Child Health Research Institute

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Transcriptome-based drug repurposing in group 3 medulloblastoma

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Medulloblastoma (MB) is the most common malignant brain tumor of childhood, accounting for 20% of pediatric brain tumors. Recent advances in the field have identified four molecular subgroups of MB: WNT, SHH, group 3 (G3) and group 4 (G4). Despite this, current treatment protocols consist of surgical resection followed by craniospinal radiation and chemotherapy, often uninformed by the "molecular grades" in part due to poor understanding of the high-risk subgroups. Subgroup-specific prognoses vary widely across the molecular spectrum of MB from >95% five-year survival in WNT MB to <50% five-year survival in G3MB. There is an unmet need to address these outcome disparities, specifically in G3MB, the most aggressive subgroup. By comparing patient-derived gene expression signatures with transcriptomic drug signature databases, we have identified FDA-approved compounds with cytotoxic effects against G3MB. Differential expression analysis was performed with two cohorts of RNA-seq data: a local cohort of pediatric MB samples (GSE148389) and an external validation cohort (GSE164677). These data were analyzed against the Library of Integrated Network-based Cellular Signatures (LINCS) database, containing expression signatures of cell lines treated with over 42,000 chemical compounds. FDA-approved, CNS-bioavailable drugs that could reverse the gene expression profile of G3MB were chosen for further analysis. Confirmatory functional assays were performed in HDMB03, a G3MB cell line, to assess cytotoxic effects on survival/proliferation, clonogenicity, wound healing, and apoptosis *in vitro*. 81 CNS-available, FDA-approved drugs were identified; 13 were selected for further testing based on favorable safety profiles in children. Our top candidates based on IC50, fluoxetine (48h IC50 ~11uM), sertraline (48h IC50 ~7uM), nortriptyline (48h IC50 ~7uM), and simvastatin (48h IC50 ~8uM), fell into three classes of drugs: selective serotonin reuptake inhibitors, tricyclic antidepressants, and statins. All compounds attenuated wound healing and clonogenicity in HDMB03. Nortriptyline induced a dramatic dose-dependent increase in apoptosis (2 to 30%) by flow cytometry. Western blotting confirmed these findings with increases in cleaved caspases 3 and 9, cleaved PARP, and Bcl-2. We have successfully identified compounds cytotoxic to G3 MB *in vitro* using our *in silico* drug discovery pipeline. These drug classes have all been validated in other cancers to possess anti-tumorigenic effects. Our pipeline serves as a relatively accessible method to identify repurposable FDA-approved candidates against high-risk MB, with a promise to mitigate current drug toxicity and quality of life in survivors.