those of parental tumors. The organoids exhibited consistent histological features and molecular profiles with those of the parental tumors. Using a public database of meningioma, we identified that upregulated *forkhead box M1* (*FOXM1*) was correlated with increased tumor proliferation. Overexpression of *FOXM1* in benign meningioma organoids increased organoid proliferation; depletion of *FOXM1* in malignant organoids decreased proliferation. Additionally, thiostrepton, a FOXM1 inhibitor combined with radiation therapy, significantly inhibited proliferation of malignant meningioma organoid models ($P<0.01$).

An organoid model for meningioma enabled us to elucidate the tumor biology of meningioma along with potent treatment targets for meningioma.

Key words: Meningioma | Organoid | *FOXM1*

TB-3

MIR-33A DEPLETION ACCELERATE MEDULLOBLASTOMA GENERATION AND INVASION

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Background and purposes: Lipid metabolism have been shown to be associated with tumorigenicity in various malignancies. The purpose of this study was to investigate the association of miR-33, a key regulator of lipid metabolism, in tumorigenicity and progression of medulloblastoma. Methods: Incidence of medulloblastoma and histopathological findings were compared between ptch1+/- mice and ptch1+/- miR-33a-/- mice. Tumors extracted from these mice were transplanted subcutaneously in nude mice (n=14 for ptch1+/-, n=19 for ptch1+/- miR-33a-/-) and in C57BL/6 mice (n=12 for each). Gene expression profile was compared between tumors from ptch1+/- mice and those from ptch1+/- miR-33a-/- mice. Results: Knockout of miR-33a in ptch1+/- transgenic mouse model increased the incidence of spontaneous generation of medulloblastoma from 34.5% to 84.0% ($p< 0.001$) at 12 months. Histopathological analysis showed infiltrative tumor borders in ptch1+/- miR-33a-/- tumors as compared with ptch1+/- ones. Tumor formation was observed in 21.4% for ptch1+/- tumors and 68.4% for ptch1+/- miR-33a-/- tumors in nude mice ($p= 0.008$). It was observed in 0% and 16.7% in immune competent mice. RNA sequencing detected that SCD1 and SREBF1 was upregulated in tumors from miR-33a knockout mice. Discussion: Our results demonstrated that depletion of miR-33a accelerated medulloblastoma generation and invasion. miR-33a may also be important for immune evasion. SCD1, which is reported to play a role in tumor stem cell maintenance and metastasis, can be a potential therapeutic target for medulloblastoma.

Key words: medulloblastoma | lipid metabolism | transcriptome

TB-4

ANTITUMOR EFFECTS OF A NOVEL CURCUMIN DERIVATIVE CURCUMIN MONOGLUCURONIDE ON GLIOBLASTOMA CELLS IN VITRO AND IN VIVO

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The therapeutic outcome of glioblastomas (GBMs) is still very poor. Therefore, invention of novel therapeutic methods against GBM cases is considered urgent. The antitumor effects of naturally-derived compounds are attracting attention recently, and therapeutic efficacy of curcumin, a plant-derived compound previously used for multiple purpose, has been indicated in many cancer systems; however, clinical application of curcumin is considered difficult because of its poor bioavailability (under 1 %). Curcumin monoglucuronide (CMG), a water-soluble prodrug of curcumin recently developed for overcoming this weakness, has been demonstrated excellent antitumor effects for several malignancies in vitro and in vivo; therefore, we investigated the effects of CMG against GBM cells. CMG induced cell death of human GBM cells lines (T98G, U251MG, and U87MG) by dose dependent manner by triggering multiple forms of cell death such as apoptosis and perthanatos. Immunoblotting of CMG-treated GBM cell lysates demonstrated activation of multiple cell death signaling. Furthermore, immunodeficiency mice harboring intracerebral U87MG cell xenografts systemically treated by CMG showed significantly prolonged survival compared with control mice. These results suggest CMG would be a novel therapeutic agent against GBM cases.

Key words: Glioblastoma | Curcumin monoglucuronide | Therapeutic model

TB-6

EXPERIMENTAL EVALUATION OF THE THERAPEUTIC POTENTIAL OF BORON NEUTRON CAPTURE THERAPY IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background: High-dose methotrexate and whole brain radiation therapy (WBRT) is the recommended treatment for primary central nervous system lymphoma (PCNSL). Although the initial treatment is successful, the recurrence rate is high and the prognosis is poor. Boron neutron capture therapy (BNCT) is a nuclear reaction-based tumor cell-selective particle irradiation that occurs when non-radioactive boron-10 is irradiated with neutrons to produce α particles (10B [n, α] 7Li). In this study, we conducted a basic research to explore the possibility of BNCT as a treatment option for PCNSL. Methods: Cellular uptake of boron using human lymphoma cell-lines after exposure to boronophenylalanine (BPA) were evaluated. The cytotoxicity of lymphoma cells by photon irradiation or neutron irradiation with BPA were also evaluated. The lymphoma cells were implanted into the mouse brain and the bio-distribution of boron after administration of BPA were measured. In neutron irradiation studies, the therapeutic effect of BNCT on mouse CNSL models were evaluated in terms of survival time. Results: The boron concentration in lymphoma cells after BPA exposure was sufficiently high, and lymphoma cells showed cytotoxicity by photon irradiation, and also by BNCT. In in vivo bio-distribution study, lymphoma cells showed enough uptake of BPA with well contrasted to the brain. In the neutron irradiation experiment, the BNCT group showed a significant prolongation in their survival time compared to the control group. Conclusions: In our study, BNCT showed its effectiveness for PCNSL in a mouse brain tumor model. PCNSL is a radio-sensitive tumor with a extremely good response rate, but it also has a high recurrence rate / a high rate of adverse events, so there is no effective treatment for recurrence after treatment. Our translational study showed that BNCT is possibly have an important role against PCNSL during the therapy lines as a new treatment option for PCNSL patients.

Key words: boron neutron capture therapy (BNCT) | primary central nervous system lymphoma (PCNSL) | radiation therapy

TB-8

GENETIC AND MOLECULAR PROPERTIES OF LONG-TERM PROLIFERATING TUMORSPHERE -FORMING GLIOMA DERIVED CELLS

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Long-term proliferating tumorsphere-forming glioma derived cells (LTP-TS-GDCs) and patient derived xenografts (PDXs) are essential tools for translational research for glioma. However, only small subsets of glioma

samples are established as LTP-TS and/or PDXs and little is known about the genetics and molecular properties of LTP-TS -forming GDCs and PDX. In this study, we aim to analyze the characteristics of LTP-TS -forming GDCs and PDXs. We tried primary sphere cultures from 56 glioma patient-derived samples and established 11 LTP-TS-GDCs out of 45 glioblastoma samples and no long-term sphere culture was isolated from grade3 and grade 2 gliomas. LTP-TS-GDCs had self-renewal ability and possessed certain multipotency. However, they significantly less expressed SOX1 FOXG1 and TUBB3, whereas they expressed LGALS1 and EN1 significantly higher than normal neural stem/progenitor cells. In addition, we found that LTP-TS-GDCs shared the same genetic profiles with original patients' tumors. Furthermore, we investigated the genetic differences between the glioma tissues which were successfully established as LTP-TS-GDCs and those which were not. We found that glioma tissues with TERT promotor mutations and triple copy number alteration (CNA) [EGFR, CDKN2A, and PTEN loci] are significantly established as LTP-TS-GDCs. Lastly, we next investigated in vivo characteristics of glioma PDXs. We have injected glioma PDXs lines into immunodeficient mice brains and histopathologically analyzed the characteristics of xenografts. Each xenograft well recapitulated histological features of original patients' tumors and tumor cells remarkably invade through subventricular zone. In conclusion, each LTP-TS-GDCs and PDXs had various gene expression profiles, reflecting intratumoral and interpatient heterogeneities of glioma. In addition, TERT promotor mutations and triple CNA significantly correlated with success rate of LTP-TS-GDCs. These findings will be of use and advance the preclinical and translational researches of glioma.

Key words: glioma | long-term proliferating tumorsphere-forming glioma derived cells | patient derived xenografts

TB-9

AN ATTEMPT TO ESTABLISH A PATIENT-DERIVED BRAIN TUMOR CULTURE MODEL BY ORGANOID CULTURE METHOD

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Background: Molecular heterogeneity among and within tumors are one of the reasons for the poor survival rate of brain tumors even with the current standard therapy. However, monolayer culture and neuro-sphere culture (NS) use exogenous growth factors, so may not show the true nature of the tumor. And the culture establishment rate is low, especially low-grade tumors. Therefore, we used the glioblastoma organoid (GBO) culture method showed by Fadi to create culture models of various brain tumors and investigated their characteristics. Methods: We examined the establishment rate in pathological and genotypic types of 56 patients who underwent brain tumor resection at our hospital between January 2020 and June 2021 and were cultured with GBO or NS. If tumor cells are increased visually at 1 month after culture, we defined establishment. Results: There were 15 cases of glioblastoma, 7 cases of anaplastic astrocytoma, 7 cases of diffuse astrocytoma, 3 cases of diffuse midline glioma, 2 cases of anaplastic oligodendroglioma, 5 cases of oligodendroglioma, and 16 cases of others. The establishment rate was 76.5% by the GBO method and 40% by the N S method. By histological type, GBO: 80% in glioblastoma, NS: 58.3% in glioblastoma, GBO: 83.3% in AA, NS: 40% in AA, and GBO: 100% in DA. The IDH mutation and pTERT mutation were investigated in GBO: IDHwt/TERT+ 87.5%, IDHwt/TERT- 64.3%, IDHmt/TERT- 100%, and in NS: IDHwt/TERT+ 75%, IDHwt/TERT- 33.3%, IDHmt/ TERT- 20% in NS. In addition, establishment was observed in GBO 2 case in medulloblastoma, 1 case in ependymoma. Discussion and Conclusion: This suggest that GBO can be used to establish culture models for low-grade tumors. In addition, GBO can establish culture earlier, so it is expected to be applicable to personalized therapies such as preclinical drug efficacy studies tailored to individual patients.

Key words: organoid | culture model | brain tumor

IMMUNOLOGY (IM)

IM-2

POSSIBILITY OF IMMUNOTHERAPY FOR THE GLIOBLASTOMA PATIENTS WITH O6-METHYL-GUANINE DNA METHYLTRANSFERASE (MGMT) EXPRESSION OR PROMOTER UNMETHYLATED

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Background: MGMT is a DNA repair protein that removes the cytotoxic O6-methylguanine (O6MG) DNA lesions generated by TMZ; thereby, MGMT expression is mechanistically linked to TMZ resistance. However, thus far, there is no effective treatment for these patients with MGMT promoter unmethylated. Therefore, a new treatment for GBM patients with MGMT expression is urgently needed. To this end, we examined the tumor microenvironment in GBM with or without MGMT expression. Methods: Based on The Cancer Genome Atlas (TCGA) primary GBM cohort, the tumor-infiltrating lymphocytes (TILs) expression level was calculated using the CIBERSORTx algorithms and the single-sample Gene Set Enrichment Analysis (ssGSEA) method. Furthermore, the differential expression gene analysis was conducted and pathway analysis was performed using Ingenuity Pathway Analysis (IPA). The results were validated using the GBM cohort from the Chinese Glioma Genome Atlas (CGGA) database. In addition, TILs were isolated from 13 surgically removed primary GBM tumors in our institution. Their responses to autologous tumors were evaluated by IFNγ ELISA. Results: T cells CD8 score by CIBERSORTx was significantly higher in the MGMT-high tumor. Similarly, ssGSEA scores for activated CD8 T cell, Macrophage, activated B cell, and Type 1 T helper cell were significantly higher in the MGMT-high tumor. Conversely, T cells CD4 naive was significantly higher in the MGMT-low tumor. Consistently, tumor-reactive TILs were detected in the MGMT-high tumor. Pathway analysis showed that Rictor was highly enriched in the MGMT-high tumor and Rictor inhibited lymphocyte activation. Coclusion: In this study, we demonstrated that macrophage was highly activated in the MGMT-high tumors. Thus, CSF-1R inhibitor can be combined with immunotherapy in these MGMT-high tumors to enhance anti-tumor immune responses. In addition, TMZ + mTOR2 inhibitors + PD-1 inhibitors may be effective against the MGMT-L group.

Key words: MGMT | CD8+T cell | tumor microenvironment

IM-4

IMPACT OF OHSV ACTIVATED NOTCH SIGNALING IN TUMOR MICROENVIRONMENT AND ITS IMPACT ON ANTI-TUMOR IMMUNITY

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Oncolytic herpes simplex virus-1 (oHSV) is novel FDA-approved immunotherapy for advanced melanoma patients in US. Also, oHSV is recently approved for the treatment of recurrent GBM in Japan. We have shown that oHSV treatment of GBM cells induces NICD cleavage and NOTCH activation in adjacent uninfected glioma cells via HSV-1 microRNA-H16 (Otani Y and Yoo JY, Clin Cancer Res, 2020), however, the consequences of NOTCH on immunotherapy in GBM is unknow. Here we have investigated the impact of oHSV-induced NOTCH signaling on the tumor microenvironment (TME). Analysis of TCGA GBM data and experimental murine models revealed NOTCH induced immunosuppressive myeloid cell recruitment and limited anti-tumor immunity. In oHSV treated tissue, viral infection educated tumor associated macrophages to secrete CCL2 which recruited monocytic myeloid derived suppressor cell (MDSC) that attenuated anti-tumor immunity. Consistent with this, CCL2 induction was also observed in serum of recurrent GBM patients treated with oHSV (NCT03152318). Importantly, blockade of NOTCH signaling reduced the oHSV induced immunosuppressive environment and activated a CD8 dependent anti-tumor memory response. These findings present the opportunities for combination therapies that can help improve therapeutic benefit and anti-tumor immunity in GBM.

Key words: Immunotherapy | Oncolytic HSV | Tumor microenvironment

IM-6

HVJ-E CONTAINING PD-L1 SIRNA INHIBITS IMMUNOSUPPRESSIVE ACTIVITIES AND ELICITS ANTITUMOR IMMUNE RESPONSES IN GLIOMA

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Inactivated Sendai virus particle, hemagglutinating virus of Japan-envelope (HVJ-E), is a non-replicating virus-derived vector, in which the genomic RNA of Sendai virus (HVJ) has been destroyed. HVJ-E is a promising vector that enables the highly efficient and safe introduction of enclosed molecules such as RNA into target cells. Moreover, HVJ-E provokes robust antitumoral immunity by activating natural killer (NK) cells and CD8+ T lymphocytes and their induction into the tumor periphery, and by suppressing regulatory T lymphocytes (Treg) locally in the tumor. In the present study, we investigated a novel combination of antitumor immunotherapy