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Differentiation therapy of childhood neuroblastoma through selective inhibition of histone deacetylase 8

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Neuroblastoma is a highly malignant tumor of childhood leading to death of advanced-stage patients in approximately 70% of cases despite intensive, multi-modal treatment. This is due to aggressive biological behavior and chemotherapy resistance. In addition, patients suffer from therapy-related immediate and long-term toxicities. Thus, more effective therapy approaches aiming at oncogenic molecular targets are strongly demanded to improve therapeutic efficacy, reduce toxicity, and avoid long-term side effects.

Histone deacetylase (HDAC) inhibitors are promising anti-tumor agents and are currently under investigation for the treatment of a broad spectrum of cancer diseases. However, one clinical drawback is the toxicity of broad-spectrum HDAC inhibitors. Selective targeting of one individual HDAC isozyme in a defined tumor entity may be an attractive alternative treatment approach to reduce unwanted side effects. Previous studies in a large cohort of clinical samples from the German Neuroblastoma Trial identified HDAC family member 8 (HDAC8) as a suitable target in childhood neuroblastoma. Targeting of HDAC8 in cultured neuroblastoma cell lines derived from neuroblastic bone marrow metastases inhibited tumor cell proliferation and clonogenicity and favored maturation into a more differentiated phenotype.

The results from this thesis verify HDAC8 as a promising target for neuroblastoma therapy *in vivo*. The toxicity profiles including body weight, blood parameters and organ toxicity, the half-lives as well as selectivity for two small molecule HDAC8 inhibitors are established.

The efficacy of HDAC8 inhibitor treatment against high-risk neuroblastoma is demonstrated in two subcutaneous xenograft mouse models of MYCN oncogene amplified neuroblastoma. Of clinical significance, the unselective broad-spectrum HDAC inhibitor SAHA (vorinostat) is more toxic in the same models and less efficacious in one of the models.

The anti-tumoral activity of HDAC8 inhibitor treatment *in vitro* and *in vivo* is characterized by the induction of cell cycle arrest and the upregulation of differentiation markers, such as neurofilament, as well as caspase3-mediated cell death.

The combination with retinoic acid significantly enhances differentiation of the immature cells and results in prolonged neurofilament positive neurites *in vitro* and markedly reduces tumor cell growth *in vivo*. These phenotypes are hypothesized to be derived from an enhanced signaling via the cAMP/PKA/CREB pathway.

The here presented thesis generated the preclinical basis for the use of selective HDAC8 inhibitors as a promising, less toxic therapy for childhood neuroblastoma. Furthermore, it introduces for the first time the selective inhibition of HDACs as an innovative therapeutic strategy for tumor diseases.