CBMS-05
COMPREHENSIVE METABOLOMICAL ANALYSIS OF IDH1R132H CLINICAL GLIOMA SAMPLES REVEALS SUPPRESSION OF β-OXIDATION DUE TO CAROTINOID DEFICIENCY.
Satsuki Miyata1, Kaoru Yotsumaga, Eiji Sakauchi, Masashi Urabe, Yoshitani Onuki1, Akira Gomi, Takashi Yamaguchi1, Makiko Mieno, Hiroaki Mizukami, Akhiro Kume, Keiya Ozawa, Eiji Watanabe1, Kenzuke Kawai,1 Hitoshi Endou1, Department of Neurosurgery, Jichi Medical University, Tochigi, Japan.

BACKGROUND: Gliomas with isocitrate dehydrogenase 1 (IDH1) mutation have alterations in several enzyme activities, resulting in various metabolic disturbances. Furthermore, the goal of this study was to investigate the mechanism of the better prognosis of gliomas with IDH1 mutation by performing metabolomic analysis.

METHODS: To comprehensively understand the metabolic state of human gliomas, we analyzed clinical samples obtained from surgical resection of glioma patients (grades II-IV) with or without the IDH1 mutation, and compared them with US7 glioblastoma cells overexpressing IDH1 or IDH1R132H cDNA. We used capillary electrophoresis and liquid chromatography time-of-flight mass spectrometry for these analyses. RESULTS: In clinical samples of gliomas with IDH1 mutation, levels of 2-hydroxyglutarate (2HG) were significantly increased compared with gliomas without IDH1 mutation. Gliomas with IDH1 mutation also showed decreased 2-oxoglutarate and downstream intermediates in the tricarboxylic acid cycle and proteins involved in production of energy, amino acids, and nucleic acids. The marked difference in the metabolic profile in IDH1 mutant clinical samples compared with that of mutant IDH expressing cells includes a decrease in β-oxidation due to acyl-carotinoids and carotinoids.

DISCUSSION: These metabolic changes may explain the better prognosis in IDH1 mutant gliomas.

CBMS-07
SERINE SYNTHESIS AND ONE-CARBON METABOLISM IN GLIOMA CELLS TO SURVIVE GLUTAMINE STARVATION.
Kazuhito Tanaka1, Takashi Sasaayama1, Takiko Uno1, Yuichi Fujita1, Mitsuhiro Hashiguchi1, Yasuhito Iino, Eiji Kohmura1, 1Department of Neurosurgery, Kobe University Graduate School of Medicine, Kobe, Japan.

Cancer cells optimize nutrient utilization to supply energetic and biosynthetic pathways. These metabolic processes also include tumor maintenance and epigenetic regulation through nucleic acid and protein metabolism, enhancing tumorogenicity and clinical resistance. But less is known about how cancer cells exhibit metabolic flexibility to sustain cell growth and survival from nutrient starvation. Here, we identify a key role for serine availability and one-carbon metabolism in the survival of glioma cells from glutamine deprivation. To identify metabolic response to glutamine deprivation in glioma cells, we analyzed metabolites using gas chromatography and mass spectrometry (GC/MS) in glioma cells cultured in glutamine-deprived medium and examined gene expression of key enzymes for one-carbon units using RT-PCR and western blotting methods. These expressions were also confirmed by immunohistochemical staining in glioma clinical samples. Metabolome studies indicated serine, cysteine, and methionine as key differentiating amino acids between glioma cells from glutamine deprivation. To identify metabolic changes may explain the lower cell division observed in IDH1 mutant gliomas and may be one mechanism of the better prognosis in IDH1 mutant gliomas.

CBMS-08
INVESTIGATION FOR NICOTINIC EFFECTS ON STEM CELL’S PROPERTY IN HSV-TK/GCV GENE THERAPY.
Haruki Kominami1, Tomohiro Yamashita, Tomoya Oshi, Makoto Horikawa, Taisuke Yamamoto, Shinchiro Koizumi, Tetsuro Sameshima, Hiroki Namba; 1Department of Neurosurgery, Seirei Mikatahara General Hospital.

BACKGROUND: Herpes simplex virus-thymidine kinase/ganciclovir (HSV-tk/GCV) system is one of feasible therapeutic strategies for defeating malignant gliomas. Stem cells with intrinsic tumor tropism are used for suicide gene vehicles, which make this therapy further realistic. Nicotine is known to affect cellular migration capacity in various types of cells but whether nicotine impacts on stem cells’ migration capacity to gliomas is not scrutinized. In this research, we investigated nicotinic impact on stem cells’ properties including tumor tropism and gap junctional intercellular communication (GJIC), which is crucial to this therapeutic strategy.

METHODS: Mouse induced pluripotent stem cell (iPSC)-derived neural stem cells (miPSC-NSCs) and human dental pulp mesenchymal stem cells (hDPSCs) were used. Nicotine cytotoxicity for 24 hours was evaluated by MTT assay for stem cells and glioma cells; GS-9L and C6 (rat), GL261 (mouse), U251 and U87 (human). Tumor tropism to glioma-conditioned medium (GCM) with or without non-toxic nicotine concentrations was assessed using Matrigel Invasion Chamber. Nicotine effect on GJIC was monitored with scrape loading/dye transfer assay (SL/DT assay) for co-culture of stem cells and glioma cells (stem cell/glioma cell or paracrine assay for glioma cells alone using high-content analysis. RESULTS: MTT assay revealed a 1 μM nicotine, equivalent to serum nicotine concentration in habitual smoking, is the maximum safe concentration for stem cells and glioma cells. Tumor tropism (miPS-NSCs to GL261-CM, hDPSCs to U251- or U87-CM) and GJC of co-culture of stem cells and glioma cells (miPS-NSC/GL261, hDPSC/U251) or glioma cells alone (GS-9L, C6, GL261 and U251) were not affected by 1 μM of nicotine. CONCLUSIONS: Physiological nicotine presence did not affect (1) stem cell’s tumor tropism to gliomas and (2) GJC between stem cells and glioma cells or within glioma cells. HSV-tk/GCV therapy may retain its therapeutic efficacy against gliomas even under physiological nicotine concentrations.

CBMS-10
FUNCTIONAL ROLE OF MYCN IN SHH TYPE TP53 MUTATED MB’S METABOLISM.
Yotaka Yokogami1, Takashi Watanabe1, Shinji Yamashita,1 Asako Mizuguchi1, Hideo Takeshma1; 1Department of Neurosurgery, Section of Clinical Neuroscience, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan.

BACKGROUND: Medulloblastoma is classified in 4 subgroups. Prognosis and therapeutic option were different from each subgroups. Thus, we need subgroup-specific in vitro models for investigating new therapeutic targets. Little established medulloblastoma cell-lines, which have been subgrouped is available. Especially, commercially available SHH type TP53 mutated cell-line is only DAOY. We established new cell lines 507CSC / 507FS from the patient with SHH type with TP53 mutated MB. This matched pair cell line showed high expression of MYCN in serum free conditioned medium. To know the functional role of N-MYC in MB, we used 507CSC and DAOY.

MATERIAL AND METHODS: Using chemical inhibitor of MYCN in 507CSC and DAOY, proliferation assay, mRNA expression and measurement of ex-vivo metabolic phenotype were performed. RESULTS: MYCN inhibition leads to cell death in both cell lines. MYCN regulated glucose, glutamine and methionine metabolism. Especially the targets were PKM2, GLS2, MAT2A, DMT1 and 3A. CONCLUSIONS: MYCN is a target of therapy in a patient with SHH type TP53 mutated medulloblastoma.

CBMS-12
PENTAMIDINE: TRANSLATIONAL RESEARCH FOR A NEW THERAPY TARGETING ON GLOMIA CELLS AND GLIOMA STEM CELLS USING DRUG REPOSITIONING.
Sho Tamai1, Sabit Hemragul,1 Jiaapa Shabierjiang1, Guangtao Zhang1, Jiaakang Zhang1, Yi Wang2, Shingo Tanaka1, Masashi Kimoshita,1 Atsushi Hirao, Mitsutoshi Nakada1; 1Department of Neurosurgery Kanazawa University.

INTRODUCTION: Glioblastoma (GBM) is primary malignant brain tumor with poor prognosis. Despite aggressive chemoradiotherapies, GBM has resistance and finally relapses. Recently, it is reported that glioma stem cells (GSCs) are forming tumors and induce the recurrence. However, there is no effective therapy for GSCs. Herein, we newly identified pentamidine, an antiprotozoal drug, is effective for not only glioma cells but also GSCs by drug repositioning approach. METHODS: We tested the nicotinic impact on stem cell lines, A172 and T98, and patient-derived glioma stem cell lines KG017, KG007 which were established at Kanazawa University. We investigated proliferation ability, stemness and intracellular signal change by proliferation assay, sphere forming assay and western blotting, respectively. We also analyzed the change of tumor microenvironment.

RESULTS: Pentamidine is known as the therapeutic drug for pneumocystis