

## ET-13

**CONTROL OF ACTIVATED MICROGLIA THROUGH P2X4 RECEPTOR IN RADIATION BRAIN NECROSIS**

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**INTRODUCTION:** Brain radiation necrosis (RN) is severe adverse event after radiation therapy for brain tumor patients, especially in case of re-irradiation. Although corticosteroids or vitamin E, etc. are clinically used for RN, the effect is limited and underlying mechanism is to be cleared. Therefore, we established RN mouse model with irradiating right hemisphere of mouse brain using proton beam at dose of 60 Gy [Kondo et al., 2015]. In this study, we investigated change of phospholipids and lipid mediators after irradiation using this RN model in correlation with microglia activation. **METHODS:** After irradiation, change of phospholipids and lipid mediators in mouse brain was investigated using imaging mass spectrometry and LC-MS. Immunohistochemistry on microglia and P2X4 receptor, a receptor for lysophosphatidylcholine (LPC) was performed. **RESULTS:** In imaging mass spectrometry, 1 and 4 months after irradiation, phosphatidylcholine (PC): (16:0/20:4), (18:0/20:4) decreased in irradiated area compared non-irradiated area. On the other hand, LPC: (16:0) increased in irradiated area compared to non-irradiated area after 1 month and 4 months irradiation. PC (16:0/20:4) is a precursor of LPC (16:0) and arachidonic acid (20:4). By LC-MS, LPC was twice higher in irradiated area compared to non-irradiated, 6 months after irradiation. Microglia was highly activated in irradiated area compared to non-irradiated from 3 months after irradiation to 8 months and strongly co-expressed P2X4 receptor was confirmed in irradiated area after 6 months. Preliminary P2X4 receptor agonist administration test prolonged the RN to 12 months after irradiation. **CONCLUSION:** In RN, LPC may continuously activated microglia through P2X4 receptor and cause chronic inflammation after irradiation. P2X4 agonist administration test including action resolution and immunohistochemistry is ongoing.

**TUMOR BIOLOGY/MODELS (TB)**

## TB-01

**HUMAN IPS CELL-DERIVED BRAIN TUMOR MODEL UNCOVERS THE EMBRYONIC STEM CELL SIGNATURE AS A KEY DRIVER IN ATYPICAL TERATOID/RHABDOID TUMOR**

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Atypical teratoid/rhabdoid tumor (AT/RT), which harbors SMARCB1 mutation and exhibits a characteristic histology of rhabdoid cells, has a poor prognosis because of the lack of effective treatments. We established human SMARCB1-deficient pluripotent stem cells (hPSCs), which enabled investigation of the pathogenesis of AT/RT. SMARCB1-deficient hPSCs and neural progenitor-like cells (NPLCs) efficiently gave rise to brain tumors when transplanted into mouse brain. Notably, the emergence of typical rhabdoid cells was significantly enhanced in tumors from SMARCB1-deficient hPSCs. An embryonic stem cell (ESC)-like gene expression signature was more prominent in hPSC-derived tumors when compared with NPLCs-derived tumors. Moreover, mice transplanted with SMARCB1-deficient hPSCs showed poor survival than NPLC-transplanted mice. Activation of the ESC-like signature by the forced expression of reprogramming factors conferred a rhabdoid histology in SMARCB1-deficient NPLC-derived tumors, suggesting that acquisition of the ESC-like signature is responsible for the rhabdoid histology. Consistently, we found activation of the ESC-like gene expression signature and an ESC-like DNA methylation landscape in clinical specimens of AT/RT. Mechanistically, c-MYC expression was sufficient to acquire the ESC-like signature and the rhabdoid histology in SMARCB1-deficient NPLC-derived tumors, which resulted in poor survival. Together, SMARCB1-deficient hPSCs offer the first human model for AT/RT, which uncovered the unappreciated role of the activated ESC-like signature in the poor prognosis and unique histology. Finally, we performed a CRISPR/Cas9 knockout screening to inhibit activation of the ESC-like signature in AT/RT. Our effort identified candidate genes as therapeutic targets, including RAD21, which encodes a key component within the cohesin complex. Notably, chemical inhibition of HDAC8, which indirectly targets the function of cohesin, with simultaneous inhibition of EZH2 efficiently suppressed activation of the ESC-like signature and inhibited the growth of AT/RT cells. Collectively, we propose that the ESC-like signature could be a crucial therapeutic target for AT/RTs with rhabdoid histology.

## TB-02

**NF-KB CANONICAL PATHWAY ACTIVATION DRIVES GLYCOLYSIS AND TUMOR PROGRESSION IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA**

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Recent genomic analyses have identified highly recurrent genetic alterations in PCNSL. However, due to the lack of clinically representative PCNSL preclinical models, the pathogenic mechanisms of these alterations remains largely unknown. Here, we established the largest panel of 12 clinically relevant PCNSL patient-derived orthotopic xenografts retained the histopathologic phenotype, lymphoma expression subtype, copy number alterations and 90% of the non-synonymous mutations of primary tumors, with 100% concordance of MYD88 and CD79B mutations, which are highly recurrent in PCNSL. Patient tumor regression with high-dose methotrexate correlated with in vitro sensitivity to methotrexate in corresponding PCNSL models. By knocking down canonical NF-kB pathway genes, we found that successful orthotopic xenograft formation was dependent on NF-kB canonical pathway activation induced by MYD88 mutation or overexpression of EBV-related LMP1. Metabolically, PCNSL xenografts phenocopied the high 18F-fluorodeoxyglucose uptake observed in patients and demonstrated glycolytic dependence, revealing new potential therapeutic strategies in PCNSL. Collectively, we found NF-kB canonical pathway activation as a crucial driver of PCNSL xenograft progression and found that NF-kB canonical pathway induced an addiction to glycolysis, revealing a novel potential therapeutic strategy. Our PCNSL xenograft panel represents a valuable and reproducible preclinical tool that has the potential to help decipher how genetic and/or epigenetic alterations contributes to lymphomagenesis and tumor maintenance and enhance the development of novel therapeutic strategies in PCNSL.

## TB-03

**THE SURVIVAL PROLONGATION EFFECT OF NOVEL BORON COMPOUND FOR BNCT USING RAT BRAIN TUMOR MODEL**

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**INTRODUCTION:** Boron neutron capture therapy (BNCT) is form of tumor-cell selective particle irradiation. Although novel boron compounds have been developed, BPA (boronophenylalanine) and BSH (borocaptate sodium) are used in the clinical practice. The development of effective boron compounds is a major theme. We used Dodecaborate-containing BPA (AAL) which is combined the characteristics of both BPA and BSH. We have been conducting research on how the new compound for BNCT will affect rat brain tumor model. **MATERIALS AND METHODS:** We evaluated the boron concentration of F98 glioma cells for BPA and AAL, and the biodistribution of these following BPA administrated intravenously (i.v.) or AAL administrated by convection-enhanced delivery (CED) in F98 glioma bearing rats. In BNCT study, the therapeutic effect was evaluated in terms of the survival time for all rats divided into six groups. **RESULTS:** The uptake of boron showed almost the same value at all exposure times in high concentration. In biodistribution study, the AAL(CED) 6h after the termination group attained the highest boron concentrations of the tumor ( $59.9 \pm 18.2 \mu\text{g/g}$ ). In the BNCT study, the median survival time in the AAL(CED) group (31(29–35) days) was shorter than that in the BPA(i.v.) group (34(33–36) days). And the combination group of AAL(CED) and BPA(i.v.) gave the most significant prolongation of survival (38(36–40) days). **DISCUSSION:** AAL(CED) and BPA(i.v.) combined group had a significant survival prolongation compared with the single-agent group. It is thought that AAL irradiated by thermal neutron had a cell-killing effect on cells in which BPA was not taken up. The combination uses of AAL (CED) provides additional BNCT effects. The mechanism by which AAL is incorporated has not been clarified, and further experiments including the influence on normal cells are in progress. **CONCLUSION:** Dodecaborate-containing BPA (AAL) is a novel boron compound for BNCT that can be expected to prolong the survival time in combination with BPA.

## TB-04

**TERT PROMOTER MUTATION AS A SUSCEPTIBLE MOLECULAR MARKER OF BCNU LOCAL THERAPY**

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**INTRODUCTION:** Alkylating agents, including Temozolomide (TMZ) and CCNU (ACNU) have been widely accepted as a standard treatment in malignant gliomas. Several studies also demonstrated that BCNU wafer placement extended survival in glioblastoma patients. However, little study demonstrated gene-specific efficacy of BCNU local therapy in malignant gliomas. Herein, we investigated BCNU sensitivity for patient-derived primary cultured glioma cells. **MATERIALS AND METHODS:** From January 2017 to July 2019, 58 gliomas (grade III, IV) were tested genomic analysis and ATP-based cell viability after BCNU treatment. IDH1/2 mutation and TERT promoter mutation status was determined by Sanger sequencing. MGMT methylation status were evaluated by methylation specific PCR. **RESULTS:** Of 58 cases, 10 cases (17.2%) and 32 (55.2%) cases harbored IDH1/2 mutation and TERT mutation (C228T, C250T), respectively. Among them, co-mutation was identified in 5/58 cases (8.6%). MGMT was methylated in 17/58 cases (29.3%). Interestingly, the presence of TERT promoter mutation was positively correlated with BCNU sensitivity, particularly in IDH1/2 wild-type tumors ( $p < 0.05$ ). In contrast, there was no significant relationship between TMZ sensitivity and IDH mutation/MGMT methylation status. **CONCLUSION:** Although sample size is small, our results imply TERT promoter mutations might be a predictive molecular marker for BCNU sensitivity in malignant gliomas. Since TERT mutations are located at two hot spot loci (C228T and C250T), vast majority of TERT promoter mutations can be evaluated during surgery, which may contribute tailored therapeutic strategy in malignant gliomas.

#### TB-06

##### MOLECULAR MECHANISM OF BRAIN TUMOUR FORMATION DRIVEN BY SUPRATENTORIAL EPENDYMOMA-SPECIFIC YAP1 FUSION GENES

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YAP1 fusion-positive supratentorial ependymomas predominantly occur in infants, but the molecular mechanisms of oncogenesis are unknown. Here we show YAP1-MAMLD1 fusions but not YAP1 wildtype are sufficient to drive malignant transformation of neural progenitors in the developing cerebral cortex in mice, and the resulting tumours share histo-molecular characteristics of human ependymomas. Nuclear localization of YAP1-MAMLD1 protein is associated with its oncogenicity and is mediated by the nuclear localization signal of MAMLD1 in a YAP1-Ser127 phosphorylation-independent manner. Chromatin immunoprecipitation-sequencing analyses of human YAP1-MAMLD1-positive ependymoma reveal enrichment of NFI and TEAD transcription factor binding site motifs in YAP1-bound regulatory elements, hypothesizing the important role of these transcription factors in YAP1-MAMLD1-driven tumourigenesis. Indeed, co-immunoprecipitation assays revealed physical interactions of TEADs and NFIA/B with the YAP1 and MAMLD1 domains of the fusion protein, respectively. Mutation of the TEAD binding site in the YAP1 fusion or repression of NFI targets prevents tumour induction in mice. Together, these results demonstrate that the YAP1-MAMLD1 fusion functions as an oncogenic driver of ependymoma through recruitment of TEADs and NFIs, indicating a rationale for preclinical studies to block the interaction between YAP1 fusions and NFI and TEAD transcription factors.

#### TB-08

##### PATIENT DERIVED XENOGRFT'S BIOBANK FROM KANSAI MOLECULAR DIAGNOSIS NETWORK FOR CENTRAL NERVOUS SYSTEM TUMORS

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Patient-derived xenografts (PDXs) are essential tools for translational research for brain tumors. However, it is sometimes difficult for each institution to establish PDXs because it needs experiences and techniques and it also takes a lot of works to establish them. Thus we aim to establish patient derived xenograft's biobank among institutions of Kansai Molecular Diagnosis Network for Central Nervous System (CNS) Tumors, Osaka, Japan. We have already began sharing two anaplastic astrocytoma PDXs, twelve glioblastoma IDH wild type PDXs, two medulloblastoma Shh sub-

group PDXs, one atypical teratoid/rhabdoid tumor (AT/RT) PDX, and three metastatic brain tumor PDXs. Furthermore these PDXs can also be cultured in vitro, except 2 medulloblastoma SHH subgroup PDXs, 1 AT/RT PDX. However, we have not yet established any PDXs from low grade glioma, ependymoma, primary central nervous system lymphoma (PCNSL), diffuse intrinsic pontine glioma (DIPG).

We began sharing these PDXs among the institutions of Kansai Molecular Diagnosis Network for CNS Tumors, Osaka, Japan. However, further improvement is necessary to succeed in establishing PDX from low grade glioma, PCNSL, DIPG, etc. and get enough number of PDXs so we can share PDXs from almost all of the brain tumors.

#### TB-09

##### MRNA-SEQ FOR PERICYTES FROM IN VITRO BRAIN METASTASIS AND BLOOD-BRAIN BARRIER MODEL.

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**BACKGROUND:** Metastatic brain tumors associated with poor prognosis and limited treatment options. The blood-brain barrier (BBB) is supposed to play a major role in brain metastasis. However, little is known about the role of pericytes in brain metastasis formation. This study aimed to reveal the expression profile of interaction between pericytes, endothelial cells, and cancer cells. **METHODS:** The Institutional review board approved this study. We established an in vitro BBB model with rat primary cultured BBB-related cells (endothelial cells and pericytes) and investigated the gene expression of pericytes under the lung cancer cell's coculture circumstances. Pericytes showed inhibition of the KNS-62 cell proliferation significantly ( $p < 0.05$ ). RNA was extracted from the pericytes using miRNeasy mini kit. Complementary DNA library preparation was performed with QuantSeq 3 'mRNA-Seq Library Prep Kit. RNA-seq was performed with MiSeq using MiSeq Reagent Kit v3. Sequencing reads were analyzed on the Maser (TopHat2-CuffLinks2-CummeRbund, TopHat2-HTSeq) and TCC-GUI (EdgeR, DESeq, baySeq) platform. Enrichment analysis was performed at Metascape, and the results were analyzed concerning the OMIM and KEGG databases. **RESULT:** The RIN value of RNA  $< 8.0$  was confirmed. Data quality was acceptable in Fast QC analysis. In TCC differential expressed gene (DEG) analysis, cluster analysis showed that the influence of pericyte lot difference was stronger than the change between cell lines and control. Therefore, lot-specific DEG analysis was performed; the data were pretreated and re-analyzed to try to identify genes involved in the suppression of cancer cell growth. **DISCUSSION:** This study revealed that some expression profiles of brain pericytes implemented in the prevention of metastatic lung cancer cell proliferation in the brain. Pericytes exert an anti-metastatic effect and thus have the potential for the preventive treatment of brain metastasis.

## IMMUNOLOGY (IM)

#### IM-01

##### PI3K GAMMA INHIBITOR FOR OVERCOMING TREATMENT RESISTANCE IN COMBINATION THERAPY OF TEMOZOLOMIDE AND ANTI-PDL1 ANTIBODY FOR GLIOBLASTOMA PATIENTS

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**PURPOSE:** Multidisciplinary therapies including immunotherapy in glioblastoma (GBM) patients often cause long survivor, while early relapse of GBM still remains. We should find factors associated with the immunotherapy-resistance for overcoming it. We previously reported that the infiltration of PD-1 positive cells and M2 macrophages (M2M&phi) increased in recurrent specimens compared to the initial specimens of GBMs treated with chemo-radiotherapy and autologous formalin-fixed tumor vaccine. Here we evaluate whether combination of novel immunotherapies, anti-PD-L1 antibody and M2M&phi inhibitor (IPI-549) inhibits growth of temozolomide (TMZ)-treated glioma cells rather than monotherapy. **METHODS:** Using murine glioma initiating cells (TS) and TMZ-resistant TS (TMZRTS) cells, PD-L1 expression and cytokine production associated with M2M&phi were evaluated. TMZRTS cells were implanted in mice flank, followed by anti-PD-L1 antibody and / or IPI-549 administration. **RESULTS:** Relative cell proliferation rate of TMZRTS cells was lower than TS cells, while PD-L1 mRNA expression was higher. Treatment with PD-L1 antibody caused marked infiltration of M2M&phi in glioma tissue. The