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/

or maintenance of tight junctions in blood vessel endothelial cells in human non-cancer tissues. CLIC2-expressing endothelial cells supposedly prevent hematogenous spread of cancer cells. In this study, we investigated CLIC2 expression in human brain tumor tissues and also addressed its function by employing human meningioma cells, rat glioma cells and rat malignant brain tumor model. Thirty-one meningioma cases, six SFT/HPC cases, twelve pituitary adenoma cases and twenty-three glioblastoma cases who underwent surgery at Ehime University Hospital were included. CLIC2 mRNA expression levels were investigated with immunoblotting and quantitative RT-PCR. Cells from the meningiomas were cultured and their CLIC2 expression was knockdown. Filter-based invasion assays and gelatin zymography were performed using the knocked-down meningioma cells. Rat C6 glioma cells stably expressing rat CLIC2 were established and transplanted into the right striatum of neonatal Wistar rats. Effects of CLIC2 on the survival periods of the animals were investigated. CLIC2 expression levels were high in the low-grade cases but low in the high-grade cases and highly invasive cases. Meningioma cells, of which CLIC2 expression was knocked-down, showed higher invasive activity than control cells. The CLIC2-knock down cells displayed increased activities of MMP-2 and MMP-9. Rat brain tumor models revealed that high expression of CLIC2 was correlated with smaller and less invasive brain tumors compared with those consisted of control cells. The rats transplanted with CLIC2-expressing cells survived longer periods than the rats with control C6 cells. These results suggest that CLIC2 plays a role in suppression of invasive activities of tumor cells.

ADULT CLINICAL TRIALS/THERAPEUTIC STUDIES (ACT)

ACT-01

THE SECOND GENERATION ANAPLASTIC LYMPHOMA KINASE (ALK) INHIBITOR CERITINIB EFFECTIVELY INDUCES CELL DEATH IN HUMAN GLIOBLASTOMA CELLS

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Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase which expresses only in the developmental stage of the brain during embryogenesis of human. On the other hand, a variety of ALK gene alterations, such as oncogenic fusion, activating point mutation, or wild type gene amplification, have been recently discovered as the powerful oncogene in various tumors, and these ALK mutations have also been known as the potential therapeutic targets against tumors harboring these ALK mutations. For example, ALK inhibitors have been already approved and used for the clinical treatment of non-small cell lung cancers harboring oncogenic ALK fusion.

Previously, we reported classical ALK inhibitors triggered cell death in human glioblastoma (GBM) cells, which did not express ALK, via suppression of transcription factor STAT3 activation but not in normal tissue-derived cells.

In this study, we investigated the anti-tumor effect of newly-developed ALK inhibitors in GBM cells. As a result, a second generation ALK inhibitor ceritinib induced cell death in various human GBM cell lines with lower concentration compared to other ALK inhibitors. Besides, ceritinib also suppressed STAT family activity in these GBM cell lines. From these results, we consider ceritinib might be a novel therapeutic agent against GBMs, and further investigation about the specific anti-tumor mechanism of ceritinib in GBM cells is currently on-going.

ACT-02

BORON NEUTRONS CAPTURE THERAPY FOR RECURRENT HIGH-GRADE MENINGIOMAS, FROM REACTOR TO ACCELERATOR

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INTRODUCTION: Recurrent high-grade meningiomas (rHGM) are difficult to control. We have applied tumor-selective particle radiation, reactor-based boron neutron capture therapy (BNCT) with excellent tumor control. METHODS: Forty-six recurrent and treatment-refractory high grade meningiomas were treated with reactor-based BNCT by Osaka Medical College (OMC) and Kyoto University Research Reactor team collaboratively until February 2019. Tumor shrinkage, overall survival (OS), progression free survival (PFS), Lesion/Normal (L/N) ratio in boronophenylalanine positron emission tomography (BPA-PET) and causes of treatment failures are analyzed. RESULTS: Subjects had been almost always treated heavily, high-risk patients for prognosis. They were received surgery 3 times and some radiotherapy 2 times averagely, prior to BNCT. All cases responded well and markedly shrunk by BNCT. The mean L/N ratio in BPA-PET was 4.0 which is higher than glioblastomas. Two-year PFS was 49.0% (95% CI: 28.84–66.49). Unfortunately follow-up was insufficient and 2 year OS was very similar to 2 year PFS. Treatment failures were observed as recurrence out of fields of neutron irradiation, systemic metastasis and in field local recurrence almost equally. SUMMARY AND PROSPECTS: Median PFS and OS of rHGM are 5 months and 2 years respectively in literatures. We achieved relatively favorable results by reactor-based BNCT. On the other hand, we performed accelerator-based BNCT clinical trial for recurrent glioblastomas steadily first in the world. Based on these backgrounds, we applied investigator-lead, clinical trial of accelerator-based BNCT for rHGM as RCT design. Government (PMDA and AMED) has approved our proposal. We start this trial with the primary endpoint as PFS, from August 2019. Treatment arm is BNCT and control one is best-supportive care. If the subjects in control arm show progress disease in follow-up, they can be treated by BNCT as rescue treatments. We will introduce details of this trial in our presentation.

ACT-05

PREDICTIVE FACTORS RELATING TO OUTCOME AFTER RESECTION OF LOW-GRADE GLIOMAS WITHOUT CHEMOTHERAPY OR RADIOTHERAPY

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BACKGROUND: There are several treatment options, including observation, after surgical removal of low-grade gliomas (LGG). If postoperative chemotherapy and/or radiotherapy are not provided, resection-alone approach will probably be alternative to a natural course of LGG under observation. The objective of this study was evaluation of prognostic factors associated with overall survival (OS) of patients with LGG treated with surgery alone. METHODS: A consecutive series of 236 adult patients who underwent surgery for LGG without adjuvant therapy was analyzed retrospectively. In 193 cases (82%) histopathology of the tumor was re-classified based on evaluation of IDH1 mutational status and 1p/19q co-deletion according to criteria of WHO classification 2016. Cox proportional hazards model was used for statistical analysis. RESULTS: Median extent of resection (EOR) was 95% (range, 1–100%) and in 210 cases (89%) EOR was >=90%. During postoperative follow-up tumor progression was noted in 106 patients, and 30 patients died of disease. Overall, 10-year OS rate was 82.0%. There was statistically significant difference (P <0.001) in OS among molecularly re-classified tumors, with 10-year OS rates of 90%, 79%, and 75% in cases of OD, DA IDH1-mutant, and DA IDH1-wild, respectively. In patients with EOR >=90% 10-year OS rate was 75%. Multivariate analysis revealed that only EOR >=90% (RR, 0.23; 95% CI, 0.09–0.66; P<0.007) and presence of 1p/19q co-deletion (RR, 0.41; 95% CI, 0.16–0.97; P = 0.042) are independently associated with OS. In patients with EOR >=90% such factors as type of disease manifestation, time interval between onset of symptoms and surgery, and molecular subtype of the tumor did not show significant associations with OS. CONCLUSION: Survival outcome in patients with LGG who underwent surgical resection alone may be predicted by EOR and presence of 1p/19q co-deletion. In cases with EOR >=90% molecular subtype of the neoplasm does not impact OS.

ACT-09

RETROSPECTIVE INVESTIGATION ABOUT STATUS AND RESULT OF ADMINISTRATION OF BEVACIZUMAB FOR MALIGNANT GLIOMAS IN THE REAL WORLD

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6 years have passed after approval of Bevacizumab for malignant gliomas in Japan, we analyzed the application and the results in our institution. Subjects were 56 patients who were histologically diagnosed as malignant gliomas. Bevacizumab was used in 41 patients among them. In 14 patients, Bevacizumab was introduced after initial therapy. The resection rates were below partial resection in 11 of the 14 patients. In 12 patients, administrations were finished and the average use was 7.6 times. The reason was PD in 6, and side effect in 4. Eight patients died, the average OS of those who died was 9.9 months, the average PFS after Bevacizumab was 5.4 months, and the average time from discontinuation to death was 2.1 months. In 27 patients used at the time of recurrence, the initial excision rate tended to be higher than in the former cases. In 22 patients the administrations were