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 in metabolism. 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 radiation (IR) and temozolomide (TMZ) treatment. Genetic suppression of PGAM1 in multiple GBM cell lines resulted in decrease proliferation, apoptosis and colony formation after radiation and temozolomide treatment compared to parental cells. Moreover, parental cells demonstrated DNA damage (gH2AX 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 subsequent 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 an activator of DNA damage repair pathway and link tumor metabolism to drug response 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 intensive chemotherapy. Recent genomic analyses have identified recurrent genetic alterations in Primary central nervous system lymphoma (PCNSL). However, lack of clinically representative PCNSL models has diminished our understanding of the pathogenic mechanisms of those 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 immuno-competent PCNSL patients. Notably, orthotopic xenograft formation was consistently dependent on deregulated signaling through the RelA/p65-hexokinase 2 (HK-2) axis. MYD88/CD79B mutations and Pin1 activation, or LMP1 and Pin1 activation, converge on 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 SELL

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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 aneuploid cells in tumor specimens. Here, we adopted mouse glioma stem cell lineages 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 transplant was significantly lower in diploid groups. We will discuss the role of ploidy in glioma cells populations.

CBMS-09

INTERCELLULAR COMMUNICATION AT GLIOBLASTOMA STEM CELL NICHES

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Glioblastoma multiforme (GBM) contains heterogenous 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, border niche. However, the mechanisms 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 and 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 exist intra- and extracellularly. 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 high-grade glioma. SiRNA-mediated RPS6 knock-down significantly suppressed the characteristics of GSCs, including their tumorsphere 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 compare 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 gem 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