CBMS-02
PHOSPHOGLYCERATE MUTASE 1 (PGAM1) CONTROLS DNA DAMAGE REPAIR VIA REGULATION OF WIP1 ACTIVITY
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Phosphoglycerate Mutase 1 (PGAM1) is overexpressed in different forms of cancer and has been suggested to have additional functions beyond its role as a metabolic enzyme. We here report that PGAM1 is overexpressed in GBMs and indirectly regulates activation of ATM, Chk1 and Chk2 but not ATR, thereby increasing the efficiency of DNA damage repair and resistance to irradiation (IR) and temozolomide (TMZ) treatment. Genetic suppression of PGAM1 in multiple GBM cell lines resulted in decreased proliferation, apoptosis and colony formation after irradiation and temozolomide treatment as compared to parental cells. Moreover, parental cells demonstrated DNA damage (pH2AX foci) whereas isogenic PGAM1 knockdown cells exhibited no DNA damage repair activation and a significant increase in sub-G0 apoptotic cells that expressed annexin-V, cleaved caspase-3 and cleaved PARP-1. Mechanistically, suppression of PGAM1 expression inhibited phosphorylation of ATM at s1981 and the downstream downstream phosphorylation of Chk2 and cdc25C. Moreover, PGAM1 co-immunoprecipitated with WIP1, a phosphatase reported to bind and dephosphorylate ATM, Chk1, and Chk2. Cytoplasmic binding of WIP1 with PGAM1 prevented nuclear localization of WIP1, leaving ATM and its downstream substrates phosphorylated, which is required for DNA damage repair activity. Consistent with these observations, mice intracranially implanted with PGAM1 knockdown GBM cells and treated with TMZ and IR had longer survival than similarly treated mice implanted with matched control cells. These results therefore define PGAM1 as a regulator of DNA damage repair pathways leading to tumor metabolism and drug resistance in GBM.

CBMS-04
NOVEL XENOGRAFT MODEL TO CLARIFY TUMOR PROGRESSIVE MECHANISM AND THERAPEUTIC TARGET IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA
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Primary central nervous system lymphoma (PCNSL) is a rare lymphoma of the central nervous system and has a dismal prognosis despite extensive chemotherapy. Recent genomic analyses have identified recurrent genetic alterations in primary central nervous system lymphoma (PCNSL). However, lack of clinically representative PCNSL mouse models has diminished our understanding of the pathogenic mechanisms of these genetic events. Here, we established 14 patient-derived orthotopic xenografts (PDOXs). Comprehensive analysis showed that PDOXs faithfully retained the phenotypic, metabolic, and genetic features with 100% concordance of MYD88 and CD79B mutations present in immunocompetent PCNSL patients. Notably, orthotopic xenograft formation was consistently dependent on deregulated signaling through the RelA/p65-hexokinase 2 (HK-2) axis. MYD88/CD79B mutations in immortalized human PCNSL patients. Notably, orthotopic xenograft formation was consistently dependent on deregulated signaling through the RelA/p65-HK-2 signaling in immunocompetent and EBV-positive PCNSL, respectively. Genetic and pharmacological inhibition of this key signaling axis potently suppressed PCNSL tumor growth in vitro and in vivo. Additionally, our models further offer a platform for predicting clinical chemotherapeutics efficacy. Therefore, our models provide critical insights into pathogenic mechanisms and therapeutic discovery in PCNSL.

CBMS-05
BIOLOGICAL AND PATHOLOGICAL MEANING OF ANEUPLOIDY IN MOUSE GLIOMA STEM CELL
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Chromosomal instability (CIN) is a pathological condition where cells continuously mis-segregate chromosomes, producing aneuploid cells. CIN has been recognized as a hallmark of cancer, and its correlation with biological malignancy has been pointed out. Glioma cell line often reveals karyotype aberrations, and a variety of ploidy in the tumor tissue. However, several studies have indicated that aneuploidy is disadvantageous for proliferation or tumorigenesis, and these paradox prompt us to address the role of aneuploidy in glioma stem cells. Here, we adopted mouse glioma stem cell lines and found that aneuploid population is increased in glioma stem cells in vitro. We also examined Aurora B at centromeres which is crucial for failsafe chromosome segregation and found its reduced activity in glioma stem cells, suggesting that insufficient Aurora B activity plays a causative role in CIN in glioma stem cells. Next, to investigate the tumorigenicity of aneuploid cells, we sorted the glioma stem cells depending on the karyotype pattern, and allografted into mouse brain. We found that the growth rate of diploid glioma stem cells was higher than others in vitro, and the probability of survival after allogeneic transplantation was significantly lower in diploid groups. We will discuss the role of ploidy in glioma cell populations.

CBMS-09
INTERCELLULAR COMMUNICATION AT GLIOBLASTOMA STEM CELL NICHES
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Glioblastoma multiforme (GBM) contains heterogeneous population of cells including a small population of GBM stem cells (GSCs), which potentially cause therapeutic resistance and tumor recurrence. GSCs harbored in special microenvironments, such as perinecrotic niche, perivascular niche, perytumoural niche. However, the microenvironments underlying the pathogenesis and maintenance of GSCs remain largely unknown. Stemness and chemo-radioresistance was promoted by not only additional mutation, but also microenvironment of GBM cells. Previously, we had reported that growth factors, cytokines secreted by oligodendrocyte lineage cells and macrophages/microglia induce stemness and chemo-radioresistance into GBM cells. Recently, Ito et al. reported that incorporation of ribosomes and ribosomal proteins into somatic cells promoted lineage trans-differentiation toward multipotency. Ribosomal proteins present in extra- and intracellularly. There is a possibility that ribosomal proteins promote stemness into cancer cells, we focused on 40S ribosomal protein S6 (RPS6), which is related to cell proliferation in lung and pancreatic cancer, but not reported in GBM. RPS6 was significantly upregulated in glioblastoma and gliosarcoma knock-down significantly suppressed the characteristics of GCSs, including their tumosphere potential and stemness marker expression, such as Nestin and Sox2. RPS6 overexpression enhanced the tumorsphere potential of GSCs. Moreover, RPS6 expression was significantly correlated with SOX2 expression in different glioma grades. Immunohistochemistry data indicated that RPS6 was predominant detected at GSC niches, concurrently with the data from IVY GAP databases. Furthermore, RPS6 and other ribosomal proteins were upregulated in GSC-predominant areas in this database. The present results indicate that, in GSC niches, ribosomal proteins play crucial roles in the development and maintenance of GSCs and are clinically associated with chemo-radioresistance and GBM recurrence. These results suggested that intercellular communications through growth factors, cytokines, and ribosomes are regarded as new treatment targets of GBM.

CBMS-11
PROTEIN DEUBIQUITINATION PATHWAY IS A NOVEL THERAPEUTIC TARGET AGAINST MALIGNANT CNS NON-GERMINOMATOUS GERM CELL TUMORS
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Central nervous system germ cell tumors (CNSGCTs) are rare intracranial neoplasm usually developed in adolescents and young adults. However, in East Asia including Japan, incidence of CNSGCTs is considerably higher compared with other regions of the world. Whereas germinomas generally respond to chemo-radiotherapy well, malignant subtypes of non-germinomatous germ cell tumors (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 CNSGCT tumor tissues (including 55 NGGCT), which were registered to the Intracranial Germ Cell Tumor Consortium (iGCT), 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 knockdown in an established human seminoma cell line Tcam2 for 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 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 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 egrf-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 mutations and intracellular signaling. In this study, we focused on 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 activation of 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