degradation. The Ki-67/MIB-1 index decreased and the number of macrophages increased after chemotherapy. Moreover, the ratio of GSCs to total tumor cells increased after chemotherapy. GSCs and macrophages constitute the mechanism of resistance to and recurrence after alkylating agent chemotherapy in oligodendrogliomas.

Key words: 1p/19q | neoadjuvant chemotherapy | glioma stem cell

GENETICS/EPIGENETICS (GEN)

GEN-7

LIQUID BIOPSY IN BRAIN TUMOR PATIENTS -THE PRESENT AND FUTURE-

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We have previously published liquid biopsy for the diagnosis of brain tumors including PCNSL (JCO Precision Oncology, 2019; Leukemia and Lymphoma, 2019) and diffuse midline gliomas (DMG) (Diagnostics, 2021). We used the Maxwell RSC cfDNA extraction kit to extract circulating tumor DNA (ctDNA from) 1 milliliter of cerebrospinal fluid (CSF), and droplet digital PCR to detect MYD88 L265P mutations in PCNSL and H3F3A K27M mutations in DMG. From our initial experience, we were able to detect a high rate of MYD88 mutations in PCNSL, but not H3F3A mutations in DMG. We also observed that higher concentrations of ctDNA were obtained when prompt centrifugation and storage were done after obtaining CSF. Application of liquid biopsy to early detection of relapse and monitoring of treatment relapse are highly anticipated. In cases of PCNSL, we perform liquid biopsy when relapse is suspected on post-contrast MRI. However interestingly, the rate of MYD88 mutations detected is lower than that of newly-diagnosed cases. We would also like to share our experience performing liquid biopsy in conjunction with CSF cytology in brain tumor patients with evidence of leptomeningeal disease. From our initial experience, we would like to discuss the present limitations and future prospects of liquid biopsy in brain tumor patients.

Key words: Liquid biopsy | MYD88 | H3F3A K27M

GEN-8

REAL-TIME PCR BASED INTRAOPERATIVE GENETIC ANALYSIS FOR GLIOMAS

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An individual therapeutic strategy based on the genetic characterization is important in gliomas. However, it has been difficult to obtain genetic features during surgery. In this study, we present an overview of intraoperative genetic analysis using modified real-time PCR method. The tumor specimen was crushed with liquid nitrogen, then extract DNA within 60 minutes. Reagents of real-time PCR for detecting IDH, TERT, and BRAF hot spot mutations were stocked and real-time PCR was performed after mixing the extracted DNA. We used PNA and LNA to detect single nucleotide variant (SNV). The average time from tumor extraction to intraoperative tentative judgement was approximately 100 minutes. Using this system. we preliminary performed intraoperative genomic analysis in10 glioma patients. We confirmed that 8 of 10 cases (80%) of intraoperative genomic diagnosis were consistent with post-operative diagnosis by Sanger sequencing. However, we experienced 2 (20%) unmatched cases due to low allele of SNV, which indicates that more advanced system is required for clinical application.

Key words: glioma | Real-time PCR | intraoperative genomic analysis

EXPERIMENTAL THERAPEUTICS (ET)

ET-1

TRANSLATIONAL RESEARCH PLATFORM FOR MALIGNANT BRAIN TUMORS

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Introduction: The standard therapy for malignant brain tumors includes surgery and combination therapy with radiation and chemotherapy, but to provide individualized treatment based on the biological and molecular genetic background of the tumor, integrate genetic information with various functional data are required. In this study, we present an overview of our integrated approaches for translational research and clinical management. Methods: In glioma, pre-and intra-operative clinical information, including intraoperative genetic diagnosis, and intraoperative rapid immunohistochemistry is obtained, then a multidisciplinary treatment approach is started based on these integrated data. Specimens collected intraoperatively are cryopreserved for future analysis, and primary cultured cells are routinely collected. The cultured cells are transplanted into the brain of immunodeficient mice to establish patient-derived xenograft model (PDX). Genetic screening, such as IDH, TERT, BRAF, H3F3A mutation and MGMT methylation analysis are routinely assessed within a few days after surgery and used as information for integrated diagnosis. In case of PDX establishment or recurrence, we perform whole exon sequencing or comprehensive genomic assessment to identify genetic abnormalities. If genomic alterations for possible molecular targeted therapy are identified, we assess drug sensitivity test in vitro and in vivo, which are utilized for research to develop optimal molecular targeted therapy. The results, such as the therapeutic effects of molecular targeted drugs, are used for clinical applications. Results: Since the platform was established, we have treated a total of 286 patients, including 189 gliomas and 37 central nervous system lymphomas based on the integrated information. We are currently collecting clinical data to examine if this integrated approach could provide clinical benefit.Conclusion: The translational research system for malignant brain tumors plays an important role in the promotion of clinical and basic research.

Key words: translational research | brain tumors | research platform

ET-5

BIOLOGICAL EFFECTS OF SIMULTANEOUS USE OF MULTIPLE DRUGS IN NEUTRON CAPTURE THERAPY USING RAT BRAIN TUMOR MODEL

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The world's first clinical trial of boron neutron capture therapy (BNCT), which treats malignant brain tumors with a single dose of neutron irradiation using multiple boron drugs simultaneously, was performed at our institution, and its excellent results have stimulated BNCT research around the world. BNCT is a particle irradiation therapy that biologically targets cancer cells, and is expected to be a "new option for cancer treatment" because it can deliver a dose of radiation at the cellular level. In the case of BNCT using a combination of multiple drugs, a method to appropriately consider the biological effects of the combination in the dose calculation has not been established. At present, BNCT based on an accelerator-based irradiation system and a boron drug (BPA) based on essential amino acids has been approved by the regulatory approval for head and neck cancer and has shown good results in brain tumors. As basic research, we have continued to develop new boron drugs, which will be essential in the future, and have explored the interpretation of the biological effects of multiple boron drugs in combination and the optimal conditions required for drug development. The survival curve of BNCT in a rat brain tumor model showed that the effect of the new drug alone was comparable to that of BPA, and the effect of the combination was improved, but the effect of the combination did not match the prediction of the combined biological effect derived from each drug. However, it has been found that the effect of the combination does not match the prediction based on the combination of biological effects derived from each drug. In other words, even if the equivalent X-ray equivalent dose (Gy-Eq) is calculated, the combined effect of some drugs exceeds the prediction, while the combined effect of other drugs is poor.

Key words: glioma | neutron capture therapy | biological effectiveness

ET-6

GEMCITABINE RADIOSENSITIZATION PRIMES IRRADIATED MALIGNANT MENINGIOMA CELLS FOR SENOLYTIC ELIMINATION BY NAVITOCLAX

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BACKGROUND: Malignant meningioma is an aggressive tumor that requires adjuvant radiotherapy after surgery, yet there has been no standard systemic therapy established so far. We have demonstrated that malignant meningioma cells are exquisitely sensitive to gemcitabine due to their increased expression of hENT1 and dCK, which play critical roles in the intracellular transport and activation of gemcitabine, respectively (Takeda et al. Oncotarget 8:90996, 2017; Yamamoto et al., Neuro-Oncol 23:945, 2021). Significantly, in support of our findings, the efficacy and safety of gemcitabine have recently been documented in a small case series of patients with recurrent meningiomas, which has further led to a phase 2 clinical trial to evaluate the efficacy of gemcitabine in recurrent high-grade

meningiomas (Khaddar et al., South Asian J Cancer 9:261, 2020). Besides its efficacy as a single agent, gemcitabine reportedly has a radiosensitizing effect in pancreatic cancer. However, it remains unknown whether or how gemcitabine interacts with ionizing radiation (IR) in malignant meningioma cells. METHODS: We examined radiosensitization effects of gemcitabine using malignant meningioma cell lines and xenografts (s.c. and i.c.) and explored the underlying mechanisms. RESULTS: Gemcitabine sensitized malignant meningioma cells remarkably to IR through the induction of senescence both in vitro and in vivo. Gemcitabine augmented the intracellular production of reactive oxygen species (ROS) by IR, which, together with cell growth suppression/senescence induced by this combination, was inhibited by N-acetyl-cysteine, suggesting a pivotal role for ROS in these combinatorial effects. Navitoclax, a senolytic drug, further enhanced the effects of the combination of gemcitabine and IR in vitro and in vivo by strongly inducing apoptotic cell death in senescent cells. CONCLUSION: These results suggest that gemcitabine is not only a promising radiosensitizer for malignant meningioma but also creates in combination with IR a therapeutic vulnerability of senescent meningioma cells to senolytics. (submitted for publication)

Key words: meningioma | gemcitabine | senescence

ET-7

ROLES FOR HENT1 AND DCK IN GEMCITABINE SENSITIVITY AND MALIGNANCY OF MENINGIOMA

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Background: High-grade meningiomas are aggressive tumors with high morbidity and mortality rates that frequently recur even after surgery and adjuvant radiotherapy. However, limited information is currently available on the biology of these tumors, and no alternative adjuvant treatment options exist. Although we previously demonstrated that high-grade meningioma cells were highly sensitive to gemcitabine in vitro and in vivo, the underlying molecular mechanisms remain unknown. Methods: We examined the roles of hENT1 (human equilibrative nucleoside transporter 1) and dCK (deoxycytidine kinase) in the gemcitabine sensitivity and growth of meningioma cells in vitro. Tissue samples from meningiomas (26 WHO grade I and 21 WHO grade II/III meningiomas) were immunohistochemically analyzed for hENT1 and dCK as well as for Ki-67 as a marker of proliferative activity. Results: hENT1 and dCK, which play critical roles in the intracellular transport and activation of gemcitabine, respectively, were responsible for the high gemcitabine sensitivity of high-grade meningioma cells and were strongly expressed in high-grade meningiomas. hENT1 expression was required for the proliferation and survival of high-grade meningioma cells and dCK expression. Furthermore, high hENT1 and dCK expression levels correlated with stronger tumor cell proliferative activity and shorter survival in meningioma patients. Conclusions: The present results suggest that hENT1 is a key molecular factor influencing the growth capacity and gemcitabine sensitivity of meningioma cells and also that hENT1, together with dCK, may be a viable prognostic marker for meningioma patients as well as a predictive marker of their responses to gemcitabine.

Key words: meningioma | gemcitabine | hENT1

ET-8

INTEGRATED DIAGNOSTIC APPROACH TO PREDICT PROGNOSIS FOR MALIGNANT GLIOMAS

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Previous studies indicated that MGMT promoter methylation status with IDH and TERT promotor mutation are major prognostic factors in glioma. In addition to these molecular features, we have been assessing drug sensitivity against several chemotherapeutic agents, including temozolomide (TMZ). Here, we examined if this combined information could strongly predict drug sensitivity and the prognosis in glioma patients. One hundred and twenty-five IDH wild-type gliomas (WHO grade III and grade IV) were included in this study and retrospectively analyzed. Among them, we focused on 37 patients with partial surgical resection and biopsy to assess radiological difference on MRI. The primary cultured tumor cells were exposed with several compounds for 72 hours, then ATP based cell viability assay was performed. The favorable radiological therapeutic effect was found in 6 out of 8 (75%) with MGMT promoter methylated cases, while unfavorable in 23 of 29 (79.3%) with MGMT promoter unmethylated cases (p=0.008). The drug screening assay demonstrated that 7 of 10 cases with favorable TMZ sensitivity in vitro showed response on MRI, whereas 22 of 27 (81.5%) cases with TMZ resistance in vitro indicated tumor progression (p=0.006). Of note, all 5 cases with sensitive to TMZ and methylated MGMT promoter demonstrated favorable radiological response (p=0.002). These 5 cases tended to survive longer (median survival time, 697 days) as compared to others (median survival time, 391 days, p=0.13). These data indicate that integrated approach with genomic assessment and drug screening test may predict therapeutic response to chemotherapy and contribute selecting optimal therapy in glioma patients.

Key words: Prognostic prediction | Temozolomide | MGMT

ET-9

DEVELOPMENT OF PHOTOSENSITIVE ANTIBODIES FOR NEAR-INFRARED LIGHT IMMUNOTHERAPY TARGETING EGFR AND IL13RA2 OF MALIGNANT GLIOMAS AND INVESTIGATION OF THEIR PHOTODYNAMIC CYTOTOXIC ACTIVITY IN VITRO

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Introduction: Near-Infrared Photoimmunotherapy (NIR-PIT) is a recently developed hybrid cancer therapy based on photodynamic cytotoxicity and anti-tumor immunopotentiation, utilizing a photosensitive antibody drug (PSAD). A global Phase III trial of NIR-PIT with an anti-EGFR-PSAD in patients with recurrent head and neck squamous cell carcinoma (HNSCC) is already underway, and NIR-PIT is expected to have therapeutic applications also in malignant gliomas. Methods: In this study, monoclonal antibodies to EGFR and IL13Rα2 were conjugated to the photosensitive dye IRDye700DX (IR700) to produce PSADs (EGFR-Ab/IR700 and IL13Rα2-Ab/IR700) and in vitro PDT assays using these PSADs were performed on four human glioma cell lines (U87MG, U251, U138, A172).Five groups were studied: EGFR-Ab/IR700 monotherapy: 5 μg/ml or 10 μg/ml, IL13Rα2-Ab/IR700 monotherapy: 5 μg/ml or 10 μg/ml, and EGFR-Ab/IR700: 5 μg/ml + IL13Rα2-Ab/IR700: 5 μg/ml combination therapy. The cytotoxic activity of each group was compared after irradiation with 690 nm light at 16 J/cm2. Results: Significantly higher cytotoxic activity was observed in all four glioma cell lines when EGFR-Ab/IR700 and IL13Rα2-Ab/IR700 were used in combination at 5 μg/ml each, than when each PSAD was treated with a doubled dose (10 μg/ml).Conclusion: Malignant gliomas show extensive cellular heterogeneity with diverse expression of cell surface antigens. The present results suggest that a therapeutic strategy using several different photosensitive antibodies simultaneously may lead to the release of tumor antigens from a greater number of tumor cells, resulting in a more efficient host immune response for therapeutic purposes.

Key words: Photoimmunotherapy | EGFR | IL13Rα2

TUMOR BIOLOGY/MODELS (TB)

TB-2

PATIENT-DERIVED MENINGIOMA ORGANOID MODEL DEMONSTRATES *FOXM1* DEPENDENT TUMOR PROLIFERATION

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Recent comprehensive studies have revealed several molecular alterations that are frequently found in meningiomas. However, effective treatment reagents targeting specific molecular alterations have not yet been identified because of the limited number of representative research models of meningiomas.

We established 18 organoid models comprising of two malignant meningioma cells (HKBMM and IOMM-Lee), 10 benign meningiomas, four malignant meningiomas, and two solitary fibrous tumors (SFTs). Using immunohistochemistry and molecular analyses consisting of whole exome sequencing, RNA-seq, and DNA methylation analyses, we compared the histological findings and molecular profiling of organoid models with