

sion levels of immune regulatory molecules such as Foxp3, CD163, PD-1, PD-L1, CD4, and CD8 were not significantly changed after the treatment using the Stupp regimen, compared with combinational usage of Bev. In addition, expressions of VEGF/VEGFR, hypoxic markers, and stem cell marker were not altered before and after Stupp regimen, either. Bev persistently inhibited immune suppressive cells and immune checkpoint molecules via down-regulation of VEGF pathway. In contrast, Stupp regimen did not affect immune regulations and tumor microenvironment. CONCLUSION: These results suggested that immunosupportive effect was caused by Bev administration, leading to the novel combinational treatment strategies, in addition to Stupp regimen.

#### ANGI-05

##### PATHOGENESIS OF RESISTANCE (MIMICRY AND CO-OPTION) TO ANTI-ANGIOGENIC TREATMENT FOR GLIOBLASTOMA

Shingo Takano<sup>1</sup>, Toshihide Tanaka, Eiichi Ishikawa<sup>1</sup>, Youhei Yamamoto, Jun Takai, Masahide Matsuda<sup>1</sup>, Takao Tsurubuchi<sup>1</sup>, Hiroyoshi Akutsu<sup>1</sup>, Akira Matsumura<sup>1</sup>; <sup>1</sup>Department of Neurosurgery, Faculty of Medicine, University of Tsukuba

**PURPOSE:** Vessel co-option and vascular mimicry are important resistant factors with anti-angiogenic treatment for glioblastoma, but those precise evaluation is not clear. We had three types of glioblastoma surgically removed specimens treated with / without bevacizumab (Bev). Using these samples, pathogenesis of co-option and mimicry was morphometrically clarified. **MATERIALS / METHODS:** Three types of glioblastoma specimens were analyzed; 1) Bev naive (N group, n 14), 2) Bev effective that was treated pre-operative neoadjuvant Bev (E group, n 5), 3) Bev refractory that recurred with continuous Bev treatment for paired E group (R group, n 5). Vascular density was defined as a number of type IV collagen covered lumen. Vascular mimicry was measured as a ratio of CD34 negative / type IV collagen positive lumen. Vessel co-option was graded to 3 degrees (-), (+), (++) at tumor margin. **RESULTS:** (1)Vascular density was significantly lower with E group (p<0.01) and R gr up (p<0.02) compared to N group. (2)Mimicry was significantly higher with R group compared to N and E group (p<0.01). Between paired samples, refractory case was constantly higher than effective sample. (3) Co-option was increases with R group compared to N group. **DISCUSSION/CONCLUSION:** The effect of Bev for glioblastoma was investigated on three points (vascular density, vascular mimicry and vessel co-option) and two pathogenesises were clarified. In Bev refractory case, density was decreased, but mimicry and co-option were increased compared to Bev naive case. In Bev effective case, density was decreased, but mimicry and co-option were unchanged. Anti-angiogenic treatment for initial and Bev refractory glioblastoma should consider targeting co-option and mimicry in addition to Bev.

#### CELL BIOLOGY/METABOLISM/STEM CELLS (CBMS)

##### CBMS-01

##### AGE-DEPENDENT GLIOBLASTOMA PROGRESSION SUPPRESSED BY NAD+

Daisuke Yamashita<sup>1</sup>, Saya Ozaki<sup>1</sup>, Satoshi Suehiro<sup>1</sup>, Kazuhiro Sonomura, Toru Kondo, Taka-Aki Sato, Takeharu Kunieda<sup>1</sup>, Ichiro Nakano; <sup>1</sup>Department of Neurosurgery, Ehime University Graduate School of Medicine, Toon, Japan

The rise in population aging worldwide is causing an unparalleled increase in death from many cancers, including glioblastoma (GBM). Here, we have explored the impact of aging and rejuvenation on GBM tumorigenesis. Compared with old GBM, young GBM displayed elevated neuronal/synaptic signaling via brain-derived neurotrophic factor (BDNF) and SLIT and NTRK like-family member 6 (SLITRK6), promoting favorable survival rates. These effects were attributed to the rise in nicotinamide adenine dinucleotide (NAD<sup>+</sup>) levels, as brain rejuvenation by parabiosis or administration of nicotinamide mononucleotide (NMN) in mice elicited a younger phenotype with activated neuronal/synaptic signaling and improved outcomes. Our data indicate that age-associated NAD<sup>+</sup> loss contributes to the highly aggressive GBM in the elderly. These findings have therapeutic implications in GBM and provide mechanistic insights into the exacerbation of GBM tumorigenesis with age.

##### CBMS-02

##### CROSSTALK WITH ASTROCYTES ESTABLISHES TUMOR EDGE IN GLIOBLASTOMA

Daisuke Yamashita<sup>1</sup>, Saya Ozaki<sup>1</sup>, Toru Kondo, Takeharu Kunieda<sup>1</sup>, Ichiro Nakano; <sup>1</sup>Department of Neurosurgery, Ehime University Graduate School of Medicine, Toon, Japan

Clinical outcomes for patients with glioblastoma (GBM) are extremely poor due to inevitable tumor recurrence even after extensive treatments. These recurrences are thought to manifest from cells located within the

tumor edge. Despite this, the precise molecular mechanism governing GBM spatial phenotypic heterogeneity (e.g. edge vs. core) and subsequent tumor recurrence remains poorly elucidated. Here, using patient-derived GBM core and edge tissues, we analyzed transcriptional and metabolic signatures in an effort to determine how GBM facilitates the edge phenotype and its associated recurrence-initiating cells (RICs). In so doing, we unexpectedly identified CD38 as an essential protein in the formation of the edge phenotype and found a CD38-driven interaction between edge GBM cells and neighboring astrocytes that communally develops a GBM edge that is unresectable by surgery and retains RICs. These findings have profound implications for future clinical therapies and provide new mechanistic insights into both tumor progression and recurrence.

##### CBMS-03

##### PHOSPHORYLATION STATE OF OLIG2 REGULATES PROLIFERATION OF GLIOMA STEM CELLS.

Norihiko Saito<sup>1</sup>, Nozomi Hirai<sup>1</sup>, Kazuya Aoki<sup>1</sup>, Sho Sato<sup>1</sup>, Ryo Suzuki<sup>1</sup>, Yu Hiramoto<sup>1</sup>, Satoshi Fujita<sup>1</sup>, Haruo Nakayama<sup>1</sup>, Morito Hayashi<sup>1</sup>, Takatoshi Sakurai<sup>1</sup>, Satoshi Iwabuchi<sup>1</sup>; <sup>1</sup>Department of Neurosurgery, Toho University Ohashi Medical Center

The Cancer Genome Atlas project described a robust gene expression-based molecular classification of glioblastoma with the functional and biological significance of the subclasses yet to be determined. Here, we show that a comprehensive analysis of a panel of glioma initiating cell (GIC) lines can identify a group of stem cells with high OLIG2 expression as in Proneural-like GBM subtype. In vitro differentiation studies showed that proneural GIC lines possess the potential to differentiate into astrocytic, neuronal, and oligodendrocytic lineages, whereas mesenchymal GICs exhibited limited potential for neural lineage differentiation following retinoic acid induction. A considerable decline of OLIG2 in proneural GIC lines was observed following retinoic acid treatment. We also showed that OLIG2 is a functional marker associated with cell proliferation in Olig2-high GIC lines. In addition, OLIG2 inhibition disrupted cell-cycle control mechanism by decreasing CDK2 and CDK4 and elevating apoptosis-related molecules. Mechanistic investigations revealed molecular interactions between CDK2/CDK4 and OLIG2. Inhibition of CDK2/CDK4 activity disrupted OLIG2-CDK2/CDK4 interactions and attenuated OLIG2 protein stability. Further investigation on these mechanisms may lead to novel targeted therapy on GBMs with high OLIG2 expression.

##### CBMS-04

##### SIGNIFICANT ROLE OF HYPOXIA IN THE EXPRESSION AND FUNCTION OF OSTEOPONTIN IN CD44-HIGHLY EXPRESSED GLIOMA STEM-LIKE CELLS IN TUMOR PROGRESSION OF GLIOBLASTOMA

Masahiro Nishikawa<sup>1</sup>, Akihiro Inoue<sup>1</sup>, Yoshihiro Ohtsuka<sup>1</sup>, Saya Ozaki<sup>1</sup>, Shirabe Matsumoto<sup>1</sup>, Satoshi Suehiro<sup>1</sup>, Hajime Yano, Yonehiro Kanemura, Junya Tanaka, Takanori Ohnishi, Takeharu Kunieda<sup>1</sup>; <sup>1</sup>The Department of Neurosurgery, Ehime University Graduate School of Medicine, Toon, Japan

The poor prognosis of glioblastoma multiforme (GBM) may be due to the surviving glioma stem-like cells (GSCs) in the tumor periphery after tumor resection. We demonstrated that CD44-expressed GSCs existed much more in the tumor periphery of high invasive (HI) type GBM than low invasive (LI) type GBM. The HI type was significantly associated with worse outcome, but how GSCs with high CD44 expression relate to tumor progression remains unknown. In this study, we investigated effects of hypoxia on CD44-directed signal pathways, leading to tumor invasion and proliferation in GBM. We focused on the CD44 ligand osteopontin (OPN) because it is known hypoxia affects the interaction of CD44 and OPN which promotes stemness and proliferation of cancer stem cells. We examined mRNA expressions of hypoxia inducible factor (HIF)-1a, HIF-2a, CD44 and OPN in tumor tissues of GBM and investigated effects of hypoxia (1% O<sub>2</sub>:severe or 5% O<sub>2</sub>:moderate) on the expression of these molecules using cultured GSCs that were established from tumor tissues showing high CD44 expression in the periphery of GBMs. In addition, we analyzed the effects of OPN on invasive, migratory and proliferative activities of GSCs under the hypoxic conditions. OPN was much higher expressed in the tumor periphery of LI type GBM than HI type GBM. Severe hypoxia significantly increased the expressions of HIF-1a and CD44 but did not OPN. On the other hands, moderate hypoxia promoted the expressions of HIF-2a and OPN. Knockdown of HIF-2a significantly inhibited OPN expression. In addition, the more OPN was expressed in the cultured GSCs under moderate hypoxia, the more the GSCs proliferated and decreased their invasive and migratory activities. In conclusion, GSCs existing in the tumor periphery of GBM can migrate or proliferate by changing CD44-directed signal pathways. Moderate hypoxia promoted HIF-2a/OPN/CD44 pathway, resulting in phenotypic transition to high proliferative tumors.