CBMS-05

COMPREHENSIVE METABOLOMIC ANALYSIS OF IDH1R132H CLINICAL GLIOMA SAMPLES REVEALS SUPPRESSION OF B-OXIDATION DUE TO CARNITINE DEFICIENCY.

Satsuki Miyata[1], Kaoru Tominaga, Eiji Sakashita,
Masashi Urabe, Yoshiyuki Onuki[1], Akira Gomi,
Takashi Yamaguchi[1], Makiko Mieno, Hiroaki Mizukami,
Akihiro Kume, Keiya Ozawa, Eijyu Watanabe[1],
Kensuke Kawai[1], Hitoshi Endou; [1]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 changes. The aim of this study was to investigate the mechanism for the better prognosis of gliomas with IDH 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 U87 glioblastoma cells overexpressing IDH1 or IDH1^{R132H} 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 IDH mutation. Gliomas with IDH mutation also showed decreased 2-oxoglutarate and downstream intermediates in the tricarboxylic acid cycle and pathways involved in production of energy, amino acids, and nucleic acids. The marked difference in the metabolic profile in IDH mutant clinical glioma samples compared with that of mutant IDH expressing cells includes a decrease in β-oxidation due to acyl-carnitine and carnitine deficiencies. CONCLUSIONS: These metabolic changes may explain the lower cell division observed in IDH mutant gliomas and may be one mechanism of the better prognosis in IDH mutant gliomas.

CBMS-07

SERINE SYNTHESIS AND ONE-CARBON METABOLISM IN GLIOMA CELLS TO SURVIVE GLUTAMINE STARVATION

Kazuhiro Tanaka[1], Takashi Sasayama[1], Takiko Uno[1],
Yuichi Fujita[1], Mitsuru Hashiguchi[1], Yasuhiro Irino, Eiji Kohmura[1];
[1]Department 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 redox maintenance and epigenetic regulation through nucleic acid and protein methylation, enhancing tumorigenicity 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 spectroscopy (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 control and glutamine-deprived groups. Serine synthesis was mediated through autophagy rather than glycolysis. Gene expression analysis identified upregulation of Methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) to regulate serine synthesis and one-carbon metabolism. Importantly, suppression of this metabolite impaired glioma cell survival in glutamine deprivation. In human glioma samples. MTHFD2 expressions were highest in poorly nutrient regions around "pseudopalisading necrosis". Serine-dependent one-carbon metabolism has a key role for glioma cells to survive glutamine starvation. These results may suggest the new therapeutic strategies targeting critical glioma cells adapting the tumor microenvironment.

CBMS-08

INVESTIGATION FOR NICOTINIC EFFECTS ON STEM CELL'S PROPERTY IN HSV-TK/GCV GENE THERAPY

Hiroaki Kenmochi[1], Tomohiro Yamasaki,
Tomoya Oishi, Makoto Horikawa,
Taisuke Yamamoto, Shinichiro Koizumi,
Tetsuro Sameshima, Hiroki Namba; [1]Department 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 variety 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 (miPS-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 (CM) with or without non-toxic nicotine concentrations was assessed using Matrigel Invasion Chamber. Nicotine effect on GJIC was assessed with scrape loading/dye transfer assay (SL/DT assay) for co-culture of stem cells and glioma cells (stem cell/glioma cell) or parachute assay for glioma cells alone using high-content analysis. RESULTS: MTT assay revealed 1 μM of 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 GJIC 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) GJIC 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

Kiyotaka Yokogami[1], Takashi Watanabe[1],
Shinji Yamashita[1], Asako Mizuguchi[1], Hideo Takeshima[1]; [1]Department 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 505CSC / 507FBS 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 measurements 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, DNMT1 and 3A. CONCLUSION: MYCN is a target of therapy in a patient with SHH type TP53 mutated medulloblastoma.

CBMS-12

PENTAMIDINE; TRANSLATIONAL RESEARCH FOR A NEW CHEMOTHERAPY TARGETING ON GLIOMA CELLS AND GLIOMA STEM CELLS USING DRUG REPOSITIONING

Sho Tamai[1], Sabit Hemragul[1], Jiapaer Shabierjiang[1],
Guangtao Zhang[1], Jiakang Zhang[1], Yi Wang[1],
Shingo Tanaka[1], Masashi Kinoshita[1], Atsushi Hirao, Mitsutoshi Nakada[1];
[1]Department 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 revealed 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 using drug repositioning approach. METHOD: We used two glioma cell lines, A172 and T98, and patient-derived glioma stem cell lines KGS01, KGS07 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. RESULT Proliferation ability was prohibited by pentamidine in both glioma cell lines and GSC lines. The half maximal inhibitory concentrations were 5–10 μM in glioma cell lines and 1–5 μM in GSC lines. Sphere forming assay revealed that size and number of spheres were reduced in both GSC lines, depending on concentration of pentamidine. In all cell lines, phosphorylation of extracellular signal-related kinase (ERK) and signal transducer and activator of transcription 3 (STAT3) were suppressed by pentamidine. DISCUSSION: Pentamidine is known as the therapeutic drug for pneumocystis