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 immunotherapy-resistance in GBM. Combination therapy with anti-PD-L1 antibody and M2M&phi inhibitor could overcame 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 Set 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 T 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. TNFA signaling via NFκB in Treg group, IFNα response, IFNγ response and ALLOGRAFT response in M2 group, G2M CHECKPOINT, GLYCOLYSIS, WNTβ catenin signaling, MITOTIC SPINDLE and TGFβ signaling in M1 group were upregulated. In conclusion, tumor microenvironment of glioblastoma can be divided into 4 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 $6x10^7$ 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 sulcus. 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^6-isopentenyl modification of adenosine (ms^2i^6A) is an evolutionally conserved modification that is found in mitochondrial (mt)-tRNAs. Cdk5 regulatory subunit-associated protein 1 (CDK5RAP1) specifically converts N6-isopentenyladenosine (i^6A) to ms^2i^6A at position A37 of four mt-DNA-encoded tRNAs, and the modification regulates efficient mitochondrial translation and energy metabolism in mammals. Here, we report that the ms^2 conversion mediated by CDK5RAP1 in mt-tRNAs 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 mt-proteins. CDK5RAP1 abrogated the antitumor effect of i^6A by converting i^6A to ms^2i^6A 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 hypoxic microenvironment 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 endogenous 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 HMRG fluorescent scaffold combined with various types of dipeptides. 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 (isoelectric 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/