

TUMOR BIOLOGY/MODELS (TB)

TB-02

COMPREHENSIVE ANALYSIS OF EXPANDABLE BENIGN PITUITARY ADENOMAS WITHOUT GENETIC MANIPULATIONS

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BACKGROUND: Since most pituitary adenomas are benign, there is no model of pituitary adenomas that can be continuously cultured without genetic manipulation. In this study, we analyzed the genetic characteristics of the established cell lines by continuous culture of benign pituitary adenomas without genetic manipulation. **METHOD:** The pituitary adenoma was continuously cultured by modifying the conditional reprogramming (CR) method, and the morphology was observed and fluorescent immunostaining (IF) was performed. In addition, genetic characteristics were comprehensively analyzed, differences from the surgical samples were analyzed by enrichment analysis using GSEA, and network analysis was performed using cytoscape based on the protein-protein interaction database. (Results) 1) Of the 14 cases of pituitary adenoma that had undergone primary culture (median MIB-1 labeling index 1.5%), 12 cases were capable of continuous culture for more than 2 months. (6 months or more: 5 cases) 2) After 2 weeks of culturing, the cell morphology changed dramatically. These cultured cells were positive for follicular cell marker s100, ectodermal nervous system markers Nestin, Notch1, and mesodermal marker Vimentin. In these cells, synaptophysin, which was strongly positive in the surgical specimen collected at the same time as the culture, disappeared. 3) Enrichment analysis revealed that gene expression such as epithelial mesenchymal transition (EMT), angiogenesis, IL6 signaling via NFkB, extracellular degradation, integrin cell surface interaction increased in cultured cells, and gene expression such as neurotransmitter including synaptophysin decreased significantly. (Conclusion) 1) The modified CR method allowed continuous culture of pituitary adenoma cells. 2) The cultured cells showed characteristic change of EMT, which was seen in invasive pituitary adenoma.

TB-03

NEWLY ESTABLISHED MENINGIOMA ORGANOID MODEL ELUCIDATED AN IMPORTANT ROLE OF *FOXM1* IN MENINGIOMA PROGRESSION

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Meningioma is the most frequently occurring intracranial neoplasms in adults. Tumor removal surgery and radiotherapy were the widely accepted standard treatment for meningioma. Most meningioma cases were cured by extended total removal. However, some tumors develop in locations less amenable to resection, resulting in tumor recurrence after incomplete tumor removal followed by radiotherapy. Although several comprehensive studies have revealed frequently found molecular alterations of meningiomas, effective treatment reagents targeting specific molecular alterations have not been identified yet because of limited number of representative research models such as tumor cell lines or animal models of meningiomas. Recently developed 3D culture technologies have led to the development of novel cancer models, termed organoid models, due to their quite high efficiency of establishment. In this study, we established primary organoid culture methods using malignant meningioma cell lines (e.g. HKBMM and IOMM-Lee) and patient-derived meningioma tissues. Using this novel method, we have been able to establish six organoid models (four WHO grade I meningiomas, one WHO grade III one and one solitary fibrous tumor (SFT)) using tumor tissues derived from six consecutive patients with 100% success rate. Histological analyses, whole exome sequencing and copy number analyses revealed that these organoids exhibited consistent histological features and molecular profiling with those of parental tumors. Using public database, we identified upregulated *FOXM1* was correlated with increased tumor proliferation. Over-expression of *FOXM1* in benign meningioma organoids increased organoid proliferation, while depletion of *FOXM1* in malignant ones decreased their proliferation. We revealed that novel organoid model for meningioma enable to shed light on the tumor biology of meningioma.

TB-06

ERIBULIN PROLONGS SURVIVAL IN AN ORTHOTOPIC XENOGRAFT MOUSE MODEL OF MALIGNANT MENINGIOMA

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Meningioma is the most common intracranial tumor, and its prognosis is typically favorable. However, patients of malignant meningioma (WHO grade III) most often experience recurrence, undergo multiple surgical treatments, and have poor prognosis. No effective therapy for malignant meningioma has been established yet. We recently reported an efficacy of eribulin (Haraven®) for glioblastoma. Eribulin is considered to target TERT, which is frequently mutated in its promoter. Since TERT promoter mutation is also found in malignant meningioma, this study aims at investigating the anti-tumor effect of eribulin against TERT promoter mutation-harboring human malignant meningioma cell lines in vitro and in vivo.

Two meningioma cell lines IOMM-Lee and HKBMM were used in this study. In the viability assay and the flow cytometry, eribulin strongly inhibited cell proliferation by cell cycle arrest. Apoptotic cell death in malignant meningioma cell lines was confirmed by vital dye assay and immunoblotting. Moreover, wound healing assay revealed the suppression of tumor cell migration after eribulin exposure. To assess the effect of eribulin in vivo, orthotopic xenograft mouse models of both malignant meningioma cell lines were constructed. The intraperitoneal administration of eribulin significantly prolonged the survival of meningioma cell lines implanted in the brain ($p < 0.0001$). Furthermore, apoptosis was histologically observed in brain tumor tissue by immunohistochemistry.

Thus, this study suggests that eribulin is a potential therapeutic agent for treating malignant meningioma.

IMMUNOLOGY (IM)

IM-03

CD206 EXPRESSION IN PERIPHERAL BLOOD-DERIVED INDUCED-MICROGLIA-LIKE CELLS AS A SURROGATE BIOMARKER FOR THE SPECIFIC IMMUNE MICROENVIRONMENT OF GLIOMA

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INTRODUCTION: As recent advancement of multimodal treatments including immune-check point inhibitors have not led to massive outcome improvement of glioma. Targeting the peculiar immune microenvironment of glioma is a promising approach to innovate a breakthrough treatment, however, there remains to be technical and ethical burdens for monitoring the bioactivities of immune cells in neural tissues. Herein, we examined the feasibility of non-invasive monitoring of glioma-associated microglia/macrophages (GAM) properties through utilization of our originally developed induced-microglia-like (iMG) cells technique. **METHODS:** We isolated primary microglia (pMG) from surgically-obtained brain tissues of 15 patients with neurosurgical diseases. We induced iMG cells from monocyte extracted from their corresponding peripheral blood by MACS treatment. Expression profiles of representative markers for an M1 and M2 microglia phenotype were analyzed in both pMG and iMG cells by qPCR. **RESULTS:** q-PCR revealed that a significant correlation of expression level of microglial markers between pMG and the corresponding iMG in each patient. Synchronous upregulations of CD206 were exclusively detected in patients with glioma. **CONCLUSION:** The present study suggested that iMG cells can be a less-invasive monitor tool for disease-related bioactivity of microglia, thereby seem to be utilized as an intermedium for investigation of relationship between microglia and neuronal diseases including glioma. CD206 upregulation detected by iMG technique can surrogate the specific microenvironment of glioma surrounding tissues and might be utilized as a future biomarker of glioma

ADULT CLINICAL TRIALS/THERAPEUTIC STUDIES

(ACT)

ACT-02

CHANGES IN RECURRENCE PATTERN AND PROGNOSIS OF GLIOBLASTOMA AFTER APPROVAL OF BEVACIZUMAB AS FIRST-LINE APPLICATION

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INTRODUCTION: There exist controversies on recurrence and aggressiveness after use of first-line bevacizumab (BEV) which has been approved in Japan and proven to be beneficial. Therefore, we analyzed the clinical impact of BEV approval by investigating the overall clinical course and glioblastoma (GBM) relapse pattern.

METHODS: We included 100 patients with IDH-wildtype GBM between September 2006 and February 2018 from our institution. They were subdivided into pre-BEV (n=51) and post-BEV (n=49) groups. Overall, progression-free, deterioration-free, and post-progression survivals (OS, PFS, DFS, and PPS, respectively) were compared. We analyzed the relapse pattern of 72 patients, whose radiographic progressions were confirmed.

RESULTS: Significant improvements in DFS (median DFS in the pre-BEV and post-BEV eras: 8.5 and 13.8 months, $P=0.0046$), and PFS (7.5 and 9.9 months, $P=0.0153$) after BEV approval were observed. These survival prolongations were strongly correlated ($r: 0.91, P<0.0001$). Non-enhancing tumor emerged as a novel recurrence pattern in the post-BEV era (five of 33; 15.2%). Changes in relapse pattern did not significantly impact OS, PFS, and DFS. No significant difference in PPS between pre-BEV and post-BEV eras was observed (6.7 and 5.5 months, $P=0.2319$). The rate of early (within 6 months) focal recurrence was significantly lower ($P=0.0155$) in the post-BEV era (four of 33; 12.1%) than in the pre-BEV era (18 of 39; 46.2%). A significant decrease in early focal recurrence after BEV approval was observed exclusively in patients with unresectable tumors ($P=0.0110$). Treatment era was the only parameter significantly correlated with decreased early focal recurrence rate ($P=0.0021$, univariate analysis; $P=0.0144$, multivariate analysis).

CONCLUSIONS: We found that, first-line BEV in Japan for unresectable tumors has a positive impact on the prevention of early progression and clinical deterioration of GBM without accelerating the clinical course after recurrence.

ACT-03

CLINICAL OUTCOME AND RADIOLOGICAL FINDINGS OF PATIENTS WITH RECURRENT GLIOBLASTOMAS TREATED BY BEVACIZUMAB

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OBJECT: Seven years have passed since the approval of bevacizumab (BEV) in Japan. We retrospectively reviewed the clinical outcome and radiological findings of patients with recurrent glioblastomas (GB) treated by BEV. **METHOD:** We reviewed 116 patients, including 27 cases of newly diagnosed GB and 89 cases of recurrent GB, treated by BEV during the study period between 2013 June and 2019 September. Cumulatively, 116 patients received 1672 cycles of BEV. Among those, we focused on 74 patients with newly diagnosed GB treated by BEV at recurrence to examine clinical characteristics, outcome, and radiological findings of T2-circumscribed or double-positive proposed by Nowosielski et al. or Bahr et al., respectively. **RESULT:** The study cohort comprised median age of 66.8 years (range 10 to 81), median KPS of 60% (range, 20 to 100), median cycles of administration 11 (1 to 59), median period of treatment 172 days (0 to 1413), median post-BEV survivals 266 days, and overall survival 693 days. Patients without progressive disease at 6 months post-BEV MRI (n = 23) presented favorable post-BEV survival of 713 days than those with progressive disease (n = 8) ($p=0.0003$). The radiological findings varied by patients, tumor lesions, and sequential imaging; thus, it was difficult to correlate with survival. Our data implied that the T2-circumscribed lesion was accompanied by no enhancement at T1 but hyperperfused at arterial spin labeling imaging, indicating that blood-brain barriers were intact and vascularization is activated. **CONCLUSION:** Although our cohort included patients with relatively high age, some had prolonged post-BEV survival. T2-circumscribed or double-positive was not useful to predict the survival; however, MRI at 6 months post-BEV can be an indicator for two years of post-BEV survival.

ACT-05

PRESENT AND FUTURE OF PRECISION-BASED MEDICINE USING CANCER GENOME PANELS

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BACKGROUND: Two cancer genome panels were approved for use in Japan in 2019, and their application in brain tumors are awaited. We have used CANCERPLEXR and FoundationOne CDx (F1) panels for the realiza-

tion of precision-based medicine in brain tumors. Patients and **METHODS:** From August 2017 to present, we have applied cancer genome panels in 11 times to tumors in 9 patients. We assessed patient data including age, sex, pathology, reason for using the panel and actionability. **RESULTS:** The range of age of 4 to 69 years (mean 45.2 years), and 5 men and 4 women were studied. Pathological diagnosis was epithelioid glioblastoma (GBM), giant cell GBM, anaplastic ependymoma, anaplastic meningioma, anaplastic large cell type lymphoma, meningeal melanomatosis, enterogenous carcinoma, choroid plexus carcinoma and pineoblastoma. CANCERPLEXR was performed 7 times and F1 panel 4 times and the reasons included confirmation specific gene mutations such as BRAF V600E and TP53, young (pediatric) age and patient request. In one patient, by analyzing primary and recurrent tissue, we were able to assess genetic hits involved in malignant transformation. Actionable targets were found in 4 (44%) of cases, and action was taken in only 1 epithelioid GBM patient with BRAF V600E mutation, albeit with dramatic response (Kanamaru et al., Acta Neuropathol Commun, 2019). All tumors were microsatellite stable. **CONCLUSIONS:** We were able to understand tumor biology in rare brain tumors using 2 genome panels. We need to increase the percentage of patients actually treated. I will also like to touch briefly on how use genome panels for translational research on brain tumors.

ACT-07

CLINICAL TRIALS OF 11C-METHIONINE PET FOR BRAIN TUMORS

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BACKGROUND: Although 11C-Methionine (MET) PET has widely used, 11C-MET tracer has not been approved in Japan. We conducted multi-center prospective clinical trials using MET for drug approval in diagnosis of brain tumors. **Methods** Two trials using 11C-MET PET were performed in Hokkaido University, Osaka University and Fukushima Medical University; 1) Diagnostic accuracy in differentiating tumor recurrence from radiation injury after radiotherapy in brain tumors, 2) The diagnostic efficacy in newly-diagnosed gliomas. 1) The patients with suspected brain tumor recurrence underwent MET and 18F-Fluorodeoxyglucose (FDG) PET. When the target lesion showed MET and/or FDG uptake, the patients underwent target resection for pathological confirmation. Positive prediction values of each tracer uptake were assessed as primary outcome measure, and the sensitivities and specificities of each PET exams were also assessed. 2) The patients with suspected gliomas underwent MET PET. Tissue samplings were performed from MET uptake lesions without contrast-enhancement on MRI in each patient, and evaluated the existence of tumor cells. Diagnostic additional values of MET PET on contrast-enhanced MRI was also investigated. Safety of MET PET was also assessed in each trial. **Result** 1) 57 cases were investigated. 38 cases underwent surgery and 32 cases (84%) were confirmed tumor recurrence histopathologically. MET and FDG uptake in 32 recurrence cases were 100% and 50%, respectively. Sensitivities and specificities of tumor recurrence were 84% and 89% in MET, and 100% and 56% in FDG. 2) 53 glioma cases were enrolled. Viable tumor cells were proven in 98% in MET uptake lesion without contrast-enhancement. In 42 out of 53 cases (78%), MET PET depicted tumor area beyond the contrast-enhancement area on MRI. No severe adverse events were observed in both trials. **Conclusions** MET PET were effective in diagnosis of brain tumors, and safety of MET was demonstrated.

ACT-17

PROTOCOL DESIGN OF A MATRIX-TYPE OF NOVEL CLINICAL TRIAL FOR LOWER-GRADE GLIOMAS

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INTRODUCTION: Differentiation between glioma grade 2 and 3 was performed based on histological findings. The current grade is an important prognostic factor due to its widespread use, economic efficiency, and data accumulation, but analog elements remain and the genetic marker is unknown. The concept of Lower-grade glioma including G2/3 is spreading. On the other hand, WHO grade is the criteria of clinical trials, and evidence is established for G2 with low risk and high risk, G3 alone or with G4. In Japan, JCOG 1303 and 1016 have been implemented for high-risk G2 and G3, respectively and will be finished next year. Therefore, we examined the feasibility and design of novel clinical trial for patients with grade 2/3 glioma.