ANGI-2

IDENTIFICATION AND FUNCTIONS OF CD44 AS A PREDICTOR FOR BEVACIZUMAB-RESISTANT GLIOBLASTOMA TO OPTIMALLY TREAT THE TUMOR WITH BEVACIZUMAB

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Anti-angiogenic therapy with bevacizumab (Bev), a monoclonal antibody targeting vascular endothelial growth factor (VEGF), is a common treatment for recurrent glioblastoma (GBM), but its survival benefit is limited. Resistance to Bev is thought to be a major cause of ineffectiveness on Bev therapy. To optimize Bev therapy, identification of a predictive biomarker for responsiveness to Bev is required. Based on our previous study, we focused on the expression and functions of CD44 and VEGF in the Bev therapy. Here, we analyze a relationship between CD44 expression and responsiveness to Bev and elucidate the role of CD44 in anti-VEGF therapy. CD44 and VEGF expression in the tumor core and periphery of 22 GBMs was examined, and the relationship between expression of these molecules and progression-free time on Bev therapy was analyzed. The degree of CD44 expression in the tumor periphery was evaluated by the ratio of the mRNA expression in the tumor periphery to that in the tumor core (P/C ratio). VEGF expression was evaluated by the amount of the mRNA expression in the tumor periphery. To elucidate the roles of CD44 in the Bev therapy, in vitro and in vivo studies were performed using glioma stem-like cells (GSCs) and a GSC-transplanted mouse xenograft model, respectively. GBMs expressing high P/C ratio of CD44 were much more refractory to Bev than those expressing low P/C ratio of CD44, and the survival time of the former was much shorter than that of the latter. In vitro inhibition of VEGF with siRNA or Bev activated CD44 expression and increased invasion of GSCs. Bev showed no anti-tumor effects in mice transplanted with CD44-overexpressing GSCs. The P/C ratio of CD44 expression may become a useful biomarker predicting responsiveness to Bev in GBM. CD44 reduces the anti-tumor effect of Bev, resulting in much more highly invasive tumors.

Key words: glioma stem cell | bevacizumab | CD44

CELL BIOLOGY/METABOLISM/STEM CELLS (CBMS)

CBMS-1

TARGETING AMINO ACID METABOLIC VULNERABILITIES IN IDH-MUTANT AND IDH-WILDTYPE GLIOMAS

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IDH-wildtype glioma and IDH-mutant glioma have different genetical and metabolic background although their histological appearances are similar. To reveal the difference in metabolites between IDH-wildtype and IDH-mutant glioma, and to find the effective treatment targeting cancer metabolism according to the status of IDH in gliomas, two artificial cell lines made from normal human astrocyte, NHAE6E7hTERTRas (IDH-wildtype) and NHAE6E7hTERTIDHmut (IDH-mutant), were investigated. RNA-seq analysis revealed that about 10% of changed genes were involved with metabolism. Capillary electrophoresis- and ion chromatography-coupled mass spectrometry revealed that the amount of asparagine was lower in NHAE6E7hTERTRas cells compared with NHAE6E7hTERTIDHmut cells. L-asparaginase, which converts asparagine into aspartate, was more effective in former cells. L-asparaginase induced autophagy and inhibition of autophagy by 3-MA suppressed L-asparaginase-induced antitumor effect. Adding asparagine into the culture medium rescued the antitumor effect of L-asparaginase. L-asparaginase increased the expression of asparagine synthetase (ASNS) and inhibition of ASNS enhanced the antitumor effect of L-asparaginase. Metabolic assay also showed the lower amount of glutamine, glutamate and 2-oxoglutarate in NHAE6E7hTERTIDHmut cells than NHAE6E7hTERTRas cells. Inhibition of GLUD1 which converts glutamate to 2-oxoglutarate, suppressed proliferation of the cells by inducing ROS and apoptosis in NHAE6E7hTERTIDHmut cells. Exogeneous dimethyl 2-oxoglutarate rescued the cytotoxicity by GLUD1 inhibitor, suggesting decreased 2-oxoglutarate was associated with GLUD1 inhibitor-induced cytotoxicity. ROS inhibitor, NAC suppressed GLUD1 inhibitor-induced ROS, apoptosis, and cytotoxicity in NHAE6E7hTERTIDHmut cells, revealing that cytotoxicity by GLUD1 inhibitor was at least partially due to the inhibitor-induced ROS. Other IDH-wildtype glioma cells, U251 and U87 showed similar sensitivity to L-asparaginase and GLUD1 inhibitor to NHAE6E7hTERTRas, whereas U251 expressing mutant IDH1 showed similar sensitivity to GLUD1 inhibitor to NHAE6E7hTERTIDHmut, which suggested that the difference of sensitivity to each reagent was due to the status of mutant IDH. L-asparaginase and GLUD1 inhibitor will be new therapeutic options for IDH-wildtype glioma and IDH-mutant glioma, respectively.

Key words: cancer metabolism | glioma | IDH

CBMS-3

POTENT BYSTANDER EFFECT IN SUICIDE GENE THERAPY USING GENETICALLY ENGINEERED STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

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HSV thymidine kinase (TK)/ganciclovir (GCV) has a long history of application in malignant glioma and we have previously demonstrated its bystander effect on gliomas using several stem cell types as a vehicle. The main reason for applying stem cells is that they have a unique tumor-trophic activity that allows them to deliver TK genes efficiently to nearby the tumor. Stem cells from human exfoliated deciduous teeth (SHED) are mesenchymal stem cells easily harvested from dental pulp and no studies have reported suicide gene therapy using SHED as a carrier for malignant gliomas. For transduction of SHED with the HSVTK gene (SHEDTK), we used HSVTK retrovirus-producing cells.In vitro experiments showed a significant migration ability of SHEDTK toward tumor-conditioned medium and representative tumor growth factors. We also detected a significant bystander effect of SHEDTK on gliomas in the presence of GCV. In vitro time-lapse imaging showed that both SHEDTK and glioma cells underwent gradual morphological apoptosis and activation of caspase 3/7 was observed in both cell types. In intracranial tumor models using nude mice, SHEDTK migrated around the U87 cell mass implanted in the contralateral hemisphere. Additionally, coculture suspensions of SHEDTK and U87-luciferase cells were xeno-transplanted followed by intraperitoneal administration of GCV for 10 days. All mice of treatment group survived for more than 100 days, whereas those treated without GCV died of tumor growth with median survival of 42 days after tumor implantation. Furthermore, pre-existing intracranial U87 model mice were injected intratumorally with SHEDTK followed by GCV administration as described above. The tumor volume was significantly reduced during the treatment period, and over-all surivial in treatment group is prolonged significantly to that of control groups. These results indicate that SHEDTK-based suicide gene therapy might offer a new promising therapeutic modality for human malignant gliomas.

Key words: suicide gene therapy | stem cells from human exfoliated deciduous teeth | glioma

CBMS-5

ONE-CARBON METABOLISM PROTECT GLIOMA CELLS UNDER GLUTAMINE STARVATION THROUGH UPREGULATION OF MTHFD2

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Cancer cells optimize nutrient utilization to supply energetic and biosynthetic pathways. However, less is known about how cancer cells exhibit metabolic flexibility to sustain cell growth and survival from nutrient starvation. Here, we find that serine and glycine levels were higher in low-nutrient regions of tumors in glioblastoma multiforme (GBM) patients than they were in other regions. Metabolic and functional studies demonstrated that serine availability and one-carbon metabolism support glioma cell survival following glutamine deprivation. Serine synthesis was mediated through autophagy rather than glycolysis. Gene expression analysis identified upregulation of methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) to regulate one-carbon metabolism. In clinical samples, MTHFD2 expression was highest in the nutrient-poor areas around pseudopalisading necrosis. Genetic suppression of MTHFD2 and autophagy inhibition caused

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

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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

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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

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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

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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