GEN-9

WHOLE GENOME SEQUENCING ANALYSIS REVEALS STRUCTURAL VARIANTS CONTRIBUTE TO TUMOR EVOLUTION IN IDH-MUTANT GLIOMA

Yusuke Funakoshi[1,2], Takuma Nakashima[1,3], Atsuhito Uneda[1], Shohei Nambu[4], Shota Tanaka[4], Joji Ishida[5], Ryuta Saito[3], Ryosuke Hanaya[6], Koji Yoshimoto[2], Yoshitaka Narita[7], Hiromichi Suzuki[1]; [1]Division of Brain Tumor Translational Research, National Cancer Center Research Institute, Tokyo, Japan, [2]Department of Neurosurgery, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan, [3]Department of Neurosurgery, Nagoya University Graduate School of Medicine, Nagoya, Japan, [4]Department of Neurosurgery, The University of Tokyo, Tokyo, Japan, [5]Department of Neurosurgery, Okayama University Graduate School of Medicine, Okayama, Japan, [6]Department of Neurosurgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan, [7]Department of Neurosurgery and Neuro-Oncology, National Cancer Center Hospital, Tokyo, Japan

Introduction: Recent large-scale sequencing projects for IDH-mutant adult-type diffuse glioma (Astrocytoma, IDH-mutant and Oligodendroglioma, IDH-mutant and 1p/19q-codeleted) have revealed the genetic landscape of coding mutations. However, little is yet known about the non-coding regions and structural variants (SVs). We analyzed deep (> x120) whole-genome sequencing (WGS) to delineate the comprehensive genetic lesions of IDH-mutant gliomas. **Methods:** We investigated WGS of 228 cases with IDH-mutant glioma (204 cases in our cohort along with 24 publically available cases). **Results:** The median tumor mutational burden in astrocytoma and oligodendroglioma was 2.0/Mb and 1.7/Mb, respectively. The median number of SV per case was 15.0 and 4.5, respectively. SV was involved in known driver genes in 7.7% of cases, supporting a model in which accumulation of SV as well as mutation drives tumor initiation and/or progression. The distribution of SV is biased on each chromosome, suggesting that each chromosome has a distinct susceptibility for SV. In IDH-mutant astrocytoma, complex SVs are significantly enriched on chromosome 12 which frequently involves *CDK4*, suggesting that SVs could lead to tumor evolution. The numbers of SVs and single nucleotide variants (SNVs) per case are correlated, presuming that IDH-mutant glioma can progress by acquiring both SNV and SV in a time-dependent manner. **Conclusion:** SV could contribute to the development of IDH-mutant gliomas as well as mutations. Since WGS has a great resolution for genetic alterations, further analysis would enable us to uncover glioma pathogenesis.

GEN-10

WHOLE GENOME LANDSCAPE OF GLIOBLASTOMA, IDH-WILD TYPE

Takuma Nakashima[1,2], Yusuke Funakoshi[1,3], Atsuhito Uneda[1], Shohei Nambu[4], Shota Tanaka[4], Joji Ishida[5], Ryuta Saito[2], Ryosuke Hanaya[6], Koji Yoshimoto[3], Yoshitaka Narita[7], Hiromichi Suzuki[1]; [1]Division of Brain Tumor Translational Research, National Cancer Center Research Institute, Tokyo, Japan, [2]Department of Neurosurgery, Nagoya University Graduate School of Medicine, Nagoya, Japan, [3]Department of Neurosurgery, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan, [4]Department of Neurosurgery, The University of Tokyo, Tokyo, Japan, [5]Department of Neurosurgery, Okayama University Graduate School of Medicine, Okayama, Japan, [6]Department of Neurosurgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan, [7]Department of Neurosurgery and Neuro-Oncology, National Cancer Center Hospital, Tokyo, Japan

Introduction: Glioblastoma, IDH-wild type (GBM) is the most common malignant brain tumor with a dismal prognosis. Although its coding region is well-analyzed, little is yet known about the landscape of whole-genome in GBM. Here, we analyzed whole-genome sequencing data from large cohorts to completely uncover the genetic aberrations in GBM. Methods: We analyzed 281 whole-genome sequencing data of patients with GBM, of which 152 cases are from our cohort with deep coverage (×120) and 129 cases are from a public database. Results: The median numbers of somatic mutations and structural variants (SVs) per case are 3.0/Mb and 62.5, respectively. While a complex SV is rare in other malignant brain tumors such as IDH-mutant glioma (35% of samples), a large proportion of GBM cases (85%) have complex SV with ≥10 breakpoints. *CDKN2A/B* homozygous deletions (HDs) are usually comprised of a simple deletion in IDH-mutant glioma whereas about a quarter of *CDKN2A/B* HDs in GBM are induced by complex SVs. In addition, 30.5% of extrachromosomal DNA (ecDNA) involves multiple chromosomes. Taken together, complex SVs could play a key role in the initiation and progression of GBM. Our deep WGS enables us to delineate a fine view of clonal architecture, where mutational signature varies between clonal and subclonal mutations. The majority of clonal mutations consist of the clock-like signature, whereas subclonal mutations have a relatively low proportion of the clock-like signature. Instead, several other signatures including the APOBEC signature significantly increase in subclones, presuming that various mutational processes along with the clock-like signature contribute to the GBM pathogenesis in its progression phase. Conclusions: GBM evolves through exploiting complex structural variants involving multiple driver genes and the accumulation of genetic mutations caused by distinct mechanisms depending on its developmental stage.

GEN-13

PAIRED MUTATIONAL ANALYSIS IN SECONDARY NERVOUS SYSTEM LYMPHOMA AND PCNSL SYSTEMIC RELAPSE REVEALS DRIVER MUTATION CANDIDATES IN THE CENTRAL NERVOUS SYSTEM.

Nobuyoshi Sasaki[1], Satoshi Kume[2], Kensuke Tateishi[3], Taishi Nakamura[3], Kenji Ibayashi[4], Yuki Yamagishi[1], Kuniaki Saito[1], Keiichi Kobayashi[1], Yuko Matsushita[5], Yuko Hibiya[5], Mai Kitahara[6], Saki Suzuki[1], Reiko Nagano[7], Satoshi Yamashita[8], Hirofumi Nakatomi[1], Yoshiaki Shiokawa[1], Koichi Ichimura[5], Motoo Nagane[1]; [1]Department of Neurosurgery, Kyorin University Faculty of Medicine, [2]Department of Neurosurgery, Akiru Municipal Medical Center, [3]Department of Neurosurgery, Yokohama City University, [4]Department of Neurosurgery, Jichi Medical University [5]Department of Brain Disease Translational Research, Juntendo University Facility of Medicine, [6]Department of Brain Tumor Translational Research, National Cancer Center Research Institute, [7]Department of Epigenomics, National Cancer Center Research Institute, [8]Department of Informatics and Biotechnology Engineering, Maebashi Institute of Technology

Primary central nervous system lymphoma (PCNSL) is an aggressive extranodal non-Hodgkin lymphoma confined to the brain, eyes, and the spinal cord. The mechanism of central nervous system (CNS) tropism in PCNSL has not been fully elucidated. Diffuse large B cell lymphomas (DLBCLs) occasionally present with distant recurrence, which can involve inside and outside the CNS. Secondary central nervous system lymphomas (SCNSLs) are CNS relapse of systemic lymphoma. PCNSLs also rarely present with systemic relapse. We have previously reported in our study of whole exome sequencing that PCNSLs harbor frequent mutations in genes of B cell receptor pathway members and aberrant somatic hypermutation (aSHM) target genes. Although several genetic alterations were identified as more frequent in PCNSLs compared with systemic lymphomas, specific genetic alterations which serve as the driver for CNS tropism in PCNSLs has not been identified. In order to search for mutations which might serve as driver mutations in the CNS, we have performed targeted sequencing in paired samples from patients with recurrent lymphomas, either SCNSLs or PCNSL systemic relapses, using Ion Torrent multiplex PCR. Mutational profiles were compared between the primary and recurrent tumor. Six cases (four SCNSL cases and two PCNSL systemic relapse cases) were analyzed. Of note, in the SCNSL cases, several de novo mutations were enriched only among the recurrent CNS tumors. Among these mutations, BTG2 mutations were observed in 3/4 (75%), and B2M and KLHL14 mutations were observed in 2/4 (50%) cases. In the two PCNSL systemic relapse cases, KMT2D mutations were enriched only in the recurrent systemic tumors. It is suggested that these de novo mutations in the recurrent CNS tumors might serve as driver mutations in the CNS. Further analysis in larger cohorts, and functional studies are required in order to validate these findings.

EXPERIMENTAL THERAPEUTICS (ET)

ET-1

STEM CELL-BASED GENE THERAPY FOR MALIGNANT GLIOMA USING GENOME-EDITED HUMAN INDUCED PLURIPOTENT STEM CELLS

Ryota Tamura[1], Masahiro Yo[2], Hiroyuki Miyoshi[2], Oltea Sampetrean[4], Hideyuki Saya[3], Hideyuki Okano[2], Masahiro Toda[1]; [1]Department of Neurosurgery, Keio University School of Medicine, [2]Department of Physiology, Keio University School of Medicine, [3]Fujita Cancer Center, Fujita Health University [4]Department of Microbiology and Immunology, Keio University School of Medicine

Glioblastoma is the most aggressive primary brain tumor, and is characterized by diffuse infiltration into the normal brain parenchyma. New therapeutic approaches targeting invasive biological behaviour are warranted. In the present study, we show that neural stem cells (NSCs) derived from CRISRP/Cas9-edited induced pluripotent stem cells (iPSCs) have high tumor-trophic migratory capacity and stable constitutive therapeutic transgene expression, which leads to strong anti-tumor effects against glioma stem cell (GSC) models. The present study provides answers to some important research questions associated with stem cell-based gene therapy. First, the tumor-trophic migratory capacities of human iPSC-derived NSCs (iPSC-NSCs), fetal NSCs, and mesenchymal stem cells (MSCs) were quantitatively

evaluated by spatiotemporal methodologies. We demonstrated that iPSC-NSCs have a higher tumor-trophic migratory capacity than MSCs in the brain. Self-repulsive action and pathotropism were important for the migration of iPSC-NSCs: ephrin ligand/receptor mediated repulsion of iPSC-NSCs and CXCL12-CXCR4 interactions between GSCs and iPSC-NSCs. Second, a prodrug converting enzyme fusion gene was selected as a therapeutic gene in human iPSCs. In general, stable constitutive transgene expression by viral vectors was difficult in human iPSCs. Furthermore, viral vectors integrate randomly into the host genome, which raises concerns about transgene silencing, insertional mutagenesis, and oncogene activation. In the present study, several common insertion sites including GAPDH, ACTB, and AAVS1, were compared. The most appropriate gene locus that achieved stable constitutive transgene expression was determined via CRISPR/Cas9-mediated genome editing. Third, we revealed the novel mechanism of action using iPSC-NSCs expressing CD-UPRT, in which ferroptosis was associated with enhanced anti-tumor immune responses. We demonstrated that the established iPSC-NSCs had strong therapeutic efficacy in GSC animal models. Finally, predictive biomarkers for the efficacy of the present treatment strategy were established. We will conduct a clinical trial of this treatment strategy. This research concept can disseminate biological, medical and engineering advances.

ET-4

BASIC RESEARCH OF BORON NEUTRON CAPTURE THERAPY USING A NOVEL BORON COMPOUND TARGETED TO INTEGRIN

Kohei Tsujino[1], Shinji Kawabata[1], Hideki Kashiwagi[1], Kohei Yoshimura[1], Ryo Kayama[1], Yusuke Fukuo[1], Takuya Kanemitsu[1], Ryo Hiramatsu[1], Naonori Hu[2], Shin-Ichi Miyatake[2], Kai Nishimura[3], Takushi Takata[4], Hiroki Tanaka[4], Minoru Suzuki[4], Hiroyuki Nakamura[3], Masahiko Wanibuchi[1]; [1]Department of Neurosurgery, Osaka Medical and Pharmaceutical University, Takatsuki, Japan [2]Kansai BNCT Medical Center, Osaka Medical and Pharmaceutical University [3]Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology [4]Institute for Integrated Radiation and Nuclear Science, Kyoto University

Background: Boron neutron capture therapy (BNCT) is a particle radiation modality capable of selectively destroying tumor cells. The most commonly used boron compound for BNCT is boronphenylalanine (BPA). BPA is taken up into the tumor cell via the L-type aminoacid transporter (LAT-1). However, there are some BPA-refractory situations. Therefore, a novel boron compound is expected to improve the therapeutic performance of BNCT. We focused on integrin$\alpha v\beta 3$, which is overexpressed in malignant gliomas as in many cancer cells, and have developed cRGD-MID-AC, a conjugate of cyclic RGD (cRGD), which selectively inhibited integrin$\alpha v\beta 3$, and MID-AC, which we have already reported as effective on BNCT as BPA as a boron compound in F98 rat glioma models. We evaluated the efficacy of BNCT using this novel compound.Methods: F98 glioma cells were exposed to BPA, cRGD-MID-AC, and cRGD-MID for cellular uptake and neutron irradiation experiment. Intracellular boron concentrations and compound biological effectiveness (CBE) for each boron compound was calculated. After intravenous administration (i.v.) of cRGD-MID-AC or BPA, the biodistribution of boron compounds was measured and neutron irradiation experiment were performed in F98 rat glioma models. **Results:** Intracellular boron concentrations of BPA and cRGD-MID-AC were increased gradually at all exposed time, and CBE for cRGD-MID-AC was comparable to that for BPA. In cRGD-MID-AC, the boron concentration in the tumor was the highest at 8 h after i.v. and tended to be retained longer at 24h. In vivo neutron irradiation experiment, long-term survival was observed only in the group irradiated 8 h after cRGD-MID-AC i.v.. These experiments suggest that cRGD-MID-AC has sufficient cell-killing effect and may be more effective in vivo. **Conclusion:** cRGD-MID-AC has a tumor accumulation mechanism different from that of BPA, and could be an effective boron carrier in BNCT for malignant gliomas.

ET-5

POTENT BYSTANDER EFFECT IN SUICIDE GENE THERAPY USING TK-EXPRESSING STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

Shinichiro Koizumi[1], Makoto Horikawa[1], Taisuke Yamamoto[1], Tomoya Oishi[1], Tomohiro Yamasaki[1], Satoru Kida[1], Hiroki Namba[2], Kazuhiko Kurozumi[1]; [1]The Department of Neurosurgery, Hamamatsu University School of Medicine, [2]JA Shizuoka Kohseiren Enshu Hospital

Introduction: We investigated HSVTK/GCV suicide gene therapy for malignant glioma, and demonstrated the migration ability and antitumor effect of various tissue-derived pluripotent stem cells. In recent years, stem cells from human exfoliated deciduous teeth (SHED), which have excellent ethical and self-renewal ability, have attracted attention, especially in regenerative medicine. In this study, using SHEDTK transfected with TK, we examined the migration ability and antitumor effect against malignant glioma and metastasis models. **Methods:** In vitro assay: Using Matrigel chamber, the migration ability of SHEDTK to conditioned medium (CM) of glioma cells, lung carcinoma cells, and various tumor growth factors (TGF) was examined. The antitumor effect was examined for cell viability by co-culturing SHEDTK and each tumor cell under the addition of GCV. in vivo assay: Using glioma model mice, SHEDTK migrating around the tumor was confirmed in brain sections. In addition, we co-implanted SHEDTK and administered GCV to glioma and metastasis model as therapeutic models, evaluated tumor reduction effect by bioluminescence, and confirmed survival curves. **Results:** In vitro assay: SHEDTK significantly migrated to CM and TGF compared to control. The antitumor effect was observed even when the ratio of each tumor cell to SHEDTK was 128-256 times. in vivo assay: In glioma model mice, SHEDTK migrated around contralateral tumor. In the treatment experiment, the control group died of tumors, whereas the tumor in the treatment group disappeared within the period of GCV administration, and they survived without neurological deficits until 100 days later, and the overall survival time was improved with a statistically significant difference. **Conclusions:** We confirmed the migration ability and antitumor effect of SHEDTK on malignant glioma and metastasis models. Suicide gene therapy using SHEDTK was suggested as novel gene therapy. In the future, we plan to conduct translational research such as preclinical studies and physician -initiated clinical trials.

ET-6

EFFICACY OF NEAR-INFRARED PHOTOIMMUNOTHERAPY TARGETING PODOPLANIN IN MALIGNANT GLIOMAS

Manabu Natsumeda[1], Jotaro On[1], Jun Watanabe[1], Kazuhiro Ando[1], Yoshihiro Tsukamoto[1], Masayasu Okada[1], Ryosuke Ogura[1], Makoto Oishi[1], Yukihiko Fujii[1]; [1]Department of Neurosurgery, Niigata University, Niigata, Japan

Background: Near-infrared photoimmunotherapy (NIR-PIT) is a new cancer treatment based on a conjugate of photosensitizer IR700 and an antibody which selectively binds to the surface antigen of cancer cells. Upon exposure to near infrared light, which does not harm normal tissues, cancer cells are selectively killed. Clinical application for EGFR amplified head and neck cancer has already been realized but is still in the preclinical stages of research for brain tumors. Finding a surface antigen which is selective to cancer cells is key to the effective application of NIR-PIT. In IDH-wildtype gliomas, we found that podoplanin (PDPN) is a promising candidate. PDPN is a transmembrane sialoglycoprotein highly expressed in IDH-wildtype gliomas and other cancers. Methods: Three PDPN-positive (NGT-11, NGT-41, LN319) and three PDPN-negative (T98G, U87MG, U251MG) glioblastoma cell lines were treated with PDPN-IR700 conjugate and exposed to near infrared light. Cytotoxicity was assessed by cell viability assays. Next, we treated subcutaneous mouse xenografts by NIR-PIT. Results: Cytotoxicity was only observed after NIR-PIT using PDPN-conjugate in cell lines expressing PDPN. This cytotoxicity was dose-dependent to near infrared light exposure, and most pronounced in LN319 cell line, which expressed the highest levels of PDPN. Time lapse videos revealed instantaneous expanding, followed by rounding of dead cells after treatment. NIR-PIT treated subcutaneous xenografts showed significantly smaller tumor sizes compared to control, and pathological investigation revealed selective and extensive necrosis of tumor tissue. Discussion/conclusion: PDPN is highly expressed in IDH-wildtype gliomas, and we found that NIR-PIT was highly effective in treating PDPN expressing glioblastoma cell lines. NIR-PIT is anticipated to be more selective and cytotoxic than photodynamic therapy and is a promising new treatment strategy for IDH-wildtype gliomas.

ET-7

EVALUATION OF HYPOXIA-TARGETING RADIOPHARMACEUTICAL ^{64}CU-ATSM FOR PET MONITORING WITH LOCAL THERAPY IN HIGH-GRADE GLIOMA MODEL

Yukie Yoshii[1], Fukiko Hihara[1], Hiroki Matsumoto[1], Chika Igarashi[1], Tomoko Tachibana[1], Mitsuhiro Shinada[1], Zhang Ming-Rong[1], Akito Oshima[2], Hidemitsu Sato[3], Yoshitaka Narita[4], Hiroaki Kurihara[5], Tetsuya Yamamoto[2], Tatsuya Higashi[1], Kensuke Tateishi[2]; [1]National Institutes for Quantum Science and Technology, Chiba, Japan, [2]Department of Neurosurgery, Yokohama City University Graduate School of Medicine, Yokohama, Japan, [3]Department of Neurosurgery, Kanagawa Cancer Center, Yokohama, Japan, [4]Department of Neurosurgery and Neuro-Oncology, National Cancer Center Hospital, Tokyo, Japan, [5]Department of Radiology and IVR, Kanagawa Cancer Center, Yokohama, Japan

World Health Organization (WHO)-defined central nervous system (CNS) grade 4 high-grade gliomas (HGGs) are highly aggressive brain cancers characterized by the presence of hypoxia within a rapidly-growing tumor mass. Due to invasion to the surrounding brain parenchyma, these tumors commonly recur locally, despite aggressive surgical resection, and new therapeutic