Dominik Sturm
Dr. med.

**Integrative Genomics Identifies Biological Subgroups, Molecular Markers, and Novel Therapeutic Targets in Pediatric Brain Tumors**

Promotionsfach: DKFZ (Deutsches Krebsforschungszentrum)
Doktorvater: Prof. Dr. med. S. M. Pfister

Survival rates of children with brain tumors have significantly improved over recent decades due to developments in diagnostic procedures, multimodal therapies, and supportive cancer care. However, they remain the leading cause of cancer-related deaths in children. The steady progress in outcome to date is largely the result of the systematic use of empirically based treatment regimens, and has not been specifically related to the increasing understanding of tumor biology, which, for example, formed the basis of the rapid improvement in patient outcomes observed in the treatment of children with leukemias.

To identify novel genetic and epigenetic alterations in the two most common malignant childhood brain tumors, a multilevel screening approach of DNA, RNA, and protein technologies was used to molecularly characterize 136 glioblastoma (GBM) and 64 medulloblastoma (MB) tumor samples. DNA sequence, methylation, and copy-number aberrations were integrated with changes in mRNA and protein expression and correlated with clinical patient data, followed by functional analyses of promising candidate genes in appropriate *in vitro* models.

In GBM, this integrated analysis identified six distinct biological subgroups based on their genome-wide DNA methylation patterns. Three of these subgroups were defined by one of two recently discovered hotspot mutations in *H3F3A* (K27 or G34), or mutations in *IDH1*. These alterations were further shown to be mutually exclusive in a large cohort of 460 GBMs from patients across all ages, and frequently co-occurred with mutations in *TP53*. While mutations in *IDH1* were associated with a CpG-Island Methylator Phenotype (CIMP), a novel CpG Hypomethylator Phenotype (CHOP) was observed in samples carrying G34 mutations in *H3F3A*. The remaining three subgroups were enriched for known hallmark cytogenetic events in adult GBM, such as high-level amplifications of *EGFR* or *PDGFRA*, and homozygous deletions of *CDKN2A*. Placing these subgroups into the context of previous adult GBM molecular classification schemes revealed a striking correlation with previously described DNA methylation clusters and a corresponding enrichment for established gene expression signatures in different epigenetic subgroups.

Epigenetic GBM subgroups showed clear correlations with clinicopathological variables, such as patient age, tumor location, and survival data. Tumors of the *H3F3A* K27 mutant subgroup were enriched in midline locations including the pons, thalamus, basal ganglia, and spinal cord, were mainly found in younger children, and were associated with very poor outcome. In contrast, G34 mutations in *H3F3A* were only detected in hemispheric tumors, and affected older children and adolescent patients, displaying a better outcome than patients with wild-type tumors. Mutations in *IDH1* were largely restricted to young adult patients, predominantly located in the frontal and temporal lobes, and were associated with improved overall survival.

Integrating DNA methylation and gene expression data allowed the identification of marker genes that are differentially expressed between GBM subgroups. An immunohistochemistry-based approach using antibodies against OLIG2, FOXG1, and mutated IDH1 was able to reliably subclassify GBM samples with known mutations in *H3F3A* or *IDH1*, and was used to validate results from the screening cohort in an independent set of 143 pediatric GBM samples.

Following a similar strategy, DNA copy-number and transcriptomic data were integrated to search for potential candidate genes targeted by aberrations of chromosome 6q in medulloblastoma, which have previously been shown to define distinct subgroups of MB patients. This identified *Serum- and Glucocorticoid-regulated Kinase 1 (SGK1)* as a functionally relevant gene in this genomic region, being almost exclusively expressed in high-risk MB subgroups. Further investigation of primary tumor methylome data plus functional studies in MB cell lines established a role for SGK1 as a novel subgroup marker and important regulator of cell cycle progression and apoptosis in high-risk MB. Administration of the synthetic glucocorticoid Dexamethasone at concentrations similar to patients' peak plasma levels in neurooncology settings strongly up-regulated SGK1 mRNA and protein levels within a short period of time. At the same time, pharmacological inhibition of SGK1 using a small molecule antagonist was able to attenuate SGK1-mediated effects on MB cell lines at comparably low concentrations.

In conclusion, the work presented here describes a variety of novel findings that enhance our understanding of the biology of malignant childhood brain tumors, and sheds new light on potential cellular origins and oncogenic pathways leading to tumorigenesis. It identified potential prognostic biomarkers which may be further exploited for molecular diagnostic purposes, and also provides a focus for future work at a basic and translational/targeted therapeutic level, particularly in a pediatric and young adult population.