CBMS-05
COMPREHENSIVE METABOLOMICAL ANALYSIS OF IDH1R132H CLINICAL GLIOMA SAMPLES REVEALS SUPPRESSION OF Β-OXIDATION DUE TO CARNITINE DEFICIENCY.
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BACKGROUND: Gliomas with isocitrate dehydrogenase 1 (IDH1) mutation have alterations in several enzyme activities, resulting in various metabolic changes. The purpose of this study was to investigate the mechanism in better prognosis of gliomas with IDH1 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 expressing IDH1 or IDH1.R132H dDNA. 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 amino acids involved in production of energy, amino acids, and nucleic acids. The marked difference in the metabolic profile in IDH1 mutant clinical glioma samples compared with that of mutant IDH1 expressing cells includes a decrease in β-oxidation due to acyl-carnitine and carnitine deficiency. CONCLUSIONS: These metabolic changes may explain the lower tumor growth and survival from nutrient starvation. Here, we identify a key metabolic pathway for cancer cells to survive glutamine starvation and may be one mechanism of the better prognosis in IDH1 mutant gliomas.

CBMS-07
SERINE SYNTHESIS AND ONE-CARBON METABOLISM IN GLIOMA CELLS TO SURVIVE GLUTAMINE STARVATION
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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 glioma cells alone using high-content analysis. MTT assay revealed that size and number of spheres were reduced in both GSC lines, A172 and T98, and patient-derived glioma stem cell lines KGS01, by using drug repositioning approach. METHOD: We used two glioma cell lines, A172 and T98, and patient-derived glioma stem cell lines, A172 and T98, and patient-derived glioma stem cell lines, KGS01, by using drug repositioning approach. MATERIAL AND METHODS: Using chemical inhibitor of MYCN in glioma cells, we assessed the effect of serine deprivation on glioma stem cells migration capacity to gliomas. RESULTS: MTT assay revealed that size and number of spheres were reduced in both GSC lines, A172 and T98, and patient-derived glioma stem cell lines, KGS01, by using drug repositioning approach. CONCLUSIONS: These metabolic changes may explain the lower tumor tropism to gliomas and within glioma cells. HS-virk-GCV therapy may retain its therapeutic efficacy against gliomas even under physiological nicotinone concentrations.

CBMS-10
FUNCTIONAL ROLE OF MYCN IN SHH TYPE TP53 MUTATED MB’S METABOLISM
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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. Especially, commercially available SHH type TP53 mutated MB cell-line is only DAOY. We established new cell lines 305MSC/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 305MSC and DAOY. MATERIAL AND METHODS: Using chemical inhibitor of MYCN in 305MSC 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, G6PGL, MCTA2, DMN1 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 GLOMMA STEM CELLS AND GLOMMA STEM CELLS USING DRUG REPOSITIONING
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INTRODUCTION: Glioblastoma (GBM) is primary malignant brain tumor with poor prognosis. Despite aggressive chemotherapies, GBMs have recurrence and finally relapse. 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. METHODS: We repositioned nicotinic impact on stem cell properties including tumor tropism and gap junctional intercellular communication (GJIC), which is crucial to this therapeutic strategy. RESULTS: Mouse induced pluripotent stem cell (iPSC)-derived neural stem cells (iPS-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 revealed with scrape loading/dye transfer assay (SL/DT assay) for co-culture of stem cells and glioma cells (stem cellglioma cell) or parasite assay for glioma cells alone using high-content analysis. RESULTS: MTT assay revealed a 1 μM nicotine, equivalent to the minimal nicotine concentration in habitual smoking, is the maximum safe concentration for stem cells and glioma cells. Tumor tropism (iPS-NSCs to GL261-CM, hDPSCs to U251- or U87-CM) and GJIC of co-culture of stem cells and glioma cells (iPS-NSCS/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. HS-virk-GCV therapy may retain its therapeutic efficacy against gliomas even under physiological nicotinone concentrations.

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protections. In vivo, combination treatment of TMZ with Veliparib demonstrated inactivated TMZ resistant cells whereas only subtle combination effects in glioma xenografts, we tested the in vivo efficacy of veliparib in combination with TMZ. PARPi Veriparib or Olaparib, and combination. Using MSH6-deficient isogenic pairs of MSH6 wild type and MSH6-inactivated cells with TMZ, derived glioblastoma neurosphere lines to knockdown MSH6 expression, MSH6-deficient chemoresistant gliomas and assessed the role of the base predict benefit from this combination treatment have not been identified in non-germinomatous glioblastoma cell lines (NGGCT) are refractory, and development of novel therapy against NGGCTs is urgently needed. To develop a new therapeutic strategy against aggressive NGGCTs, we have investigated novel molecular targets for NGGCT treatment. We screened a total of 120 NGGCT tumor tissues (including 55 NGGCT), which were registered to the Intracranial Germ Cell Tumor Consortium (iGCC), and discovered multiple mutations of a molecule that regulates protein ubiquitination and degradation specifically in NGGCT cases (5 of 55 cases; 1 immature teratoma, 3 mixed germ cell tumors, and 1 embryonal carcinoma). An in vitro ubiquitination assay revealed the mutations of this molecule discovered in NGGCT cases were loss of function mutations. Reduced expression of this molecule by knockdown in an established human seminoma cell line Tcam2 or a human yolk sac tumor cell line YST1, which was recently established in our institute, resulted in enhanced proliferation as well as upregulation of MEK-ERK activation. Importantly, treatment of these two GCT cell lines with reduced expression of this molecule by MEK inhibitor trametinib suppressed augmentation of proliferation of these cells. Taken together, these results suggest that protein ubiquitination-related pathways as well as MEK-ERK cascade may serve as a novel therapeutic target against NGGCTs.

**SPDR-09**

**CHANGES IN CELL CYCLE-RELATED GENE EXPRESSIONS OF Glioblastomas BEFORE AND IMMEDIATELY AFTER CHEMORADIATION THERAPY.**

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**PURPOSE/OBJECTIVE:** The molecular responses of glioblastomas (GBMs) and Nongerminomatous Malignant CNS GTs (NGGCTs) were investigated to elucidate the molecular targets included in the resistance of these tumors to chemoradiation therapy. MATERIALS/METHODS: Phase I study of neo-adjuvant IMRT (72Gy/12F.s./TMZ for the treatment of patients with GBMs had been performed previously in our institution. In this trial, stereotactic biopsy of the tumor to confirm the pathological diagnosis prior to treatment was required, and tumor removal was scheduled within 10 days after completion of IMRT/TMZ. Therefore, both the tumor samples before and immediately after IMRT/TMZ were available. By comparing the gene expression profiles before and after IMRT/TMZ using the total mRNA sequencing (RNAseq) analysis, molecular responses of GBMs against IMRT/TMZ were investigated. More than two-fold change of expression levels was defined as significant. RESULTS: Tumor sample sets from five patients with GBMs were investigated. Among the 17,532 Groningen categories, 11 genes were found to show significant changes in gene expression in all cases, and 450 genes in more than half of the cases. Among the DNA repair related genes, DDR2 was the only gene that showed significant up-regulation in all cases. On the other hand, among the cell cycle checkpoint related genes, gene expressions of CDKN1A/CDC25A were decreased in all cases. Although the expression of TP53 was not changed, the expressions of CDKN1A/GADD45/Reprimo/SFN were also reduced. Moreover, although the expression change of CHK1 was not significant, the expressions of CDC25A/PLK1/Atm11 genes were decreased in more than half of the cases. From these results, it was considered that GBM arrested the cell cycle at the G2/M checkpoint without regulation of TP53 or CHK1 after IMRT/TMZ. CONCLUSIONS: Our results suggested that cell cycle arrest in G2/M plays a significant role in survival of GBM cells after IMRT/TMZ.