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