with reduced expression of this molecule by MEK inhibitor trametinib suppressed augmented 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.

CBMS-12

PRO RENIN RECEPTOR ANTIBODY REGULATES GLIOBLASTOMA STEMNESS

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OBJECTIVE: Glioblastoma multiforme (GBM) is characterized by a strong self-renewal potential and poor differentiated state. We previously reported that (pro)renin receptor (PRR) was a potential target for glioma therapy by silencing the gene of PRR. Here, we have developed the monoclonal antibody of PRR and examined their effects on GBM. Materials and METHODS: We performed immunohistochemical analysis to detect the protein expression of PRR and SOX-2 in human sample of 56 gliomas. We used human glioma cell lines (U251MG and U87MG) and glioma stem cell line (MGG23) in vitro study. PRR antibody was designed to target the extracellular domain of the PRR with the rat lymph node method. Expression of the Wnt signaling components and stem marker (SOX-2, Oct3/4) in human glioma cell lines and glioma stem cell line treated with PRR antibody were measured using Western blotting. The effects of PRR antibody on cell proliferation, sphere formation, apoptosis, invasion were also examined. Subcutaneous xenografts with U87MG were induced in nude mice. RESULTS: PRR expression showed a positive correlation with SOX-2 expression in glioma samples. Treatment with PRR antibody significantly reduced expression of Wnt signaling components and stem marker. We observed that PRR antibody significantly reduced cell proliferation and decreased sphere formation. Furthermore, PRR antibody suppressed invasion and induced apoptosis. In a subcutaneous U87MG xenograft model, systemic administration of the PRR antibody significantly reduced the size of the tumor volume. CONCLUSION: PRR has important role for the maintenance of stem cells and contribute to stem cell proliferation. PRR antibody inhibits cell proliferation and cell invasion and induces apoptosis. Treatment with PRR antibody could be an attractive therapeutic strategy for GBM.

SIGNALING PATHWAYS/DRUG RESISTANCE (SPDR)

SPDR-01

PAIRED EPITHELIOID GLIOBLASTOMA PATIENT-DERIVED XENOGRAFT MODELS TO EVALUATE RESISTANT MECHANISM FOR MOLECULAR TARGET THERAPY

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Epithelioid glioblastoma (E-GBM) arises at younger age, commonly disseminates to cerebrospinal fluid, and results in dismal prognosis. About half of E-GBM harbors *BRAF* V600E mutation, thus BRAF/MEK inhibitors are expected to be specifically sensitive to E-GBM like other *BRAF* V600E mutant carcinomas. However, therapeutic effect is limited by the emergence of drug resistance. To overcome this issue, it is crucial to elucidate the treatment resistance mechanisms by clinically representative models. Herein, we establish 2 paired E-GBM patient-derived xenograft (PDX) models from young adult patients (YMG62 and YMG89) with *BRAF* V600E, *TERT* promoter mutations and *CDKN2A* homozygous deletions. The YMG62 patient received dabrafenib with trametinib, while YMG89 patient received dabrafenib monotherapy after recurrence with standard treatment. The YMG62 patient was refractory to combination therapy. The YMG89 patient was initially responded to dabrafenib, but gradually became resistant and the 2 patients died due to CNS dissemination. Paired PDX models were established from tumors prior and after molecular target therapy. All PDXs were formed as CNS dissemination model, which were recapitulated to the patient characteristics. BRAF/MEK inhibitors strongly suppressed cell viability in primary tumor (YMG89P). However, BRAF/MEK inhibitors became resistant in recurrent tumor (YMG89R). YMG62 paired PDXs were resistant to molecular target therapy. Western blotting indicated retained MAPK signaling pathway and/or increased AKT phosphorylation after BRAF/MEK inhibitors treatment in refractory and recurrent cells, which indicates crucial role of re-activation in the MAPK signaling pathway and/or PI3 kinase pathway for tumor maintenance in *BRAF* V600E mutant E-GBM. We have done high throughput drug screening to identify compounds to overcome resistant to molecular target therapy. Our established E-GBM paired PDX models recapitulate patient characteristics, which may uncover treatment resistant mechanism and novel therapeutic target in E-GBM.

GENETICS/EPIGENETICS (GEN)

GEN-09

PURSUING THE FUNCTION OF MICRORNA TARGETING (PRO) RENIN RECEPTOR AGAINST GLIOMA

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(Pro)renin receptor((P)RR) is a part of the Wnt receptor complex. Wnt/β-catenin signaling pathway (Wnt signaling) plays important role in pathogenesis and self-renewal of glioblastoma (GBM), or differentiation of glioma stem cell. We previously reported that (P)RR activated Wnt signaling, (P)RR expression correlated with malignancy of glioma, and treatment with (P)RR siRNA reduced the proliferative capacity. This time, we have searched for over 2632 microRNAs by microRNA microarray that its expression is affected by (P)RR whether overexpressed or suppressed and examined their effects in GBM cell lines or its glioma stem cells.

GEN-14

DUAL REGULATION OF HISTONE METHYLATION BY MTOR COMPLEXES DRIVES THE PROGRESSION OF EGFR-MUTANT GLIOBLASTOMA

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Trimethylation of histone H3 on lysine 27 (H3K27me3) is essential for ensuring proper gene expression and chromosomal function, and its aberration is associated with the pathogenesis of various brain tumors. However, it remains unclear how histone methylation is regulated in response to genetic mutation and intracellular metabolic status to facilitate the cancer cell survival in the most malignant IDH-wildtype glioblastoma (GBM). We herein report a novel mechanism of the specific regulation of H3K27me3 by cooperative action of two mechanistic target of rapamycin (mTOR) complexes in EGFR-mutant GBM. The level of H3K27me3 is significantly associated with the mutant EGFR signaling (EGFRvIII and EGFR amplification), and integrated analyses with histopathological, NGS and metabolome examinations revealed that both mTOR complexes (mTORC1 and mTORC2) upregulate H3K27me3 downstream of aberrant EGFR signaling. mTORC1 facilitates the protein translation of enhancer of zeste homolog 2 (EZH2), which is known as H3K27-specific methyltransferase. The other mTOR complex, mTORC2, remodels the metabolism of S-adenosylmethionine (SAM), an essential substrate for histone methylation. This synergistic mechanism causes H3K27 hypermethylation which subsequently promotes tumor cell survival both in vitro and in vivo mouse tumor model via regulation of the cell cycle-related tumor suppressor genes. The findings indicate that activated mTORC1 and mTORC2 complexes under aberrant EGFR signaling cooperatively contribute to the progression of IDH-wildtype GBM through specific epigenetic regulation, nominating them as an exploitable therapeutic target against cancer-specific epigenetics.

EXPERIMENTAL THERAPEUTICS (ET)

ET-03

CONVECTION-ENHANCED DELIVERY OF EZH2 INHIBITOR FOR THE TREATMENT OF DIFFUSE MIDLINE GLIOMA

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BACKGROUND: Diffuse midline glioma (DMG) is a fatal childhood brain tumor and the majority of patients die within 2 years after initial diagnosis. Factors that contribute to the dismal prognosis of these patients include the infiltrative nature and anatomic location in an eloquent area of the brain, which precludes total surgical resection, and the presence of the blood-brain barrier (BBB), which reduces the distribution of systemically administered agents. Convection-enhanced delivery (CED) is a direct infusion technique to deliver therapeutic agents into a target site in the brain and able to deliver a high concentration drug to the infusion site without systemic toxicities. OBJECTIVE: This study aims to assess the efficacy of enhancer of

zeste homolog-2 (EZH2) inhibitor by CED against human DMG xenograft models. METHODS: The concentration of EZH2 inhibitor (EPZ-6438) in the brainstem tumor was evaluated by liquid chromatography mass spectrometry (LC/MS). We treated mice bearing human DMG xenografts with EPZ-6438 using systemic (intraperitoneal) or CED administration. Intracranial tumor growth was monitored by bioluminescence image and the therapeutic response was evaluated by animal survival. RESULTS: LC/MS analysis showed that the concentration of EPZ-6438 in the brainstem tumor was 3.74% of serum concentration after systemic administration. CED of EPZ-6438 suppressed tumor growth and significantly extended animal survival when compared to systemic administration of EPZ-6438 (P = 0.0475). CONCLUSION: Our results indicate that CED of an EZH2 inhibitor is a promising strategy to bypass the BBB and to increase the efficacy of an EZH2 inhibitor for the treatment of DMG.

ET-04

ENHANCING DRUG DELIVERY WITH MRI-GUIDED FOCUSED ULTRASOUND FOR DIFFUSE INTRINSIC PONTINE GLIOMA MODEL

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Diffuse intrinsic pontine glioma (DIPG) is surgically unresectable and one of the most devasting tumours in children. To date, there have been no effective chemotherapeutics against DIPG, despite a myriad of clinical trials. The intact blood-brain barrier (BBB) is partly responsible for the limited clinical response to chemotherapy. MRI-guided focused ultrasound (MRgFUS) is a promising non-invasive tissue ablation method for treating CNS tumours. Moreover, MRgFUS allows for temporary and repeatable BBB disruption. Our first objective was to determine the feasibility and safety of temporary BBB disruption within the brainstem using MRgFUS following intravenous administration of microbubbles in vivo. Our second objective was to select effective chemotherapeutics against DIPG cell lines, and to examine their therapeutic effects with MRgFUS in a murine model of DIPG which exhibits an intact BBB. Non-invasive opening of the BBB was determined in the brainstem of normal rodents using physiological monitoring and histological analysis. Doxorubicin was selected from a drug screen consisting of conventional chemotherapeutics tested against DIPG cell lines. We established SU-DIPG17 orthotopic xenografts which demonstrated diffusely infiltrative tumour growth. By LC-MS/MS analysis, MRgFUS led to a 4-fold increase in doxorubicin concentrations within the brainstem tumours as compared to controls. Moreover, the volumetric tumour growth rate was significantly suppressed in MRgFUS-treated animals, which also exhibited decreased Ki-67 expression. We demonstrated the feasibility and safety of MRgFUS in the rodent brainstem and have shown that MRgFUS increases doxorubicin uptake in the brainstem of a rodent model of DIPG. This preclinical data provides critical support for clinical trials investigating MRgFUS-mediated BBB opening, which may greatly improve chemotherapeutic efficacy against DIPG in children.

ET-05

ALECTINIB AND CERITINIB, THE SECOND-GENERATION ALK INHIBITORS, EFFECTIVELY INDUCE GLIOBLASTOMA CELL DEATH

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Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that only expresses in the developmental stage of the central and peripheral nervous system. A variety of ALK gene alterations, such as oncogenic fusion, activating point mutation, or wild type gene amplification, have been recently discovered as the powerful oncogene in various tumors. These ALK mutations are expected as potential therapeutic targets. Some ALK inhibitors have already been approved and used for the clinical treatment of non-small cell lung cancers harboring oncogenic ALK fusion.

Previously, we reported classical ALK inhibitors triggered cell death in human glioblastoma (GBM) cells, which did not express ALK, via suppression of transcription factor STAT3 activation but not in normal tissue-derived cells.

In this study, we investigated the anti-tumor effect of newly-developed ALK inhibitors in GBM cells. As a result, second-generation ALK inhibitors, alectinib and ceritinib, induced cell death in various human GBM cell lines with lower concentrations than other ALK inhibitors. Also, alectinib and ceritinib suppressed STAT family activity in these GBM cell lines. We consider alectinib and ceritinib might be a novel therapeutic agent against GBMs. Further investigation about the specific anti-tumor mechanism of these second-generation ALK inhibitors in GBM cells is currently on-going.

ET-06

SUPPRESSION OF GLIOBLASTOMA THROUGH NOVEL DRUG BASED ON "GENE SWITCH TECHNOLOGY"

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Glioblastoma (GBM) is the most common and aggressive malignancy primarily affecting adults. Despite intensive multimodal therapies, the prognosis of GBM is dismal and a novel therapy is needed. Here, we focused on RUNX, a transcription factor involved in the malignant transformation of GBM, and developed a novel Chlorambucil-conjugated PI-polyamides (Chb-M'), which "switches off" RUNX family. Chb-M' specifically recognizes the consensus RUNX-binding sequences (TGTGGT) and alkylates it to inhibit transcription of the downstream gene of RUNX family. Chb-M' has been shown to induce apoptosis and suppress proliferation in a variety of cancers including leukemia, and in this study, similar results were found for glioblastoma cells in vitro. Specific inhibition of RUNX1 led to a marked inhibition of tumor growth through cell cycle arrest and apoptosis. By using apoptosis array, we isolated several candidate genes which regulated by RUNX1. And some types of glioblastoma cell lines treated with Chb-M' showed elevated expression of p21 and decreased survivin. From in silico analysis using glioma patient cohorts, survivin expression was significantly higher in GBM and it was possibly involved in maintaining the malignancy of GBM. Mechanistically survivin was found to be directly transcriptionally regulated by RUNX1 through ChIP assay and reporter assay. In addition, survivin K/D cells upregulated p21 expression and accelerated apoptosis. Taken together, we hypothesized that the RUNX1-survivin-p21 pathway can potentially be exploited in the management of this malignancy. Chb-M' mediated regulation of RUNX1 can be a novel therapeutic strategy against GBM.

COMPUTATIONAL OMICS (CO)

CO-01

PREDICTION OF PATHOLOGICAL AND RADIOLOGICAL NATURE OF GLIOMA BY MASS SPECTROMETRY COMBINED WITH MACHINE LEARNING

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BACKGROUND: We have previously developed a medical diagnostic pipeline that employs mass spectrometry and machine learning. It does not annotate molecular markers that are specific to cancer but uses entire mass spectra for predicting the properties of glioma. OBJECT: To validate the power of our diagnostic method in predicting the pathological and radiological properties of glioma with a simple sample preparation procedure. METHODS: Ten patients with glioma and 4 non-glioma patients who went through surgical resection were enrolled in our hospital. A total of 1020 mass spectra were acquired from 88 specimens. In order to examine the prediction power of the diagnostic pipeline that we have developed, we performed ten-fold cross-validation for pathological and radiological findings and calculated agreement rates with the conventional methods such as pathological diagnosis (WHO grading, MIB-1 labeling index (LI), mutations in the isocitrate dehydrogenase (IDH)-1 gene and positive 5-ALA fluorescence) and radiological information (gadolinium (Gd)-enhanced area, high-intensity area on fluid-attenuated inversion recovery (FLAIR) imaging,). RESULTS: Prediction accuracy for WHO malignant grade was 91.37%. Those for MIB-1 LI more than 10% and IDH-1 mutation-positive were 82.84% and 87.75%, respectively. Our method achieved an accurate prediction of 95.00% for the 5-ALA-positive lesion. The present method displayed an accuracy of 82.36% in predicting the area of FLAIR hyperintensity and 81.27% for the Gd enhanced area. CONCLUSION: Our methodology achieved a higher rate of prediction of glioma in terms of pathology and radiology. Research is ongoing to develop a validation cohort to verify the biological profiles of glioma specimens.