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 FFPE-based cell viability after BCNU treatment. IDH1/2 mutation and TERT promoter mutation 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, 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 C230T), vast majority of TERT promoter mutations can be evaluated during surgery, which may contribute tailored therapeutic strategy in malignant gliomas.

TB-09
MRNA-SEQ FOR PERICYTES FROM IN VITRO BRAIN METASTASIS AND BLOOD-BRAIN BARRIER MODEL. Kenta Ujifuku1, Takashi Fujimoto, Kei Satoh2, Yoshii Morofuji, Hideki Muto, Hiroshi Masumoto, Shinshu Nakagawa, Masami Niwa, Takayuki Matsuoi; 1Department of Neurosurgery, Nagasaki University Graduate School of Biomedical Sciences.

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 metastasis. However, the role of pericytes in brain metastasis formation is not 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 miRNAasy 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 MiSeq. Differential gene expression (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
PSK GAMMA INHIBITOR FOR OVERCOMING TREATMENT RESISTANCE IN COMBINATION THERAPY OF TEMOZOLOMIDE AND ANTI-PDL1 ANTIBODY FOR GLIOBLASTOMA PATIENTS. Eiichi Ishikawa1, Tsusaba Miyazaki1, Masahide Matsuda1, Shingo Takano1, Akira Matsumura1; 1Department of Neurosurgery, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan.

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 (M2Mφ) increased in recurrent specimens compared to the initial specimens of GBMs treated with chemo-radiotherapy and autologous plasmid-modified tumor vaccine. Here we evaluate whether combination of novel immunotherapies, anti-PD-L1 antibody and M2Mφ inhibitor (IP-549) inhibits growth of temozolomide (TMZ)-treated glioma cells rather than monotherapy.

MATERIALS AND METHODS: Using murine glioma immortalizing cells (TS) and TMZ-resistant TS (TMZRTS) cells, PD-L1 expression and cytokine production associated with M2Mφ were evaluated. TMZRTS cells were implanted in mice flank, followed by anti-PD-L1 antibody and / or IP-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 M2Mφ in glioma tissue. The
In combination therapy strongly inhibited tumor growth in TMZRTS murine model. CONCLUSION: The anti-PD-L1 antibody treatment altered tissue microenvironment including marked infiltration of macrophages in glioma tissue, probably associated with clinical immuno-therapy-resistance in GBM. Combination therapy with anti-PD-L1 antibody and M2M@phi inhibitor could overcome it.

IM-03 IMMUNOLOGICAL SUBTYPES OF GliOBLASTOMA BASED ON TUMOR INFILTRATING CELLS
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To discover novel biological targets in glioblastoma, genomic and immunological analysis were performed using The Cancer Genome Atlas (TCGA) data set. The RNA-seq data of 156 primary glioblastoma cases were subjected to CIBERSORT to detect tumor infiltrating cell fractions. Principal component analysis was performed on this data to detect factors that strongly contribute to the first principal component, and hierarchical clustering was performed. Survival curves were compared for each of the derived clusters. Finally, Gene Set Enrichment Analysis (GSEA) using Hallmark Gene Sets was performed. In the principal component analysis, we detected seven factors (NK cells resting, T cell regulatory, NK cells activated, Macrophage type 0, T cell gamma delta, Macrophage type 2, Macrophage type 1) which strongly contribute to the first principal component. Based on these seven factors, hierarchical cluster analysis resulted in 3 cell regulatory (Treg), Macrophage type 0 (M0), Macrophage type 2 (M2) and Macrophage type 1 (M1) clusters. There was no significant difference between these groups in CD8 T cell, M2 and M1 clusters displayed better OS with a significant difference. TGFs signaling via NFkβ in Treg group, IFNα response, IFNγ response and ALLOGRAFT response in M2 group. G2M CHECKPOINT, GLYCOSYLIS, Wntβ catenin signaling, MITOTIC SPINDLE and TGFβ signaling in M1 group were upregulated. In conclusion, tumor microenvironment of glioblastoma can be divided into 14 immunological subtypes, Treg, M0, M1, and M2. Because of the contribution of innate immunity for shaping the tumor microenvironment of glioblastoma, immunotherapies targeting these innate immune cells are anticipated.

BASIC OTHERS (BOT)

BOT-01 BLOOD-BRAIN BARRIER OPENING USING 220-KHZ TRANSCRANIAL MRI-GUIDED FOCUSED ULTRASOUND AND MICROBUBBLES IN MOUSE AND RAT
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OBJECTIVE: In neuro-oncology, it is believed that one major obstacle to effective chemotherapy is the high vascularity and heterogenous permeability of brain tumors. Focused ultrasound (FUS) exposure with the microbubbles has been shown to transiently open the blood-brain barrier (BBB) without depositing thermal energy, and thus may enhance the delivery of various therapeutic drugs into brain tumors. The aim of this study was to evaluate the BBB opening using 220-kHz transcranial MRI-guided FUS (TcMRgFUS) device and microbubbles in mouse and rat. METHODS: The experiments were performed with the 220-kHz ExAblate Neuro TcMRgFUS system (InSightec) and novel lipid bubbles (LB, Teikyo Univ.). Normal mouse and rat brains were irradiated with TcMRgFUS (output power, 5W; duration of irradiation, 30 s; duty cycle 100%) following intravenous injection of 6x10^10 LB per mouse and rat, respectively. On irradiation, target temperature rise & cavitation signal were monitored by MR thermometry and cavitation receiver, respectively. Immediately after irradiation, BBB opening and complications were detected based on T1, T2, T2*, and Gadolinium (Gd) enhanced T1-weighted images. RESULTS: The maximum temperature of brain tissue was under 42°C. There were no risky-cavitation signals causing hemorrhage. The FUS-LB exposure induced successful BBB opening effect in both mouse and rat, confirmed by Gd enhancement in the target region, lateral ventricles, and sulci. In addition, there were no complications such as edema, coagulation, and hemorrhage. CONCLUSIONS: Although there remain many conditions to be optimized, BBB opening using a 220-kHz TcMRgFUS device and LB can offer a non-invasive and feasible drug delivery for brain malignancies.

BOT-02 2-METHYLTHIO MODIFICATION OF N6-ISOPENTENYLADENOSINE IN MITOCHONDRIAL TRNAs BY CDK5RAP1 PROMOTES THE MAINTENANCE OF GLIOMA-INITIATING CELLS
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2-Methylthio-N'-isopentenyl modification of adenosine (m^2A) is an evolutionarily conserved modification that is found in mitochondrial (mt-) RNA of all eukaryotic species. m^2A is a regulatory-associated protein 1 (CDK5RAP1) specifically converts N6-isopentenyadenosine (i^6A) to m^2A at position A37 of four mt-RNA-encoded tRNAs, and the modification regulates efficient mitochondrial translation and energy metabolism in mammals. Here, we report that the m^2A conversion mediated by CDK5RAP1 in mt-RNAs is required to sustain glioma-initiating cell (GIC)-related traits. CDK5RAP1 maintained the self-renewal capacity, undifferentiated state, and tumorigenic potential of GICs. This regulation was not related to the translational control of m-protiens. CDK5RAP1 abrogated the antitumor effect of i^6A by converting i^6A to m^2A and protected GICs from excessive autophagy triggered by i^6A. The elevated activity of CDK5RAP1 contributed to the amelioration of the cytotoxic effect of i^6A and promoted GIC maintenance. The hyperactivation in the tumor core activated CDK5RAP1, whose activity was inversely correlated with the oxygen concentration because of two (4Fe-4S) clusters in the enzyme. This work demonstrates that CDK5RAP1 is crucial for the detoxification of endogeneous i^6A and that GICs readily utilize this mechanism for survival.

BOT-03 INVESTIGATION OF NOVEL SPRAY TYPE FLUORESCENT PROBE FOR GliOBLASTOMA DETECTION
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PURPOSE: 5-ALA is commonly used as an intraoperative tool in malignant glioma surgery, which has been proven effective for radical tumor resection and extended progression-free survival. However, there are some limitations in its use, such as false positivity, false negativity, and inability of re-administration. We aim to develop a novel fluorescent labeling system which can be repeatedly administered by spray during surgery, using hydroxymethyl rhodamine green (HMRG) as fluorescent scaffold originally designed at our university for cancer detection. METHODS: Primary probe screening was performed using the homogenized glioblastoma (GBM) samples with the fluorescent probe library comprised of more than 320 kinds of FRET fluorescent scaffold combined with various types of dipoptides. Second probe screening was performed using fresh GBM specimens and the selected probes in primary screening. To identify the responsible enzymes, diced electrophoresis gel (DEG) assay was performed. This method utilizes the combination of two dimensional electrophoresis (isolectric point and molecular weight) and a multiwell-plate-based fluorometric assay to find protein spots with the specified activities. RESULTS: The prominent probes were selected based upon the above two-step screenings. We identified two enzymes by proteome analysis and experiments using inhibitors, which was further confirmed with real-time PCR and western blotting. DISCUSSION: This screening methodology is innovative in that it is based on selecting probes from the probe library that respond to clinical samples rather than creating probes from the responsible enzymes. Practical fluorescent probes can be established even for low-grade gliomas, which would be a breakthrough for rapid intraoperative diagnosis in glioma surgery. CONCLUSION: HMRG-based aminopeptidase fluorescent probes may be effective for GBM detection.

BOT-04 POSSIBLE INVOLVEMENT OF CHLORIDE INTRACELLULAR CHANNEL PROTEIN 2 (CLIC2) IN THE SUPPRESSION OF INVASIVE ACTIVITY OF BRAIN TUMORS
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Chloride intracellular channel protein 2 (CLIC2) belongs to the CLIC family of conserved metazoan proteins. However, CLIC2 is the least studied among its family members, and its function remains to be elucidated. Recently, we have shown that CLIC2 is correlated with the development and...