and U87MG) and primary human glioma stem cell line (MGG23). Glioma stem-like cells were cultured and isolated by neurosphere method from U251MG and U87MG. PRR antibody was made targeting the extracellular domain of the PRR with rat lymph node method. WST-1 assay or MTT assay were performed to determine the cell proliferation. Apoptosis was examined by FITC labeled annexin V and propidium iodide with flow cytometry. We analyzed molecules of Wnt signaling and stem cell markers with qRT-PCR. RESULTS: We observed that PRR antibody significantly reduced cell proliferation, decreased sphere formation. Antibody suppressed cell adherent in stem-like cell. Flow cytometry showed that antibody induced apoptosis. Antibody inhibited Wnt signaling and stem cell markers. CONCLUSIONS: PRR antibody reduced cell proliferation and induced apoptosis through Wnt signaling. PRR antibody also suppressed stemness. Our results demonstrated that PRR was a potential target for future glioma therapy.

ET-05

PRECLINICAL STUDY OF AN ANTI-HUMAN TISSUE FACTOR ANTIBODY-DRUG CONJUGATE IN A MALIGNANT GLIOMA XENOGRAFT MODEL

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Whereas macromolecules such as antibody hardly extravasate from normal blood vessels compared with low molecular agents, macromolecules leak from tumor vasculature because of the enhanced permeability. As a result, macromolecules selectively accumulate at tumor sites. We apply this phenomenon, known as the enhanced permeability and retention effect (EPR effect), to drug delivery for cancer therapy. Drug delivery system (DDS) based on the EPR effect is called the passive targeting. On the other hand, DDS based on antigen-antibody or ligand-receptor interaction is the active targeting. Antibody-drug conjugate (ADC), antibody conjugated with antitumor agents, retains both of the passive and active targeting functions. Tissue factor (TF), an initiator in the extrinsic pathway of blood coagulation, is overexpressed in various cancers including malignant glioma. To target the molecule in tumor sites, we have produced several anti-TF monoclonal antibodies. Previously, we evaluated tumor accumulation of an indium-111-labeled anti-TF antibody in an orthotopic glioma xenograft model with high expression of TF by single photon emission computed tomography/computed tomography (SPECT/CT). The imaging study showed that anti-TF antibody significantly accumulated in the tumor compared with control antibody ($P < 0.01$). The finding suggests that blood-brain barrier in brain tumors is broken and antibodies accumulate in tumors by utilizing both of the passive and active targeting. In this study, to prepare anti-TF ADC, we conjugated monomethyl auristatin E (MMAE), a microtubule inhibitor, to humanized anti-TF antibody. The anti-TF ADC recognized TF expressed in glioma cells and showed potent cytocidal activity against human glioma cell lines depending on TF expression. In addition, we evaluated in vivo antitumor effect of the ADC in a mouse model subcutaneously inoculated with TF-overexpressing glioma cells. Anti-TF ADC showed a significant higher antitumor effect compared with control ADC ($P = 0.015$).

ET-09

ACQUIRED MALIGNANT BEHAVIORS OF NPE6-PDT-SURVIVED GLIOBLASTOMA CELLS ARE SUPPRESSED BY USING MEK1/2 INHIBITOR TRAMETINIB

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INTRODUCTION: In this study, we tried to investigate alteration of oncogenic properties and their molecular regulatory mechanism of talaporfin sodium (NPe6)-mediated photodynamic therapy (NPe6-PDT)-survived glioblastoma (GBM) cells. METHODS: As the in-vitro NPe6-PDT model, human GBM cell lines (T98G, U87MG, U343), and patient derived GBM stem cells (GSY03, GSC23, MGG152) were pretreated with 0-30ug/ml NPe6 for 4 hours followed by laser irradiation (wave length 664 nm, laser-power 33 mW/cm2, total amount of irradiation 10 J/cm2) using a semiconductor laser irradiator (Panasonic Healthcare Co., Ltd., Tokyo, Japan). Cell death after PDT was evaluated by vital dye exclusion assay using Hoechst3342 and propidium iodide or CellTiter-Glo. Survived cells after NPe6-PDT (PDT-R cells) were repropagated, and alteration of intracellular molecular signaling or migration/invasion capability were analyzed by immunoblotting or Boyden chamber assay. RESULTS: In both human GBM cell lines and patient derived GBM cells, cellular viability after NPe6-PDT was decreased with dose-dependent manner of pretreated NPe6. PDT-R cells showed not only resistance against NPe6-PDT-induced cell death but also higher invasiveness and migration capability compared with pre-PDT treated cells (PDT-Con cells), and immunoblot analysis demonstrated upregulation of ERK1/2 phosphorylation in PDT-R cells in comparison with PDT-Con cells. Furthermore, these acquired malignant behavior of PDT-R cells were repressed by concomitant use of MEK1/2 inhibitor Trametinib with NPe6-PDT. CONCLUSION: We discovered PDT-R cells demonstrated higher malignant phenotypes via ERK1/2-dependent machinery compared with parent pre-PDT-treated cells. It was also suggested concomitant treatment with MEK1/2 inhibitor during PDT therapy in GBM cases would contribute to better outcome.

ET-11

ANALYSIS OF ANTI-GLIOMA EFFECT BY PROINFLAMMATORY CYTOKINES

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OBJECT: Antiglioma activity of proinflammatory cytokines, (TNF-alpha, IL-2, IL-12 related cytokines, IL-18, IL-32) are analyzed. Most effective combinations of cytokines are investigated. MATERIAL & METHOD: Antitumor activity against U373MG, U87MG were measured by co-culture with PBMC and by nude mouse subcutaneous transplantation model. Cytokine receptors on PBMC and glioma cell lines were examined by IHC and mRNA expression. Anti-tumor activity was measured by local injection and systemic administration of proinflammatory cytokines. Cell cycle alteration and expression of apoptosis-related genes after cytokine administration was analyzed. Serum concentraion of cytokines is measured by ELISA. RESULT: Cytokine receptors were not expressed on glioma cells but were present on intratmoral mononuclear cells. Anti-tumor activity against transplanted tumor is strongly observed by focal administration. Expression of apoptosis-related genes were augmented. IFN-gamma was strongly induced by TNF-alpha, IL-2 and IL-12 administration. IFN-gamma, IL-17, TNF-alpha were also induced. IL-27 and IL-32 per se did not induce IFN-gamma. Simultaneous IL-27 and IL-12 induced strong IFN-gamma induction. Anti-glioma activity of IL-12 and IL-23 were higher than the same dose of exogenous IFN-gamma. IFN-gamma, IL-2 plus IL-12 in U373MG, and IFN-gamma, IL-2 plus IL-18 in U87MG seemed to be the best combination. CONCLUSIONS: Strong anti-glioma activity was induced by proinflammatory cytokines at least partially through IFN-gamma. There may be another factors. IL-2 and IL-23 showed anti-tumor activity through IFN-gamma, IL-17, TNF-alpha. IFN-gamma + IL-2 + IL-12/-18 seems to be the best combination.

ET-12

ANTI-VEGF THERAPY WITH KETOGENIC DIET AGAINST GLIOBLASTOMA IN MOUSE MODEL

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NTRODUCTUION: Malignant glioma cells critically depend on glucose as the main energy source to survive and sustain their aggressive properties. The ketogenic diet (KD) has been proposed as a complementary therapy for treatment of malignant gliomas. VEGF inhibitor (bevacizumab) decreases blood supply to tumor and clinically used for glioblastoma treatment. Therefore, we examined anti-tumor effect of the combination of bevacizumab (Bev) and KD using mouse model. METHODS: U87MG cells were implanted into the right brain of nude mice. One week after the implantation, mice were randomized into four treatment groups: control group, KD group, Bev group, and combination (K+B) group. KetoCal 4:1 was administered to the mice for KD. Bev (10mg/kg) was injected from tail vein twice a week. Metabolic and histological analysis of the tumor, and survival analysis of the mice were performed. RESULTS: 3-hydroxy-butyrate, one of the ketone bodies, was significantly increased in the tumor of KD group, however, the metabolic enzymes of ketone bodies were not found an increased expression in immunostaining experiments. Principal component analysis (PCA) analysis demonstrated distinct clustering or a clear separation of the four groups. In K+B group, several TCA cycle-related enzymes (succinate dehydrogenase (SDH), fumarate-hydratase (FH)) were decreased, suggesting a repression of TCA cycle. In addition, several amino acids (tyrosine, valine, alanine, glutamic acid) were decreased in K+B tumor, however, alpha-ketoglutarate was significantly increased, suggesting dynamic metabolic remodeling. Histologically, Ki-67 index was most decreased in the K+B tumor among four groups. In survival analysis, Bev group had significant longer survival than control group ($p=0.0016$), and the K+B group had most longer survival time among four groups.

CONCLUSIONS: Drastic metabolic remodeling in the tumor occurred in the combination of Bev and KD This combination may be potentially useful for glioblastoma therapy.