tumor cell death and growth inhibition of glioma cells upon glutamine deprivation. These results suggest new therapeutic targets for glioma cells adapting to a low-nutrient microenvironment.

Key words: Glioma | Microenvironment | One-carbon metabolism

**CBMS-7**

**IGF1/N-CADHERIN/CLUSTERIN SIGNALING AXIS MEDIATES ADAPTIVE RADIORESISTANCE OF GLIOMA STEM CELLS**

Satoru Osuka[1], Dan Zhu[2], Zhaobin Zhang[2], Chaoxi Li[1], Christian T Stackhouse[3], Oltea Sampetrean[4], Jeffrey J Olson[2], Yancey Gillespie[1], Hideyuki Saya[4], Christopher D Willey[3], Erwin G Van Meir[1,2]; [1]Department of Neurosurgery, School of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA [2]Department of Neurosurgery, School of Medicine and Winship Cancer Institute, Emory University, Atlanta, Georgia, USA [3]Department of Radiation Oncology, University of Alabama at Birmingham, Birmingham, USA [4]Division of Gene Regulation, Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan

Glioblastoma (GBM) is composed of a variety of tumor cell populations including those with stem cell properties, known as glioma stem cells (GSCs). GSCs are innately less sensitive to radiation than the tumor bulk and are believed to drive GBM formation and recurrence following repeated irradiation. However, it is unclear how GSCs adapt to avoid the toxicity of repeated irradiation used in clinical practice. We established radioresistant human and mouse GSCs by exposing them to repeated rounds of irradiation in order to uncover critical mediators of adaptive radioresistance. Surviving subpopulations acquired strong radioresistance in vivo, which was accompanied by increased cell-cell adhesion, slower proliferation, an elevation of stemness properties and N-cadherin expression. Increasing N-cadherin expression rendered parental GSCs radioresistant, reduced their proliferation, and increased their stemness and intercellular adhesive properties. Conversely, radioresistant GSCs reduced their acquired phenotypes upon CRISPR/Cas9-mediated knockout of N-cadherin. Mechanistically, elevated N-cadherin expression resulted in the accumulation of β-catenin at the cell surface, which decreased Wnt/β-catenin proliferative signaling, reduced neural differentiation, and protected against apoptosis through Clusterin secretion. Restoration of wild type N-cadherin, but not mutant N-cad lacking β-catenin binding region, led to increased radioresistance in N-cadherin knockout GSCs, indicating the importance of the binding between N-cadherin and β-catenin. We also demonstrated that N-cadherin upregulation was induced by radiation-induced IGF1 secretion, and the radiation resistance phenotype can be reversed with picropodophyllin (PPP), a clinically applicable blood-brain-barrier permeable IGF1 receptor inhibitor, supporting clinical translation. Moreover, the elevation of N-cad and Clusterin are related to prognosis of GBM in the TCGA dataset. In conclusion, our data indicate that IGF1R inhibitor can block the N-cadherin-mediated resistance pathway. Our research provides a deeper understanding of adaptive radioresistance after repeated irradiation, and validates the IGF1/N-cadherin/β-catenin/Clusterin signaling axis as a novel target for radio-sensitization, which has direct therapeutic applicability.

Key words: N-cadherin | Glioma stem cells | IGF1R

**CBMS-10**

**METHIONINE METABOLISM CLOSELY RELATED WITH SELF-RENEW, PLURIPOTENCY AND CELL DEATH IN GICS THROUGH MODIFICATION OF CHOLESTEROL BIOSYNTHESIS AND RIBOSOMAL RNA**

Kiyotaka Yokogami[1], Yasutaka Nakatake[1], Takashi Watanabe[1], Asako Mizuguchi[1], Shinji Yamashita[1], Hideo Takeshima[1]; [1]Department of Neurosurgery, Section of Clinical Neuroscience, Faculty of Medicine, University of Miyazaki

Glioma initiating cells (GICs) are the source of glioma cells that have the ability to self-renew and pluripotency, which are treatment-resistant, starting point for relapse and eventual death despite multimodality therapy. Since high accumulation is observed in 11cMet-PET at the time of recurrence, it is important to understand the mechanism of tumor cell activation caused by the reorganization of methionine metabolism. We cultured cells in methionine-deprived culture medium and performed a comprehensive analysis, and found that methionine depletion markedly decreased proliferation and increasing cell death of GICs. Decreased SAM, which is synthesized intracellularly catalyzed by methionine adenosyltransferase (MAT) using methionine, triggered the following: (i) global DNA demethylation, (ii) hyper-methylation of signaling pathways regulating pluripotentcy of stem cells, (iii) decreased expression of the core-genes and pluripotent marker of stem cells including FOXM1, SOX2, SOX4, PROM1 and OLIG2, (iv) decreased cholesterol synthesis and increased excretion mainly through decreased SREBF2 (v) down-regulation of the large subunit of ribosomal protein configured 28S and ACA43, snoRNA guiding the pseudouridylation of 28S ribosomal RNA, which has crucial role for translation. In addition, inhibition of cholesterol synthesis with statin resulted in a phenotype similar to that of methionine removal and a decrease in stem cell markers and snoRNA ACA43. Moreover, suppression of FOXM1 decreased stem cell markers such as SOX4 and PROM1. The gene expression profile for cholesterol production was obtained from the Ivy Glioblastoma Atlas Project (IVYGAP) database and compared between tumour cells with relatively low methionine levels in area of pseudopalisading arrangement around necrosis and tumour cells in the infiltrating region, showing that cells cells in the infiltrating region have a higher capacity to produce cholesterol. Taken together, methionine metabolism closely related with self-renew, pluripotency and cell death in GICs through modification of cholesterol biosynthesis: especially SREBF2-FOXM1 and ACA43 axis with modification of ribosomal RNA.

Key words: glioma stem cells | methionine | cholesterol

## SIGNALING PATHWAYS/DRUG RESISTANCE (SPDR)

**SPDR-1**

**HSP90 INHIBITION OVERCOMES RESISTANT TO MOLECULAR TARGETED THERAPY IN *BRAF*$^{V600E}$ MUTANT GLIOBLASTOMA**

Jo Sasame[1], Naoki Ikegaya[1], Yohei Miyake[1], Takahiro Hayashi[1], Akito Oshima[1], Hirokuni Homma[1], Masataka Isoda[1], Katsuhiro Takabayashi[1], Tetsuya Yamamoto[1], Kensuke Tateishi[1]; [1]Department of Neurosurgery, Yokohama City University, Graduate School of Medicine, Yokohama, Japan

The *BRAF*$^{V600E}$ mutation results in the constitutive activation of downstream mitogen activated protein kinase (MAPK) pathway that promotes tumor growth. Recently, molecular targeted therapy using BRAF/MEK inhibitor has been reported for *BRAF*$^{V600E}$ mutant high-grade glioma, but the therapeutic effect is limited by the emergence of drug resistance. Herein, we established paired *BRAF*$^{V600E}$ mutant glioblastoma (GBM) patient-derived xenograft (PDX) models, which were derived from tumors at prior to and recurrence after molecular targeted therapy. These PDX models were found to extensively recapitulate the histology, genetic abnormalities, and even the clinical course of the patients. Furthermore, BRAF/MEK inhibitor gradually caused resistance in cell lines derived from specimens that initially responded to molecular targeted therapy. In this study, genomic and epigenomic changes had little effect on the resistance mechanism. On the other hand, we found that hyperactivation of the MAPK pathway through c-Raf and the AKT/mTOR pathway primarily caused resistance to molecular targeted therapy in *BRAF*$^{V600E}$ mutant GBM. Through a high throughput drug screening, we find that HSP90 inhibitor with BRAF/MEK inhibitor coordinately deactivates MAPK pathway and AKT/mTOR pathway, and mediates potent toxicity in vitro and in vivo in refractory and acquired resistant models. These findings support that this therapeutic approach can overcome the limitation of current molecular targeted therapy in *BRAF*$^{V600E}$ mutant GBM.

Key words: BRAF V600E | Patient-derived xenograft model | HSP90 inhibitor

**SPDR-2**

**HISTOPATHOLOGICAL INVESTIGATION OF THE OLIGODENDROGLIAL TUMORS RESECTED FOLLOWING ALKYLATING AGENT CHEMOTHERAPY**

Nakaya Masato[1], Tokunori Kanazawa[1], Kentaro Ohara[2], Yohei Kitamura[1,3], Kazunari Yoshida[1], Hikaru Sasaki[1]; [1]Department of Neurosurgery, Keio University School of Medicine [2]Department of Pathology, Keio University School of Medicine [3]Division of Neurosurgery, Tokyo Saiseikai Central Hospital

Oligodendrogliomas, i.e., lower grade gliomas with 1p/19q codeletion, are often responsive to chemotherapy, however, those tumors eventually recur and life-limiting in the majority of patients despite initial chemotherapeutic response. We have been treating those patients with upfront chemotherapy and subsequent resection following tumor volume decrease since 2006. This study aimed to elucidate the histological changes and the mechanism of recurrence after alkylating agent chemotherapy in oligodendrogliomas. Fifteen oligodendrogliomas (Grade 2: 12, Grade 3: 3) resected following tumor volume decrease after alkylating agent chemotherapy were included and compared with their pre-chemotherapy specimens. Histological changes were investigated using hematoxylin-eosin staining, and changes in proliferative activity, status of glioma stem cells (GSCs), and tumor-infiltrating macrophages were assessed using immunohistochemistry. The frequent histological findings following chemotherapy included a sparse glial background, abundant foamy cell infiltration, gliosis, calcification, and nuclear