Durham, NC, USA

Current therapy for glioblastoma (GBM) is incapacitating and limited by non-specific toxicity to the surrounding brain. We have developed an immunotherapeutic approach that selectively targets GBM by redirecting the patients' own T cells toward the tumor in an antigen-specific manner using a bispecific antibody. Our novel bispecific antibody ("BRITE") binds GBM-specific surface marker EGRF-VIII and the CD3 receptor on T cells, resulting in crosslinking and tumor-specific cell lysis. We previously showed in patient-derived and syngeneic murine glioma models, that treatment with BRITE leads to long-term survival in glioma-bearing mice. In this work, we demonstrate that localized treatment with the virus is effective against disseminated events. We have reported the efficacy of oncolytic adenovirus Delta-24-RGDOX in-vivo, yet the therapeutic benefit is limited to only a subset of patients with immunogenic GBM. We therefore hypothesize that localized treatment with the virus is effective against disseminated melanomas, including intracranial melanomas. We tested the hypothesis in the subcutaneous (s.c.) and s.c./intracranial (i.c.) melanoma models derived from luciferase-expressing B16-Red-Fluc cells in C57BL/6 mice. First, through monitoring tumor growth with bioluminescence imaging, we found that, in both s.c./i.c. and s.c./i.c. models, three injections of Delta-24-RGDOX significantly inhibited the growth of both melanoma tumor and untreated distant s.c. or i.c. tumor, thereby prolonging survival. Next, through cell profiling with flow cytometry, we observed that the virus increased the presence of T cells and effector T cell frequency in the virus-injected tumor and mediated the same changes in T cells from peripheral blood, tumor-draining lymph nodes (TLDNs), spleens, and brain hemispheres with untreated tumor. Moreover, Delta-24-RGDOX decreased the frequency of exhausted T cells and regulatory T cells in the virus-injected tumor, which is important for tumor recruitment and/or in situ expansion of antigen-specific T cells in tumors expressing the target antigen. Therefore, we concluded that local intratumoral injection of Delta-24-RGDOX resulted in systemic immune activity against the disseminated tumors. Furthermore, we speculate that given the immunogenecity, cancer-selective activity and intratumoral administration of the virus, Delta-24-RGDOX is expected to have an improved safety profile when compared to immune checkpoint blockade treatment strategies. This is the first report demonstrating that local administration of oncolytic adenovirus results in eradication of intracranial tumors, suggesting Delta-24-RGDOX could be used to manage brain metastases of melanoma.

EXTH-10. THE ACTIVATION AND SENSITIZATION OF GliOBLASTOMA CELLS VIA COLD ATMOSPHERIC PLASMA TREATMENT Jonathan Sherman, Dayan Yan, Eda Gjika, and Michael Keidar; George Washington University, Washington, DC, USA

BACKGROUND: Treatment of glioblastoma multiforme (GBM) continues to remain a challenge using conventional treatment. Through an in vitro study, we assessed the efficacy of our novel cold atmospheric plasma technology (CAP) to sensitize GBM cells to temozolomide (TMZ).

METHODS: The CAP jet is formed through the discharge (Pk-Pk: 5.8 kV) between a ring grounded cathode and a central anode and with He flow through a glass tube. The discharge process is driven by an AC high voltage (~316 kV) with a frequency of 12.5 kHz. Human glioblastoma (U87MG) cells were cultured in DMEM supplemented by 1% (v/v) penicillin and streptomycin solution and 10% (v/v) FBS. CAP was delivered to U87 cells in a 96-well plate for 1 min in combination with 10 and 15 μM H2O2. The cell viability was measured by using the MTT assay. We then tested TMZ concentrations of 10 and 50 μM. Cell viability was monitored with the Cell Titer Glo 2.0 luminescent assay. All experiments were performed in triplicate and were independently repeated at least 3 times.

RESULTS: We identified a dose-dependent state of U87MG. This activation state resulted in GBM cells sensitized to reactive species identified by decreased cell viability after treatment with H2O2 as compared to the H2O2 treatment alone (p<0.005). In addition, the plasma-activated TMZ cells were sensitized to CAP treatment. Due to this activation, the GBM cells were sensitized to TMZ. Cells treated with CAP in combination with TMZ displayed decreased cell viability at TMZ concentrations of 10 μM (p<0.05) and 50 μM (p<0.005) as compared to TMZ alone. CONCLUSIONS: This study demonstrates the activation phenomenon on GBM cells via direct CAP treatment. Due to this activation, the GBM cells are sensitized to both TMZ and TMZ+CAP identified via decreased cell viability. Future work looks to assess this effect of cell activation/sensitization with chemotherapy plus radiation treatment.

EXTH-11. TREATMENT WITH DELTA-24-RGDOX OF SUBCUTANEOUS TUMORS RESULTS IN ABSOCOPAL EFFECT ERADICATING INTRACRANIAL GliOMAS Hong Jiang, Dong Ho Shin, Teresa Nguyen, Marta M. Alonso, Frederick Lang, Candelaria Gomez-Manzano, and Juan Fuerdo; 1MD Anderson Cancer Center, Houston, TX, USA, 2Clinica Universidad de Navarra, Pamplona, Spain

Immune checkpoint blockade has revolutionized cancer therapy; however the therapeutic benefit is limited to only a subset of patients with immunogenic (“hot”) tumors and is compromised by immune-related adverse events. We have reported the efficacy of oncolytic adenovirus Delta-24-RGDOX (DNX-2440) in syngeneic glioma mouse models. We hypothesized that localized treatment with the virus is effective against disseminated melanomas, including intracranial melanomas. We tested the hypothesis in the subcutaneous (s.c.) and s.c./intracranial (i.c.) melanoma models

EXTH-12. RADIATION ENHANCES MELANOMA RESPONSE TO IMMUNOTHERAPY AND SYNERGIZES WITH BENZODIAZEPINES TO PROMOTE ANTI-TUMOR ACTIVITY Daniel Pomeranz Krummel1, Tahseen Nasri2, Benjamin Izar2, Lindsey Loewder1, Rebecca Peralta-Molina2, Katerina Kalamov2, and Richard Dell Kallay1

BACKGROUND: Treatment of glioblastoma (GBM) continues to remain a challenge using conventional treatment. Through an in vitro study, we assessed the efficacy of our novel cold atmospheric plasma technology (CAP) to sensitize GBM cells to temozolomide (TMZ).

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EXTH-13. NEUROSURGICAL DELIVERY OF THE POLY ADP RIBOSE POLYMERASE-1 INHIBITOR OLAPARIB FROM A THERMO-RESPONSIVE BIODEGRADABLE PASTE POTENTIATES RADIOTHERAPY AND PROLONGS SURVIVAL IN HIGH-GRADE GliOMA Stuart Smith1, Ricardo Serra2, Jonathan Rowlinson1, Noah Gorelick2, Gareth Vell1, Kevin Shakesheff1, Harry Brem2, Richard Grundy2, Betty Tyler1, and Ruman Rahman3; 1University of Nottingham, Nottingham, Nottingham, United Kingdom, 2Johns Hopkins University, Baltimore, MD, USA, 3Newcastle University, Newcastle, United Kingdom

There has been considerable interest in repurposing the poly ADP ribose polymerase inhibitor and purported radiosensitizer olaparib (PLGA and poly(ethylene glycol) (PEG) (PLGA/PEG) thermo-sensitive biodegradable paste. Metabolic and clonogenic assays revealed impaired proliferation and clonal growth respectively, upon acute exposure of high-grade glioma cells to olaparib (3–5 μM), an effect dramatically potentiated with 3 Gy radi-