GENETICS/EPIGENETICS (GEN)

GEN-03

GENOTYPE-PHENOTYPE CORRELATION IN 111 FAMILIES OF VON HIPPEL-LINDAU DISEASE IN JAPAN

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BACKGROUND AND AIM: von Hippel-Lindau (VHL) disease is a hereditary disease which manifest central nervous system (CNS) hemangioblastoma, retinal angioma, renal cell carcinoma (RCC), phaeochromocytoma, endolympathic sac tumor, and pancreas cyst. The VHL gene is located at 3p25 and is corresponding to 213 amino acids. Genetic-phenotype correlation analyses of VHL disease have been recently reported from several foreign countries, but the geno-type-phenotype correlation has not been characterized since above 10 years ago. Therefore, this study aimed to evaluate the VHL mutation spectrum and genotype-phenotype correlations in Japanese VHL patients. METHODS: Blood samples of 111 unrelated families of VHL disease were collected and DNAs were extracted. Direct sequencing and real-time PCR analysis were performed. Consequently, the clinical manifestations and family histories of the subjects were evaluated. RESULTS: We identified VHL mutations as follows: missense 47; deletion 17; insertion 5; nonsense 8; splice-site 9; larger deletion 25. At hot-spot codon 167, 4 missense mutations were identified, with Arg167Thr, 4 cases; Arg167Gln2, 2 cases. At codon 155, splice-site mutations were identified at 6 cases. Mutation sites were distributed in exon 1, 45; exon 2, 21; exon 3, 36. Large deletions were distributed in exon 1 & 2, 1; exon 2 & 3, 1; all exons, 11. Genotype-phenotype correlation analysis revealed that age-specific risk and number of CNS hemangioblastoma were significantly higher in subjects carrying missense mutation within HIF-α binding site or missense mutation ( P < 0.05). In addition, penetrance of RCC was significantly higher in subjects carrying non-missense mutation ( P < 0.05). CONCLUSIONS: The results of this study were similar to the previous foreign studies. This study provides insight into the genotype-phenotype correlation in that amino acids substitutions in the HIF-α binding and non-sense mutations may predispose VHL patients to age-related risk and number of CNS hemangioblastoma.

GEN-06

CLINICAL COURSE AFTER TUMOR RECURRENCE OF MGMT HYPERMETHYLATED GBM: A PROSPECTIVE LONGITUDINAL STUDY

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MGMT methylation in glioblastoma is a biomarker for determining treatment responsiveness and predicting prognosis. We analyzed whether there were differences in the prognosis between glioblastoma with MGMT hypermethylation and other glioblastomas after tumor recurrence. We enrolled 184 patients who underwent radiation therapy and temozolomide chemotherapy after tumor resection for newly diagnosed glioblastoma. MGMT methylation was quantitatively analyzed using methylation-specific high resolution melting analysis. The cut-off value for MGMT methylation had a difference of 35% from the previous values. The subjects were split into three groups according to their MGMT methylation levels, 122 in the low (L) methylation group (levels of 0-34%), 40 in the medium (M) methylation group (levels of 35-69%), and 22 in the high (H) methylation group (levels of 70% or more). We mainly focused on and compared the progression after recurrence. The progression-free survival (PFS) rate and overall survival (OS) rate were significantly longer in the M and H groups than in the L group. There was no difference in PFS between group M and group H, but OS was significantly longer in group H. The details of treatment for the 184 patients who underwent only supportive care survived for a relatively longer period of time. Biologically, MGMT hypermethylation may be associated with a moderately slow-growing tumor.

GEN-13

FIREWORK PATTERN OF CANCER GENESIS FOR Glioblastoma, IDH-wildtype

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Treatment of Glioblastoma (GBM), IDH-wildtype is a history of sequential failure. The cure for this disease is a distant story, and it is really the emperor of cancer. We believe that GBM should not be considered one of several cancers, and GBM is a cancer that occurs in a special environment called the central nervous system (CNS). What is the biggest difference between other cancers and GBM is correlated with the environment. It is thought to be neurogenesis. This presentation will review GBM genesis based on neurogenesis of CNS. It also explains what the cell of origin is, what somatic mutations occur at the cell of origin, and why these somatic mutations occur. Human glioblastoma (GBM) occurs in a place without tumor or normal tissue, that is, in the subventricular zone and have introduced the name of the firework pattern of cancer genesis, which is a metaphorical representation of the GBM genesis. So far, we have been trying to develop therapeutics focused on bulk tumors. However, in the case of GBM, IDH-wildtype, it has been found that the cell of origin is not in the tumor but is in normal SVZ, so it is now considered that the therapeutic target should also include the cell of origin.

Key words: glioblastoma, firework pattern, cell of origin

EXPERIMENTAL THERAPEUTICS (ET)

ET-02

EFFICACY OF THE SALVAGE THERAPY VIA LOmustine AND nimustine FOR RECURRENT Glioblastoma WITH TEMOZOLOMIDE Resistance

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Temozolomide (TMZ) is widely used as a part of the standard treatment of glioblastomas (GBMs). However, GBM often acquires resistance for TMZ after continuous treatment with TMZ and recures as TMZ resistance GBM (TMZ-R GBM). Other alkylating agents such as lomustine (CCNU) or nimustine (ACNU) have been sometimes used as a salvage therapy for those TMZ-R GBMs, however, their efficacy against TMZ-R GBMs has not been thoroughly investigated yet. In this study, we investigated anti-tumor effects of CCNU and ACNU for TMZ-R GBM cell lines in vitro to examine whether these agents could become alternative to TMZ in the therapy of TMZ-R GBMs. TMZ resistant clones of human GBM cell lines U87 MG (U87-R cells) and U251 MG (U251-R cells) were established as the TMZ-R GBM cell lines by cultivating U87 MG cells and U251 MG cells under continuous TMZ treatment for at least 1 year. Induction of growth arrest and apoptosis by TMZ, CCNU or ACNU against these cells were analyzed by dye exclusion assay, vital dye staining assay, and immunoblotting. The results showed that growth arrest and apoptosis were triggered upon these cells under administration of each drugs. As expected, the anti-tumor effects of TMZ for U87-R cells or U251-R cells were significantly reduced compared with those for parental U87 MG cells or U251 MG cells, respectively. On the other hand, CCNU and ACNU showed similar growth suppressive effect upon U87-R cells or U251-R cells as compared with U87 MG cells or U251 MG cells. Throughout these experiments, CCNU demonstrated strongest anti-tumor effects for all cell lines, both parental and TMZ-R GBM cell lines, and ACNU also demonstrated stronger effects than TMZ. These results suggest that CCNU or ACNU may serve as a drug of choice for salvage treatment of TMZ-R GBM.
and U87MG) and primary human glioma stem cell line (MGG23). Glioma stem-like cells were cultured and isolated by neural sphere method from U251MG and U87MG. PRK antibody was made targeting the extracellular domain of the PRK 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 RT-PCR. RESULTS: We observed that PRK 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. Col-Cl suction of PRK reduced cell proliferation and induced apoptosis through Wnt signaling. PRK antibody also suppressed migration. Our results demonstrated that PRK was a potential target for future glioma therapy.

ET-05 PRECLINICAL STUDY OF AN ANTI-HUMAN TISSUE FACTOR ANTIBODY-DRUG CONJUGATE IN A MALIGNANT GIOMA XENOGRAFT MODEL Tatsuya Tomiyama, Shun Manabe,atsu Tsuji, Tsuneo Saga, Ryu Tsumura1, Takahiro Anzai1, Yoshiyuki Koga1, Masahiro Yasunaga1, Jun-Ichi Kuroda, Akita Mukuasa, Yasuhiro Matsumura1; 1Division of Developmental Therapeutics, Exploratory Oncology Research & Clinical Trial Center, National Cancer Center, Kashiwa, Japan

Whereas macromolecules such as antibody hardly extravasate from normal blood vessels compared with low molecular agents, macromolecules leak from the blood vessels because of the increased permeability. To target 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 of the extrinsic pathway of blood coagulation, is overexpressed in various types of malignant tumors. 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 antitumor efficacy 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 Tatsuya Kobayashi1, Yoshihiro Muragaki, Masamichi Takahashi, Kojiro Wada, Kentaro Mori, Takakazu Kawamata, Koschi Ichimura1, Arata Tomiyama1; 1Division of Brain Tumor Translational Research, National Cancer Center Research Institute, Tokyo, Japan

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 line (T98G, U87MG, U87-MG), and patient derived GBM stem cells (G5Y10, G5C2, 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 cell exclusion assay using propidium iodide or CellTiter-Glo. Survived cells after NPe6-PDT (PDT-R cells) were repopulated, and alteration of intracellular molecular signaling or migration/ invasion capability were analyzed by immunoblotting or Boyden chamber assay. RESULTS: In human GBM cell line, 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 MEK1/2-dependent mechanism 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-GIOMA EFFECT BY PROINFLAMMATORY CYTOKINES KOJI ADACHI1, Fumio Yamaguchi, Tadashi Higuchi, Hiroshi Takahashi, Akio Morita; 1Department of Neurosurgery, Musashi-Kosugi Hospital, Nippon Medical School, Kawasaki, Japan

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 U873MG, 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 concentration of cytokines is measured by ELISA. RESULT: Cytokine receptors were not expressed on glioma cells but were present on intratumoral 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 U873MG, 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 Takashi Sasaayama1, Masahiro Maeyama, Kazuhiro Tanaka1, Yuschi Fujita1, Misuzu Hashiguchi1, Yoshikazu Iino, Eiji Kohmura1; 1Department of Neurosurgery, Kobe University

INTRODUCTION: Malignant glioma cells critically depend on glucose as the main energy source to survive and sustain their aggressor 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. 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 in 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 group 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.