by the antitumor immune-activating effect of HVJ-E itself with the inhibition of tumor PD-L1 molecule expression. We confirmed that intratumoral injection of HVJ-E containing siRNA targeting PD-L1 (siPDL1/HVJ-E) inhibited tumor PD-L1 protein expression in a mouse subcutaneous tumor model using TS, a mouse glioma stem-like cell. We conducted treatment experiments in the mouse brain tumor model in three groups: control group (PBS), siNC/HVJ-E group (negative control siRNA + HVJ-E), and siPDL1/HVJ-E group. We obtained a significant prolongation of overall survival in the siPDL1/HVJ-E group. Flow cytometric analyses of brain tumor models showed that the proportions of brain-infiltrating CD8+ T lymphocytes and NK cells were significantly increased after giving siPDL1/HVJ-E; in contrast, the rate of Treg/CD4+ lymphocytes was significantly decreased in HVJ-E-treated tumors (siNC/HVJ-E and siPDL1/HVJ-E). No difference was observed in the proportions of macrophages or M2 macrophages. CD8 depletion abrogated the therapeutic effect of siPDL1/HVJ-E, indicating that CD8+ T lymphocytes mainly mediated this therapeutic effect. We believe that this non-replicating immunovirotherapy may be a novel therapeutic alternative to treat patients with glioblastoma. The full article has been published (Cancer Science. 2021 Jan;112(1):81–90).

Key words: regulatory T lymphocyte | malignant glioma | PD-L1

IM-7

IDENTIFICATION OF NOVEL GLIOBLASTOMA SPECIFIC ANTIGEN USING PATIENT DERIVED TUMOR CELL FOR CAR-T CELL THERAPY

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Chimeric antigen receptor T (CAR-T) cell therapy is a newly developed antitumor immunotherapy presenting remarkable clinical response with leukemia, and is expected to be applied to other malignant solid tumors including glioblastoma (GBM). However, for development of CAR-T therapy against GBM, identification of novel and suitable tumor specific antigen is required to expect higher therapeutic efficacy. Herein, we developed our original method to detect novel GBM specific antigen using patient derived GBM (PD-GBM) cells. First, BALB/c mice were immunized by footpad injection of PD-GBM cells. B cells were extracted from lymph nodes of the mice, fused with murine myeloma cells, and then cultured to produce monoclonal antibodies for GBM cells. About 500 GBM binding monoclonal antibody lines were established, and then each antibody was again analyzed by flow cytometry with multiple PD-GBM cells and human non-tumor brain cells to find out GBM specific antibodies. Consequently, two GBM specific antibody lines were selected and genetically analyzed to identify the recognized antigen. CAR-T cells targeting the detected antigens were successfully generated, and the cytotoxicity against GBM cells was confirmed by chromium releasing assay and bioluminescent cytokine assay. Remarkably, one of the identified tumor specific antigens proved to be B7-H3, which is known pan-cancer antigen expected to be one CAR-T therapeutic target for malignant solid tumors, also expressed in most GBM cells. This result confirms that our experimental method using murine antigen-antibody reaction is feasible for detecting antigen as a novel CAR-T therapeutic target for GBM. Moreover, this method can also detect antigens derived from post-translational conformational changes such as glycosylation, which might have been overlooked by conventional methods. In addition, these results suggest our method using PD-GBM cells can identify potential targets of CAR-T therapy for each GBM patients respectively, thus leading to precision immunotherapy for GBM.

Key words: CAR T cell therapy | neurosphere | glioblastoma

IM-8

SIGNIFICANCE OF IL-1 PATHWAYS IN GLIOBLASTOMA

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Purpose: Previous studies have revealed that macrophages affect the prognosis of glioblastoma. However, there are still many unknown parts about the mechanism. In this study, we conducted an experiment with the aim of elucidating the mechanism by which tumor associated macrophages (TAM) work on tumors in the tumor microenvironment (TME). Method: Experiments were carried out using two glioblastoma cell strains, T98G, and U251. For clinical data, we analyzed it based on databases such as Protein Atlas, Ivy Glioblastoma Atlas, brain TIME database. Results: In 3D culture, we confirmed that IL-1β stimulation promoted glioblastoma cell proliferation and sphere formation. The addition of IL-1β increased mRNA expression of various cytokines such as IL-6 and CXCL8, and increased phosphorylation of STAT3 in arrays. When we administered IL-6 and CXCL8, the growth was significantly increased in cells administered with IL-6 and CXCL8. As a result, we speculated that STAT3 pathway and NFκB pathway via IL-6 and CXCL8 are involved in cell proliferation by IL-1β. In order to confirm these things, western blot was performed, and it was confirmed that phosphorylation of STAT3 and NFκB were increased. In addition, STAT3 inhibitors and NFκB inhibitors suppressed tumor growth. Clinically analysis was carried out based on the database, and it was found that IL-1β and macrophages were related. Furthermore, IL-1β was found in many cases around tumor necrosis. Discussion: This study clarifies some of the effects of IL-1β on glioblastoma. However, there are still many unknown points, and it is necessary to continue to consider them in the future.

Key words: Glioblastoma | Macrophage | IL-1β

BASIC OTHERS (BOT)

BOT-3

PROGNOSTIC FACTORS OF CNS GERM CELL TUMORS; MOLECULAR AND HISTOPATHOLOGICAL ANALYSES ON 154 CASES FROM THE IGCT CONSORTIUM

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Background: Germ cell tumors (GCTs) preferentially occurs in pediatric and young adult age groups. Chemo- and radiation therapies cause long-term sequelae in their later lives. We searched for clinical and histopathological features to predict the prognosis and affect treatment response, with a future goal of treatment stratification.Methods: A total of 154 GCT cases were included in the analysis. Total of 114 germinoma cases underwent measurement of tumor cell content on H-E specimen, and 82 GCT cases underwent 450K methylation analysis. 12p gain was determined on methylation-based copy number computation and FISH. Association with progression-free and overall survival (PFS/OS) was investigated. Results: The tumor cell content was widely distributed from <5% to 90% in the specimens, with a median value of 50%. Patients with a higher tumor cell content (>=50%) showed shorter PFS than those with a lower tumor cell content (<50 %) (p=0.03). In the multivariate analysis with tumor location, tumor cell content was the sole statistically significant prognostic factor (p=0.04). 12p gain was found in 25-out-of-82 cases (30%) and was more frequent in NGGCTs, particularly in cases with malignant components. The presence of 12p gain correlated with shorter PFS and OS, even with histology and tumor markers incorporated in the multivariate analysis. Among NGGCTs, 12p gain still had prognostic significance for PFS and OS. The 12p copy number status was shared among histological components in mixed GCTs. Whole-genome amplification was suggested by FISH.Conclusions: We found that tumor cell content significantly affected the prognosis of germinomas. 12p gain predicts the presence of malignant components of NGGCTs, and poor prognosis of the patients. Furthermore, 12p is likely to be an early event in the tumorigenesis of CNS GCT. These potentially open the possibility of leveraging these pathological and molecular factors in the future clinical trials when stratifying the treatment intensity.

Key words: Germ cell tumor | Tumor cell content | 12p gain

BOT-5

CHRYSANTHEMUM MORIFOLIUM EXTRACT IMPROVES DOXORUBICIN-INDUCED CARDIOMYOPATHY BY SUPPRESSING APOPTOSIS IN MOUSE HEART

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Background: Doxorubicin is widely used for the treatment of various malignant tumors. However, doxorubicin causes cumulative and dose-dependent cardiotoxicity, ranging from occult changes in myocardial structure and function to severe cardiomyopathy and congestive heart failure. Since this problem affects the QOL and survival of cancer patients, solutions for this problem are urgently needed. Recently, it has been reported that Chrysanthemum morifolium extracts (CME) have antioxidant and anti-inflammatory activities. The purpose of this study is to clarify whether CME decreases doxorubicin-induced cardiotoxicity and prevents the development of heart failure. Methods and Results: H9C2 cardiomyoblast cells were treated with CME (0.3, 1 mg/mL) for 2 hours and then stimulated with doxorubicin. After 24 hours incubation, surviving cells were evaluated by MTT assay. CME dose-dependently decreased doxorubicin-induced cardiotoxicity in H9C2 cells. Western blotting showed that CME significantly suppressed doxorubicin-induced increases in four markers of apoptosis: p53, phosphorylated p53, and cleaved caspase-9 and -3. Next, to investigate the effects of CME on doxorubicin-induced cardiomyopathy in vivo, C57BL6 mice were orally administered with CME (400 mg/kg/day) or vehicle daily from 2 days before doxorubicin treatment and then treated once intraperitoneally with doxorubicin (20 mg/kg). The survival ratio of the CME-treated group was significantly higher than that of the vehicle-treated group. Echocardiographic analysis at 7 days after doxorubicin stimulation revealed that CME had significantly improved doxorubicin-induced left ventricular systolic dysfunction. Apoptotic cells in mouse heart tissue were detected by TUNEL assay, which showed that CME significantly suppressed doxorubicin-induced apoptosis. Discussion: These results indicate that CME decreases doxorubicin-induced cardiotoxicity both in vitro and in vivo, suggesting that CME might possess the therapeutic potency to reduce doxorubicin-induced cardiotoxicity in cancer patients. Further studies are required to assess the effectiveness of CME for preventing doxorubicin-induced heart failure in clinical settings.

Key words: apoptosis | cardiomyopathy | doxorubicin

ADULT CLINICAL TRIALS/THERAPEUTIC STUDIES (ACT)

ACT-1

MULTICENTER INVESTIGATOR-INITIATED REGISTRATION-DIRECTED PHASE 2 STUDY OF E7090 IN SUBJECTS WITH ADVANCED OR RECURRENT SOLID TUMORS WITH FIBROBLAST GROWTH FACTOR RECEPTOR (FGFR) GENE ALTERATION: FORTUNE TRIAL

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Background: Genetic alterations of FGFRs are known to play an important role in the proliferation, survival, and migration of cancer cells as well as tumor angiogenesis and drug resistance. E7090 is an orally available selective tyrosine kinase inhibitor for FGFR1-3. A global Phase 2 study of E7090 in subjects with unresectable advanced or metastatic cholangiocarcinoma harboring FGFR2 gene fusion is ongoing (NCT04238715). We recently reported FGFR alterations that are highly sensitive to E7090 using a high-throughput functional evaluation method called MANO method (Nakamura et al. npj Precision Oncology, 2021), narrowing down the most promising FGFR alteration targets. Here, we designed a single-arm, open-label, investigator-initiated multicenter Phase 2 basket study to evaluate the efficacy and safety of E7090 in subjects with advanced or recurrent solid tumors harboring FGFR gene alterations, focusing on alterations identified by MANO method, as a sub-study under the nationwide large registry for rare cancers in Japan (MASTER KEY Project). Methods: The key eligibility criteria are: 1) Histologically confirmed metastatic or locally advanced solid tumor; 2) Ineffective to or intolerant to first line treatment, or for which standard treatment is no longer available; and 3) Confirmed FGFR gene alterations via next-generation sequencing assays that are reimbursed by insurance. Subjects will receive E7090 140 mg orally once daily until disease progression or development of unacceptable toxicity. The primary endpoint is objective response rate (ORR) by independent central review (RECIST v1.1), and the secondary endpoints include ORR by investigator assessment, progression-free survival, overall survival, disease control rate, safety, duration of response, and time to response. For primary brain tumors, RANO criteria is also applied in assessment of response. The study enrolls approximately 45 subjects. (Clinical Trial Registry: jRCT2031210043, ClinicalTrials.gov: NCT04962867)

Key words: FGFR | clinical trial | E7090

ACT-3

REACTOR-BASED BORON NEUTRON CAPTURE THERAPY WITH ADD-ON BEVACIZUMAB FOR RECURRENT MALIGNANT GLIOMA: THE FINAL REPORT

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Background: Re-irradiation had a higher rate of radiation injury because recurrent MG had already irradiated in the first-line treatment. Recently, combination therapy of re-irradiation and bevacizumab showed a lower incidence of radiation injury than re-irradiation alone. Boron neutron capture therapy (BNCT), a tumor-selective particle radiation therapy, also increased radiation injury for recurrent MG, despite the greater focus on tumor cells. In this study, we evaluated the efficacy of BNCT plus bevacizumab with early induction after BNCT. Methods: Patients with recurrent MG were prospectively enrolled in this study. BNCT was performed using Kyoto University Research Reactor as a neutron source. Bevacizumab of 10 mg/kg was initiated 1–4 weeks after BNCT and was