investigate the effect of MAPK pathway inhibitors on cancer cells and tumor-infiltrating immune cells to maximize the therapeutic efficacy in malignant gliomas. Methods. Drug concentrations in tumor, brain and plasma were assessed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). RNA sequencing and Gene Set Enrichment Analysis were performed using patient-derived BRAF-mutant glioma lines upon D+T treatment. Molecular profiles of drug-resistant clones were assessed for understanding of glioma heterogeneity and exploring new therapeutic targets. Results. BRAF-mutant stem-like glioma cells were particularly resistant to BRAF or MAPK inhibitor, along with aggressive phenotype in mice. LC-MS/MS showed effective D+T drug delivery in tumor regions. The transcriptome analysis demonstrated that D+T upregulate HLA molecules and downregulate immunosuppressive factors in patient-derived BRAF-mutant glioma lines. Consistent with these molecular changes, D+T led to changes in the proportions of tumor-infiltrating immune cells, including CD8+ cytotoxic T lymphocytes and FOXP3+ regulatory T cells. Furthermore, the therapeutic effect of D+T was further enhanced in combination with immune checkpoint inhibition. Conclusions. The present study highlights the immunomodulatory activity of MAPK pathway inhibitors in BRAF-mutant gliomas.

BIOL-06. MIR-1253 POTENTIATES CISPLATIN RESPONSE IN PEDIATRIC GROUP 3 MEDULLOBLASTOMA BY REGULATING FERROPTOSIS

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Medulloblastoma (MB), the most common malignant pediatric brain tumor and a leading cause of childhood mortality, is stratified into four primary subgroups, i.e. SHH (sonic hedgehog), WNT (wingless), and non-SHH/WNT groups 3 and 4, the latter representing high-risk MB. Haploinsufficiency of 17p13.3, which houses the tumor suppressor gene miR-1253, characterizes high-risk tumors. Despite improvements in targeted therapies, a limited proportion of these patients survive the disease. Capitalizing on the tumor suppressive properties of miRNAs as adjuncts to chemotherapy provides a promising alternative to current therapeutic strategies. In this study, we explored the potentiating effects of miR-1253 on cisplatin cytotoxicity in group 3 MB. First, *in silico* and *in vitro* analyses revealed an upregulation of ABCB7, a mitochondrial iron transporter and putative target of miR-1253, in MB cell lines and group 3 MB tumors. Overexpression of miR-1253 resulted in downregulation of ABCB7 and GPX4, a critical ferroptosis regulator, which consequently increased labile mitochondrial iron pool and, in turn, mitochondrial ROS (mtROS). Complementarily, we demonstrated, using CRISPR knockdown of ABCB7, ferroptosis induction with downregulation of GPx4 expression, liberation of free iron, mtROS generation and lipid peroxidation. Cisplatin is reported as an inducer of both apoptosis and ferroptosis-mediated cancer cell death. Therapeutically, the combination of miR-1253 and cisplatin led to an additive effect on cell viability, colony formation, apoptosis, and ROS generation. In turn, treatment with mtROS inhibitor (MnTBAP) and ferroptosis inhibitor (Ferrostatin) lead to partial recovery from the cytotoxic effects of this combination therapy. These studies identify an miR-1253-induced ferroptosis pathway targeting the ABCB7/GPX4/mtROS axis in group 3 MB. They further provide proof-of-concept in using miR-based therapeutics to augment treatment efficacy of current chemotherapeutics in the treatment of high-risk tumors.

BIOL-07. MIR-212 FUNCTIONS AS A TUMOR SUPPRESSOR GENE IN GROUP 3 MEDULLOBLASTOMA VIA TARGETING NUCLEAR FACTOR I/B (NFIB)

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Medulloblastoma (MB), the most frequent malignant pediatric brain tumor is subdivided into four primary subgroups, i.e. wingless-type (WNT), sonic hedgehog (SHH), group 3, and group 4. Haploinsufficiency of chromosome 17p13.3 and c-myc amplification distinguish high-risk group 3 tumors, which are associated with rapid metastasis, recurrence and early mortality. We sought to identify the role of miR-212, which resides on chromosome 17p13.3, in the pathophysiology of group 3 MB. RNA expression analyses revealed dramatically reduced levels of miR-212 in group 3 tumors and cell lines mainly through epigenetic silencing via histone modifications (deacetylation). Restoring *in vitro* miR-212 expression reduced tumor cell proliferation, colony formation, wound healing, migration and invasion with decreased p-AKT and p-ERK levels in group 3 MB cell lines. Interestingly, a shift in differential c-myc phosphorylation (from serine-62 to threonine-58) was also discovered with miR-212 expression, resulting in reduced total c-myc levels, concurrent with elevated cellular apoptosis. In turn, pro-apoptotic binding partners of c-myc, i.e. Bin-1 and P19ARF, were upregulated in these cells. These findings were recapitulated in stable inducible miR-212 expressing tumor cells. Using a combination of transcriptomic data and a dual luciferase assay, we isolated an important oncogenic target of miR-212, i.e, NFIB, a nuclear transcription factor implicated in metastasis and recurrence. Increased expression of NFIB was confirmed in group 3 tumors, with poor survival shown in high NFIB-expressing patients. As prior, transient NFIB silencing *in vitro* reduced not only tumor cell proliferation, colony formation, wound healing, migration and invasion, but also medullosphere formation along with decreased expression of stem cell markers (Nanog, Oct4, Sox2, CD133), confirming its role in tumor recurrence possibly via augmenting tumor stemness. Taken together, these results substantiate the tumor suppressive role of miR-212 in group 3 MB and provide a potential new oncogenic target implicated in tumor recurrence, NFIB.

BIOL-08. IGFBP2 PROMOTES TUMOR METASTASIS IN SHH MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common pediatric brain malignancy. MB comprises 5 major subgroups known as WNT, SHH p53wt, SHH p53mut, Group 3 and Group 4. Among the four MB subgroups SHH group is the most dominant molecular subgroup in infants and adults. These tumors are proposed to arise from cerebellar granule neuron precursors (CGNPs), whose developmental expansion requires SHH signaling from the neighboring Purkinje neurons. Previous reports suggest that SHH group features a unique tumor microenvironment compared with other MB groups. Recently, we performed cytokine array analysis of culture media from different MB cell lines. Interestingly, our data showed increased levels of IGFBP2 produced by SHH MB cell lines compared to others. We confirmed these results using ELISA and Western blotting from 3 human SHH MB cell lines, and Smo/A1 mouse tumor cells. IGFBP2 is a member of IGFBP super family of proteins; it plays important roles in tumor cell proliferation, metastasis and drug resistance. We analyzed the role of IGFBP2 in SHH group medulloblastoma tumor growth and metastasis. IGFBP2 knock-down stable cell lines showed phenotypic changes including reduced cell proliferation, cell migration and colony size. Our preliminary *in vitro* data suggest IGFBP2 exerts it metastasis-promoting role in SHH MB by regulating the expression of EMT marker proteins such as N cadherin, slug etc. and matrix remodeling proteins like MMPs and TIMPs. We are currently performing functional studies in organotypic tumor slice cultures to validate these findings and establish IGFBP2 as a novel regulator of aggressive tumor growth and spread in SHH MB.

BIOL-09. HNRNPA1 SPLICED VARIANT: KEY RESISTANT GENE SIGNATURE IN GLIOMAS

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Glioblastoma is inevitably a recurrent cancer. Despite of recent advancement, temozolomide remain the prescribed lifeline drug, after the surgery. Inadvertently, MGMT (O6-methylguanine-DNA-methyltransferase) expression mechanistically linked with Temozolomide (alkylating drug) glioma resistant development. To understand the resistant against Temozolomide sought to deciphered, by making invitro drug resistant glioma cell lines. RNA seq analysis over a illumina platform; drug resistant glioma cell lines showed various critical key factor such as splice factor hnRNPA1 and deubiquitinating enzymes were showed to highly upregulated in resistant cell lines. Commonly, from our previous study, the stability of hnRNPA1 in presence of USP5 were showed to promote cell survival, whereas knocking down of USP5 significantly lower down the telomerase activity and NAD/NADH ratio enlarge. Furthermore, expression of MGMT was showed significantly downregulated in hnRNPA1 knock down T98G glioma cells, as well as in U87 Temozolomide resistant cells. Extrinsic apoptosis pathway was showed more prevalent in hnRNPA1 knock down glioma cells in presence of Trail ligand. Interestingly, we found one more spliced variants of hnRNPA1 exclusively expressing in drug resistant cells is new finding. Selectively knocking down of hnRNPA1 splice variant promotes apoptosis. RNA seq analysis followed the comparison between two hnRNPA1 spliced variant knock down, drug resistant glioma cell lines showed differentially expressed transcript support our finding to be distinctly regulated by hnRNPA1 spliced variants. Spliced variant of hnRNPA1 showed a potential therapeutic candidate signature.