BIOL-10. DISTRIBUTION AND VULNERABILITY OF TRANSCRIPTIONAL OUTPUTS ACROSS THE GENOME IN MYC-AMPLIFIED MEDULLOBLASTOMA CELLS

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Myc plays a central role in tumorigenesis by orchestrating the expression of genes essential to numerous cellular processes. While it is well established that Myc functions by binding to its target genes to regulate their transcription, the distribution of the transcriptional output across human genome in Myc-amplified cancer cells, and the susceptibility of such transcriptional outputs to therapeutic interferences remain to be fully elucidated. Here, we analyze the distribution of transcriptional outputs in Myc-amplified medulloblastoma (MB) cells by profiling nascent total RNAs within a temporal context. This profiling reveals a major portion of transcriptional action in these cells was directed at the genes fundamental to cellular infrastructures, including rRNAs and particularly those in the mitochondrial genome (mtDNA). Notably, even when Myc protein was depleted by as much as 80%, the impact on transcriptional outputs across the genome was limited, with notable reduction mostly in genes of involved in ribosomal biosynthesis, genes residing in mtDNA or encoding mitochondria-localized proteins, and those encoding histones. In contrast to the limited direct impact of Myc depletion, we found that the global transcriptional outputs were highly dependent on the activity of Inosine Monophosphate Dehydrogenases (IMPDHs), rate limiting enzymes for de novo guanine nucleotide synthesis and whose expression in tumor cells was positively correlated with Myc's expression. Blockage of IMPDHs attenuated the global transcriptional outputs with a particularly strong inhibitory effect on the aforementioned infrastructure genes, which was accompanied by the abrogation of MB cell's proliferation in vitro and in vivo. Together, our findings reveal a real time action of Myc as a transcriptional factor in tumor cells, gain new insight into the pathogenic mechanism underlying Myc-driven tumorigenesis, and support IMPDHs as a therapeutic vulnerability in MB cells empowered by a high level of Myc oncoprotein.

BIOL-11. THE ROLE OF ABERRANT EXPRESSION OF PRDM6 IN THE DEVELOPING CEREBELLUM AND IN GROUP 4 MEDULLOBLASTOMA

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Group 4 medulloblastoma is the most common medulloblastoma subgroup with an intermediate prognosis and a high incidence of metastasis and late-onset relapse cases. Despite several comprehensive genomic studies in medulloblastoma, Group 4 medulloblastomas lack a unifying oncogenic driver and treatment targets. This subgroup is characterized by recurrent genetic alterations in chromatin modifiers, amplification of stemness genes, and enhancer hijacking events. 17% of Group 4 medulloblastoma cases are characterized by enhancer hijacking through tandem duplication of *SNCAIP*, resulting in high expression of PRDM6, a putative transcriptional repressor and histone methyltransferase. PRDM6 amplified medulloblastoma cases show additional mutations in other chromatin regulators, such as KDM6A, KMT2C and KMT2D, ZMYM3, and high MYCN expression. In this project, we investigate the impact and oncogenic potential of sustained PRDM6 expression in early neural stem cell populations and the developing mouse cerebellum. We drive expression of PRDM6 in human iPSC-derived neuroepithelial stem cells (NESCs) with and without high MYCN expression to study its implications in tumorigenesis. To test for tumor growth *in vivo* and changes in tumor progression as a function of PRDM6 activity, NESCs are injected into the cerebellum of adult mice. In order to elucidate impact of PRDM6 activity during embryonic cerebellar development, we also introduce PRDM6 expression into mouse embryonic stem cells (ESCs) for analysis via a new, *in vivo* cerebellar blastocyst complementation model. The latter approach is designed to ablate and repopulate early granule neural precursor cells in the embryonal cerebellum with progenitors derived from injected PRDM6-ESCs and thus to recapitulate pre- and postnatal cerebellar development *in vivo*. Together, our studies aim to understand the role of PRDM6 during normal cerebellar development and tumorigenesis and advance the understanding of the genetic drivers for Group 4 medulloblastoma.

EMBRYONAL TUMORS

EMBR-01. CLASS I HDAC INHIBITORS AND PLK1 INHIBITORS SYNERGIZE IN *MYC*-AMPLIFIED MEDULLOBLASTOMA

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Background: Medulloblastoma (MB) is one of the most common malignant pediatric CNS tumors. Patients with Group 3 MBs harboring *MYC* amplification exhibit low survival rates. Surviving patients suffer from therapy-induced sequelae, which calls for new targeted therapy strategies. We and others have previously shown the sensitivity of *MYC*-amplified MB to class I histone deacetylase (HDAC) inhibition. After demonstrating that the MYC target gene *PLK1* is significantly downregulated upon class I HDACi treatment, we hypothesized that inhibition of both HDACs and PLK1 could have synergistic effects. Methods: Cell metabolic activity changes upon HDAC and PLK1 inhibitor treatment were measured in *MYC*-amplified and non-amplified MB cell lines, as well as in an additional *MYC*-inducible cell line. The interaction effect of both inhibitors was determined by computation of the combination index (CI) using the Chou-Talalay method. Results were validated assessing cell viability, cell cycle, and apoptosis induction. Transcription profile changes after combination treatment were evaluated. Results: *MYC*-amplified MB cell lines were more sensitive than non-amplified cell lines to PLK1i treatment, showing IC50 in clinically achievable concentration ranges. Inhibition of class I HDACs and PLK1 synergistically reduced cell metabolic activity in lower concentrations in *MYC*-amplified compared to non-amplified MB cell lines. We also observed a significant loss of viability and cells in G1 phase, as well as induction of apoptosis after combination treatment in *MYC*-amplified cells. MYC target gene sets were significantly downregulated in the *MYC*-amplified cell line HD-MB03 after treatment with combination. We demonstrated reduction of MYC protein levels upon PLK1i treatment. *In vivo* evaluation of combination treatment using orthotopic Group 3 *MYC*-amplified MB PDX models is ongoing. Conclusion: Our data suggest that *MYC*-amplification is a predictive marker for PLK1i treatment in MB. The combination of HDACi and PLKi could be a candidate therapy for future clinical trials for *MYC*-amplified group 3 MB.

EMBR-02. OLIG2 REPRESENTS A PROGNOSTIC MARKER AND THERAPEUTIC TARGET IN MYC-AMPLIFIED MEDULLOBLASTOMA RELAPSE AND METASTASIS

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Medulloblastoma (MB) is one of the most common malignant pediatric brain tumors. Among the multiple MB subtypes, MB with *MYC* amplification confers an extremely poor prognosis with an overall survival rate of less than 30%. Relapse is often mediated by a small population of therapy-resistant tumor cells which expand and ultimately progress to lethal tumors. Moreover, *MYC*-amplified MB exhibits a high incidence of leptomeningeal metastases. Approximately one-third of patients with *MYC*-amplified MB present with metastases and nearly all have this complication at relapse. Metastatic *MYC*-amplified MB is highly fatal. As such, our ability to effectively treat *MYC*-amplified MB is largely dependent on our capacity to eradicate the therapy resistant tumor cells, particularly the metastatic tumor cells. The development of clinically effective therapies for this disease will be facilitated by the identification of therapy-resistant tumor cell populations and their molecular signatures involved in tumor metastasis and relapse. Using patient-derived xenograft (PDX) mouse models, we recently discovered that a subset of *MYC*-amplified MB tumors with strong OLIG2 expression ($OLIG2^{-high}$) is resistant to radiation and prone to metastasize, whereas *MYC*-amplified MB tumors without OLIG2 expression ($OLIG2^{-low}$) are sensitive to radiation without dissemination. Irradiation of $OLIG2^{-high}$ tumors led to either a small number of quiescent $OLIG2^{-}$ cancer stem-like cells (CSLCs) remaining in the cerebellar bed or to the dissemination of highly proliferative $OLIG2^{+}$ tumor cells along the leptomeninges. All mice harboring these radioresistant CSLCs succumbed to relapse. Further studies demonstrated that the quiescent $OLIG2^{-}$ CSLCs did not contribute to tumor recurrence directly, while elimination of $OLIG2^{+}$ radioresistant CSLCs with a small molecule OLIG2 antagonist significantly prevented metastatic recurrence, delayed tumor growth and prolonged animal survival. Thus, our