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Role of Histone Deacetylase 11 in Neuroblastoma

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Neuroblastoma is an embryonal childhood malignancy with primary tumors localizing to the adrenal medulla or to paraspinal ganglia. Neuroblastoma accounts for 10,8% of pediatric oncology deaths. Most neuroblastomas bear cytogenetic abnormalities reaching from chromosomal gains and losses to chromothrypsis. Because of its relevance for prognosis, amplification of the *MYCN* oncogene is the most prominent cytogenetic abnormality, causing rapid tumor cell cycling via transcriptional amplification. Germline mutations in *ALK* and *PHOX2B* are found in 1-2% of neuroblastoma patients, comprising a small group with familial predisposition to neuroblastoma. High throughput genetics only yielded mutations with a very low recurrence and did not bring further inside into the heterogeneous clinical enigma of this disease. Activating mutations in the *ALK* gene with an incidence of 6-10% presents the only druggable target in neuroblastoma evolving from molecular genetics. Molecular- and cytogenetics therefore contributed to the specification of disease stratification but have neither explained tumorigenesis nor generated druggable targets for the majority of patients. Neuroblastomas derive from sympathetic precursor cells descending from the neural crest that do not differentiate into functional sympathetic neuronal tissue. Causal events for this lack of differentiation and instead malignant transformation are poorly understood. Differentiation of neural crest cells into sympathetic neurons requires a delicate concert of signaling molecules and epigenetic regulators. This provides possible clues for disease initiation and candidates for target discovery.

Altering epigenetic and post-translational processes via HDAC inhibition showed a high potential in neuroblastoma preclinical models. First experiments of this project found, that the pre-clinical pan HDAC inhibitor HC-toxin and the clinically applied pan HDAC inhibitor panobinostat (LBH589) cause cell cycle arrest in G2/M and cell death in high-risk neuroblastoma models. In clinical trials however, effective application of pan HDAC inhibitors was shortened by dose limiting side effects. The roles of individual HDACs and the mechanisms of toxicity upon HDAC inhibition in neuroblastoma cells and healthy tissues are poorly understood. Exactly depicting these functions and processes is the precondition to use the therapeutic capacity of the acetylome in a clinically effective way.

HDAC11 epigenetically represses expression of BMP4, essential for neural crest cell differentiation and therefore is an interesting target for neuroblastoma therapy. Aim of my thesis was to further characterize HDAC11 as potential drug target in neuroblastoma and to unravel mechanisms mediating the identified anti-tumor activity.

HDAC11 depletion in *MYCN*-amplified high-risk neuroblastoma models caused a slowdown of cell cycling presenting in reduced metabolic activity, reduced proliferation and a cell cycle arrest in G2/M. Further, HDAC11 depletion induced cell death via apoptosis that was detectable correlating with the knockdown efficacy in annexin V staining, PARP western blots, caspase 3-like activity induction, and elevation of the subG_1 population of propidium iodide stainings. Notably, the induction of caspase 3-like activity via HDAC11 depletion was stronger compared to caspase 3-like activity upon class I HDAC depletion.

In genome-wide mRNA expression arrays of *MYCN*-amplified neuroblastoma models after HDAC11 depletion, HDAC11 was found to control the expression of a functionally defined group of 9 genes with canonical roles in mitosis. Regulation of the identified gene group not only contributed as expected to the described cell cycle arrest, but also to the induction of apoptosis upon HDAC11 depletion. For candidate gene RACGAP1 the apoptotic phenotype of HDAC11 could partially be rescued by RACGAP1 enforced expression.

A high expression of candidate genes strongly correlated with a worse patient outcome, when analyzing primary neuroblastoma specimen. HDAC11 depletion altered candidate gene mRNA expression correlating with expression patterns of favorable prognosis tumors. In addition, expression of the identified gene group was higher in *MYCN*-amplified tumors, also when compared to non-*MYCN*-amplified high-risk neuroblastomas.

In conclusion, HDAC11 depletion causes the downregulation of a functionally defined gene group essential for mitosis, thereby impeding proliferation. This mitotic blockade exploits proliferation dependency of *MYCN*-amplified neuroblastoma cells, inducing cell death via apoptosis. HDAC11 therefore is essential for the viability of *MYCN*-amplified neuroblastoma cells, while HDAC11 is dispensable for untransformed cells, suggesting a therapeutic window for the application of potential selective HDAC11 inhibitors. This study proposes HDAC11 as novel drug target for *MYCN*-amplified neuroblastoma due to a phenotype characterization after HDAC11 depletion, showing the induction of apoptosis and presents a corresponding mechanism.