jirovecii. In this study, pentamidine suppressed proliferation activity in all cell lines, and stemness in both GSCs. Previous papers revealed pentamidine had anti-tumor effects for some types of tumor cell lines, however, therapeutic effect for tumor stem cells have never been mentioned. CONCLUSION: These results suggest that pentamidine would be therapeutic drug for not only glioma cells but also GSCs by suppressing phosphorylation of ERK and STAT3.

SIGNALING PATHWAYS/DRUG RESISTANCE (SPDR)

SPDR-01

INHIBITION OF HOMOLOGOUS RECOMBINATION, PARP INHIBITOR, OR DIANHYDROGALACTITOL OVERCOMES TEMOZOLOMIDE-RESISTANCE IN GLIOMA CELLS.

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Glioblastoma is one of the most aggressive tumors, with 5-year survival rates of less than 10%. The standard therapy for glioblastomas is maximal safe resection, followed by radiation therapy and chemotherapy with temozolomide (TMZ). The poor prognosis is partially contributed to the acquisition of resistance to TMZ and intratumoral heterogeneity. The mechanisms of resistance to TMZ are various due to tumor heterogeneity. TMZ is a DNA-methylating agent, delivering a methyl group to DNA (O6-guanine, N7-guanine and N3-adenine). The primary cytotoxic lesion, O6-methylguanine, mispairs with thymine, leading to futile DNA mismatch repair (MMR), formation of double strand breaks (DSBs) and eventual cell death, when O6-methylguanine DNA methyltransferase (MGMT) is absent. N7-methylguanine and N3-methyladenine are repaired by base excision repair (BER). The object of the study was to reveal the mechanisms of resistance to TMZ and to find the way to overcome the resistance in glioma. Several clones of TMZ-resistant U251 or U87 were obtained and analyzed. Increased homologous recombination (HR) and deficiency of MMR system, not MGMT were revealed to be contributed to the resistance to TMZ. Inhibition of HR resensitized cells with high HR to TMZ, but it could not resensitize cells with deficient MMR. For the cells with deficient MMR, Inhibition of BER by PARP inhibitor was revealed to potentiate the TMZ-induced cytotoxicity. PARP inhibitors also potentiate the cytotoxicity of TMZ to cells with expressed MGMT. Dianhydrogalactitol (DAG) is a bifunctional DNA-targeting agent, forming N7 alkylguanine and inter-strand DNA crosslinks. DAG reduced the proliferation of cells independent of MGMT and MMR, inducing DNA DSBs, G2/M arrest, and apoptosis in TMZ-resistant glioma cells. Inhibition of chk1, or HR could enhance the cytotoxicity of DAG, increasing apoptosis cells. By selecting the appropriate treatments to the types of resistant mechanisms, these new treatments have the potential to improve the prognosis of glioblastoma.

SPDR-05

PARP INHIBITORS RESTORE TEMOZOLOMIDE SENSITIVITY IN MSH6-DEFICIENT TEMOZOLOMIDE-RESISTANT GLIOBLASTOMA CELLS

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INTRODUCTION: Mismatch repair (MMR) deficiency through MSH6 inactivation has been identified in approximately 25% of recurrent gliomas. This MMR deficiency represents a key molecular mechanism of acquired resistance to the alkylating chemotherapeutic agent temozolomide (TMZ). Potentiation of TMZ-induced cytotoxicity by PARP inhibitors (PARPi) has been reported in several cancers including gliomas. However, mechanisms that underlie the PARPi-mediated chemo-potentiation and biomarkers that predict benefit from this combination treatment have not been identified in gliomas. We investigated whether PARPis could restore TMZ sensitivity of MSH6-deficient chemoresistant gliomas and assessed the role of the base excision repair (BER) DNA damage repair pathway in PARPi-mediated effects. METHODS: We engineered glioblastoma cell lines and patient-derived glioblastoma neurosphere lines to knockdown MSH6 expression, resulting in acquired MMR-deficient resistance to TMZ. We treated these isogenic pairs of MSH6 wild type and MSH6-inactivated cells with TMZ, PARPi Veriparib or Olaparib, and combination. Using MSH6-deficient glioma xenografts, we tested the in vivo efficacy of veliparib in combination with TMZ. We used genetic and pharmacological approaches to assess the role of BER pathway in PARPi-mediated effects. RESULTS: We found that combination with PARPi restored TMZ sensitivity in MSH6-inactivated TMZ resistant cells whereas only subtle combination effects were seen in control MMR-proficient cells at the same PARPi concentrations. In vivo, combination treatment of TMZ with Veliparib demonstrated potent suppression of tumor growth of MSH6-inactivated orthotopic and flank xenografts, compared with TMZ monotherapy. Unlike PARPi, genetic and pharmacological blockage of BER pathway did not re-sensitize MSH6-inactivated cells to TMZ. CONCLUSION: PARPis restore TMZ sensitivity in MSH6-deficient glioblastoma cells. This combination treatment is a promising strategy to target acquired chemoresistance caused by MMR deficiency.

SPDR-06

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 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.

SPDR-09

CHANGES IN CELL CYCLE-RELATED GENE EXPRESSIONS OF GLIOBLASTOMAS BEFORE AND IMMEDIATELY AFTER CHEMO-RADIATION THERAPY

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PURPOSE/OBJECTIVE: The molecular responses of glioblastomas (GBMs) to hypofractionated IMRT/TMZ were investigated to elucidate the molecular targets included in the resistance of these tumors to chemo-radiation therapy. MATERIALS /METHODS: Phase I study of neo-adjuvant IMRT (72Gy/12Fx.)/TMZ for the treatment of patients with GBMs had been performed previously in our institution. In this trial, stereotactic biopsy of the tumor to confirm the pathological diagnosis prior to treatment was required, and tumor removal was scheduled within 10 days after completion of IMRT/TMZ. Therefore, both the tumor samples before and immediately after IMRT/TMZ were available. By comparing the gene expression profiles before and after IMRT/TMZ using the total mRNA sequencing (RNAseq) analysis, molecular responses of GBMs against IMRT/TMZ were investigated. More than two-fold change of expression levels was defined as significant. RESULTS: Tumor sample sets from five patients with GBMs were investigated. Among the 17,532 genes evaluated, 35 genes were found to show significant changes in gene expression in all cases, and 450 genes in more than half of the cases. Among the DNA repair related genes, DDB2 was the only gene that showed significant up-regulation in all cases. On the other hand, among the cell cycle checkpoint related genes, gene expressions of CKD1/CCNB were decreased in all cases. Although the expression of TP53 was not changed, the expressions of CDKN1A/GADD45/Reprimo/SFN were also reduced. Moreover, although the expression change of CHK1 was not found, the expressions of CDC25/PLK1/AURKA were decreased in more than half of the cases. From these results, it was considered that GBM arrested the cell cycle at the G2/M checkpoint without regulation of TP53 or CHK1 after IMRT/TMZ. CONCLUSIONS: Our results suggested that cell cycle arrest in G2/M plays a significant role in survival of GBM cells after IMRT/TMZ.