Abstracts

TBIO-26. NON-CANONICAL OPEN READING FRAMES ENCODE FUNCTIONAL PROTEINS ESSENTIAL FOR CANCER CELL SURVIVAL

John Premnath1, Oana Enache2, Victor Iurua3, Karsten Krug1, Karl Clauser2, Joshua Dempster3, Amir Karger1, Li Wang4, Karolina Stumbravite, Vickie Wang4, Genevra Botta2, Nicholas Lyons2, Amy Goodale2, Zehra Kalani6, Briania Fritchman2, Adam Brown6, Douglas Alan2, Thomas Green4, Xiaoping Yang5, Jacob Jaffe5, Jennifer Roth2, Federica Piccioni4, Marc Kirschner6, Zhe Ji4, David Root2, and Todd Golub4,6

Boston Children’s Hospital/Dana-Farber Cancer Institute, Boston, MA, USA, 1Broad Institute, Cambridge, MA, USA, 2Harvard Medical School, Boston, MA, USA, 3Harvard Medical School, Cambridge, MA, USA, 4Hospital for Sick Children, Toronto, ON, Canada, 5Stowers Institute for Medical Research, Kansas City, MO, USA, 6Department of Developmental Biology, Harvard University, Cambridge, MA, USA, 7Department of Developmental Biology, Harvard University, Cambridge, MA, USA

The brain is the foremost non-gonadal tissue for expression of non-canonical ORFs of unclear function. Yet, whether such transcripts are truly non-coding or rather the source of non-canonical protein translation is unknown. Here, we used functional genomic screens to establish the cellular bioactivity of non-canonical proteins located in putative non-coding regions of untranslated regions of protein-coding genes. We experimentally interrogated 553 open reading frames (ORFs) identified by ribosome profiling for three major phenotypes: 257 (46%) demonstrated protein translation when ectopically expressed in HEK293T cells, 401 (75%) induced a significant effect on cell cycle arrest via single-cell RNA sequencing. Analysis of the secretome of GREP1-expressing cells showed increased abundance of the oncogenic extracellular protein-1 (GREP1)—as a cancer-implicated gene with high expression in cancer cell types, such as gliomas. GREP1 knockout in >200 cancer cell lines reduced cell viability in multiple cancer types, including glioblastoma, in a cell-autonomous manner and produced cell cycle arrest via single-cell RNA sequencing. Analysis of the secretome of GREP1-expressing cells showed increased abundance of the oncogenic cytokine GDF15, and GDF15 supplementation mitigated the growth inhibitory effect of GREP1 knock-out. Taken together, these experiments suggest that the non-canonical ORFeome is surprisingly rich in biologically active proteins and potential cancer therapeutic targets deserving of further study.

TBIO-27. RASOPATHIES AND BRAIN TGORRGENESIS: ARE SOS1 MUTATIONS OF CONCERN?

Nouha Bouayed Abdelmoula, Rim Louati, Balkiss Abdelmoula, and Samir Aloulou

UR17ES36 Genomic of Signalopathies at the service of Medicine, Medical University of Max, Stax, Tunisia

Germ line gain-of-function mutations in several members of the RAS/ MAPK pathway, including PTPN11 are associated with signalopathies named Rasopathies and known as Noonan syndrome and closely related conditions. Patients harboring Rasopathies are at increased risk of myeloproliferative diseases and solid tumors, such as neuroblastoma. Mutations of SOS1, the gene encoding a guanine nucleotide exchange factor named Rasopathies and known as Noonan syndrome and closely related conditions. Patients harboring Rasopathies are at increased risk of myeloproliferative diseases and solid tumors, such as neuroblastoma. Mutations of SOS1, the gene encoding a guanine nucleotide exchange factor for Ras, represent the second most frequent genetic defect in Rasopathies. However, SOS1 mutations are rare in human malignancies and patients with germline SOS1 mutations may not be at increased risk of developing cancer. Here, we report a SOS1 variant found to segregate in a Tunisian pedigree with many members affected by brain tumors as well as epileptic disorders. Outlining our genetic counseling for congenital heart diseases, a 9-year-old female born at Stax from a consanguineous couple and having pulmonic valvular stenosis, has been investigated at the molecular level. Screening of mutations in the entire coding sequence of PTPN11, B01-473, and gene expression changes following ectopic expression across 4 cancer cell types, and 57 (10%) induced a viability defect when the endogenous ORF was knocked out using CRISPR/Cas9 in 8 human cancer cell lines. CRISPR tiling and start codon mutagenesis in >200 cancer cell lines reduced cell viability in multiple cancer types, including glioblastoma, in a cell-autonomous manner and produced cell cycle arrest via single-cell RNA sequencing. Analysis of the secretome of GREP1-expressing cells showed increased abundance of the oncogenic cytokine GDF15, and GDF15 supplementation mitigated the growth inhibitory effect of GREP1 knock-out. Taken together, these experiments suggest that the non-canonical ORFeome is surprisingly rich in biologically active proteins and potential cancer therapeutic targets deserving of further study.

VIRAL/GENE THERAPY AND OTHER NOVEL THERAPIES

TBIO-01. AWAKENING THE IMMUNE SYSTEM WITH AN IMMUNO-ONCOLYTIC VIRUS AS A THERAPEUTIC STRATEGY FOR DIPGs

Virginia Laspidua, Montse Pugdelloses, Iker Ausejo-Mauleon, Dolores Hambardzumyan, Chong Chen1, Nazan Garmine-V1, Micaela Marangulin, Mira Zalacain, Zhe Li3, and David Gomez-Manzano

The brain is the foremost non-gonadal tissue for expression of non-canonical ORFs of unclear function. Yet, whether such transcripts are truly non-coding or rather the source of non-canonical protein translation is unknown. Here, we used functional genomic screens to establish the cellular bioactivity of non-canonical proteins located in putative non-coding regions of untranslated regions of protein-coding genes. We experimentally interrogated 553 open reading frames (ORFs) identified by ribosome profiling for three major phenotypes: 257 (46%) demonstrated protein translation when ectopically expressed in HEK293T cells, 401 (75%) induced a significant effect on cell cycle arrest via single-cell RNA sequencing. Analysis of the secretome of GREP1-expressing cells showed increased abundance of the oncogenic extracellular protein-1 (GREP1)—as a cancer-implicated gene with high expression in cancer cell types, such as gliomas. GREP1 knockout in >200 cancer cell lines reduced cell viability in multiple cancer types, including glioblastoma, in a cell-autonomous manner and produced cell cycle arrest via single-cell RNA sequencing. Analysis of the secretome of GREP1-expressing cells showed increased abundance of the oncogenic cytokine GDF15, and GDF15 supplementation mitigated the growth inhibitory effect of GREP1 knock-out. Taken together, these experiments suggest that the non-canonical ORFeome is surprisingly rich in biologically active proteins and potential cancer therapeutic targets deserving of further study.

TBIO-02. EVALUATION OF THE ONCOVIRUS DELTA24-RGD AS AN ANTI-TUMOR AGENT IN PRECLINICAL MODELS OF LOCALIZED AND DISSEMINATED AT/RT

Marc Garcia-Moure1,2, Marisol Gonzalez-Huarriz1,2, Daniel de la Nava6,7, Maria Marrodain4,6, Gande Gomez-Manzano1, Juan Fueyo1, Ana Pattiño-Garcia6,7, and Marta M Alonso6,7

1University of Navarra, Pamplona, Spain, 2Health Research Institute of Navarra (IDIANA), Pamplona, Spain, 3University of Navarra, Pamplona, Spain, 4MD Anderson Cancer Center, Houston, Texas, USA

Current therapies for atypical teratoid/rhabdoid tumors (AT/RTs) are suboptimal, resulting in a 2-year OS below 20% and the development of severe side effects. Therefore, we need to explore alternative therapeutic approaches for this disease. Since the virus Delta24-RGD has already demonstrated its efficacy and safety as a therapeutic agent for brain tumors, including pediatric patients, here we propose to evaluate the anti-tumor effect of Delta24-RGD in AT/RT. In vitro, Delta24-RGD infects and replicates in AT/RT cultures followed by oncolysis, obtaining IC50 values below 1 PFU/cell. In vivo, a single local injection of Delta-24-RGD in three infratentorial AT/RT models (BT-12, CHLA-06 and CHLA-266) extended significantly the median OS (50 to 78 days BT-12; 21 to 31 days CHLA-06; 64 to 110 days CHLA-266). Delta-24-RGD also increased the survival of mice bearing supratentorial CHLA-266 tumors (from 93 to 132 days). Next, we evaluated the efficacy of Delta-24-RGD in a model mimicking metastatic disease through intraventricular injection of BT-12-luciferase cells. Administration of Delta-24-RGD inhibited tumor growth and development of metastases, leading to an increased OS and nearly 70% of long-term survivors. The interaction between Delta24-RGD and the immune system was evaluated in humanized mice models bearing CHLA-06. In this model, Delta-24-RGD treatment extended OS (from 23 to 34 days) and we characterized the anti-tumor immune response in long-term survivors. Mechanistic experiments, showed a decrease of T cell infiltration (mainly CD8), decrease of proliferating cells and a reduction of the number of vessels in FVFE brain samples in comparison with control mice. We are currently performing in vivo experiments using the expansion of activated T cells and the generation and maintenance of CD8 T memory cells. Therefore, we propose the use of Delta-24-RGD as a therapeutic approach for DIPG tumours. We observed that Delta-24-RGD is able to infect and replicate in Delta24-RGD treated mice, two DIPG murine cell lines and patients with DIPG.

TBIO-03. IN VITRO EVALUATION OF THE EFFECT OF CANNABIDIOL ON PAEDIATRIC BRAIN TUMOUR CELL LINES USING A PULSED TREATMENT REGIME

Sophie Faulks, George Lockwood, Saroote E O’Sullivan, Richard G Grundy, and Lisa C D Storer

University of Nottingham, Nottingham, Nottinghamshire, United Kingdom

Paediatric brain tumours are the second most common cancer after haemato-logical malignancies. Intermittent dosing regimens are typical for chemo-
therapy drugs in order to avoid excessive damage to organs and avoid the onset of late effects. Cannabidiol (CBD) has been shown to have cytotectic properties on paediatric brain tumour cell lines. Although CBD is far less toxic than its classical cannabis homologue, there are currently available to children suffering with brain tumours, there are some possible side effects. Given that the half-life of the drug is 24 hours, it was important to establish the nature of the effect of cumulative dosing on top of the remaining drug in the system. The poliovirus cell line, SF188 was cultured in different concentrations of CBD with either 1, 2 or 3 doses being given on consecutive days. 24 hours after the last dose the cells were analysed using the resazurin assay. It was observed that the amount of drug required for an EC50 to be obtained decreased, 17.6μM (1 dose), 9μM (2 doses), 5μM (3 doses) and that cell survival was reduced to nearly 0% in those cells which received multiple doses of CBD at 17.6μM. In order to mimic the intermittent dosing regime, the cells were returned to the incubator for 4 days before the resazurin assay was repeated. The decrease in viability was maintained over the extended culture period meaning that the ability of even the apparent “healthy” cells to proliferate had been permanently affected.

THER-04. IS THERE A ROLE FOR CANNABIDIOL IN THE TREATMENT OF CHILDHOOD BRAIN TUMOURS?
George Lockwood, Amelia Hatfield, Mohamed Mabrouk, Saorie E O'Sullivan, Richard G Grundy, and Lisa C D Storer; University of Nottingham, Nottingham, Nottinghamshire, United Kingdom

Brain tumours are the leading cause of cancer related death in children with limited treatment options and high recurrence rates. Recent evidence suggests there may be anti-tumoural properties of cannabinoids on cannabidiol (CBD) in particular. We evaluated the effect of CBD on paediatric brain tumour cell lines in 2D and 3D spheroids. pHGG (SF188), ependymoma (BxD142SEP) and human astrocytes. At the CBD EC50 concentration, astrocytic cell death was insignificant. 3D spheroids decreased in size by approximately 20% when cultured in CBD compared to cells only after 5-day exposure. Cell death increased with time after a single dose of CBD. Western Blot showed an increase in LC3b expression (autophagy) after 24 hours incubation (early cell death) in pHGG in both BxD142SEP and SF188 with PARP expression (apoptosis) increased after 5 days incubation (late cell death). Cell cycle analysis showed a decrease of cells in G2 and no change in G0 indicating cell cycle arrest. In hypoxia, SF188 and BxD142SEP cells showed decreased cell death after 24 hours and 5 days when compared to normoxia and an EC50 within acceptable limits could not be achieved. SF188 cells pre-treated with receptor antagonists indicate that CBD was not acting through CB1, CB2, GPR18, PPARα or PPARγ receptors but may act as a partial agonist of the TRPV1 and 5-HT3 receptors and a full agonist of the GPR55 receptor (resazurin assay). This provides evidence that CBD is effective at killing paediatric brain tumour cells and does not have a significant effect on normal astrocytes.

THER-05. GENETICALLY STABLE POLIOVIRUS VECTOR CARRYING H3.2K7M ANTIGEN FOR TREATMENT OF DIFFUSE MIDLINE GLIOMA BY INTRAMUSCULAR INJECTION
Margaret McManus, Daniel Landi1, Elena Dobrikova4, Michael Brown4, Yuanfan Yang1, Jana Cabbage1, Hideho Okada6, Smita Nair2,3, Darell Bigner2, David Ashley2, and Matthias Gromeier1; 1Department of Molecular Genetics and Microbiology, Duke University Medical School, Durham, NC, USA, 2Department of Neurosurgery, Duke University Medical School, Durham, NC, USA, 3Department of Pediatrics, Duke University Medical School, Durham, NC, USA, 4Department of Pathology, Duke University Medical School, Durham, NC, USA, 5Department of Neurosurgical Surgery, University of California at San Francisco, San Francisco, CA, USA, 6Parker Institute for Cancer Immunotherapy, University of California at San Francisco, San Francisco, CA, USA

BACKGROUND: H3 K27M-mutant diffuse midline glioma (DMG) is invariably lethal. Viruses naturally engage innate immunity, induce antigen presentation, and mediate CD8 T cell priming against foreign antigens. Polioviruses, in particular, are uniquely tropic for dendritic cells (DC) and potent activators DC, inducing Th1-dominant cytokine profiles, CD8 T cell immunity, and enhanced epitope presentation. Thus, poliovirus is ideally suited for vectored delivery of signatures tumour antigens.

METHODS: We created a genetically stable, polioirhinovirus chimera, ΔpoliovirusΔ61 containing a deletion of the poliovirus 3C protease that inhibits the expression of the HLA-A2 restricted H3.3 K27M antigen (RIPO (H3.3)). RESULTS: RIPO (H3.3) infects, activates, and induces H3.2K7M antigen presentation in DCs in vitro. Given intramuscularly in vivo, RIPO (H3.3) recruits and activates DCs with Th1-dominant cytokine profiles, efficiently primes H3.2K7M-specific CD8 T cells, induces antigen-specific CD8 T cell migration to the tumor site, delays tumor growth, and enhances survival in murine tumor models. CONCLUSION: This novel approach using poliovirus vectors to activate DCs would simultaneously introduce the H3.3 K27M antigen. In this way, DCs are activated optimally in situ, while being simultaneously infected to express/present tumor antigen. RIPO (H3.3), given by intramuscular injection, will be evaluated in a clinical trial for children with H3 K27M-mutant diffuse midline glioma.

THER-06. THERAPEUTIC EFFICACY OF RRV-MEDIATED PRODRUG ACTIVATOR GENE THERAPY IN CLINICAL TRIALS OF RECURRENT HIGH-GRADE GLIOMA AND IN MURINE ORTHOTOPIC MODELS OF INTRACEREBRAL GLIOMA AND INTRACEREBELLAR MEDULLOBLASTOMA
Angela Richardson1,2, Sara Collins1, Akhito Inagaki3, Valerie Armstrong4, David Robbini1, Naga Ayad6, Harry Gruber6, Douglas Jolly7, Timothy Cloughesy4, and Noriyuki Kasehara8; 1Department of Neurosurgery, University of Miami, Miami, FL, USA, 2Jackson Health System, Miami, FL, USA, 3Department of Neurosurgical Surgery, University of California, San Francisco (UCSF), San Francisco, CA, USA, 4Miller School of Medicine, University of Miami, Miami, FL, USA, 5Departments of Surgery and Biochemistry, University of Miami, Miami, FL, USA, 6Department of Psychiatry and Behavioral Sciences, Miami, FL, USA, 7Tocagen Inc., San Diego, CA, USA, 8Department of Neurology, University of California, Los Angeles (UCLA), Los Angeles, CA, USA, 9Departments of Neurological Surgery and Radiation Oncology, University of California, San Francisco (UCSF), San Francisco, CA, USA

Toca 511, a clinical-stage tumor-selective retroviral replicating vector (RRV), encodes optimized yeast cytosine deaminase (CD), which converts the prodrug 5-iododeoxyuridine (5-IDU) to the active drug 5-iodouracil (5-IU) within infected cancer cells. In preclinical models of intracerebral glioblastoma, 5-IU generated locally by Toca 511 (RRV-CD) prodruk activator gene therapy has also been shown to kill immunosuppressive myeloid cells in the tumor microenvironment, leading to anti-cancer immune activation and long-term survival. Early-phase clinical trials of Toca 511 in recurrent high-grade glioma showed highly promising evidence of therapeutic benefit, leading to a Phase III trial completed in late 2018. In the Toca-01B trial, 400 patients, randomized 1:1 vs. standard chemotherapy, which appeared to show negative results overall. However, additional analysis showed possible efficacy in prespecified subgroups, and further clinical investigation is being pursued. In preclinical studies, we have also evaluated RRV for use in medulloblastoma, the most common malignant tumor of the pediatric nervous system. Both established and primary human medulloblastoma cell lines supported efficient RRV replication in vitro, with spread to >90% of cells by day 10 post-inoculation, and RRV-CD-transduced medulloblastoma cells showed significant dose-dependent reduction in tumor viability upon exposure to 5-FC, compared to controls. In an intracerebellar HDMB03 medulloblastoma model, RRV-CD-treated mice exhibited long-term survival while on sequential cycles of 5-FC prodruk, until prodruk treatment was stopped, after which 23% long-term survival was observed (median survival 110 days) as compared to controls (median survival 28 days, 100% lethality) (p=0.00007). These results support further evaluation of RRV-mediated prodruk activator gene therapy for pediatric brain tumors.

THER-07. INHIBITION OF THE RAS SIGNALING ENHANCES VIRAL ONCOLYSIS IN MALIGNANT GLIOMAS
Yoshiki Arakawa, Makoto Yamaguchi, Masahiro Tanji, Yohei Mineharu, and Susumu Miyamoto; Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto, Japan

Pediatric malignant glioma indicates rapid proliferation, widely infiltrative properties on paediatric brain tumour cells and does not have a significant effect on normal astrocytes.

Abstracts