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**Selective targeting of histone deacetylases in *MYC* amplified Group 3 medulloblastoma**

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Medulloblastoma is one of the most frequent malignant brain tumors in children and responsible for the majority of CNS tumor-related deaths in this age group. Medulloblastoma comprises four distinct molecular subgroups, each with different genetic as well as epigenetic backgrounds as well as differing clinical characteristics, such as age and gender distribution, metastasis and clinical outcome. Especially for high-risk Group 3 patients overall survival rates remain poor despite aggressive multimodal treatment strategies including surgery, chemotherapy and irradiation leading to important long-term treatment related morbidity.

It has previously been demonstrated that Group 3 tumors are characterized by aberrations of proteins involved in chromatin organization, emphasizing the importance of epigenetics for the biology of these tumors. In this work epigenetic active histone deacetylates have been evaluated as potential drug targets in high-risk medulloblastoma.

mRNA expression pattern analysis in large cohorts of primary medulloblastoma tumor samples revealed upregulation of HDAC2 in all three high risk medulloblastoma subgroups (SHH, Group 3, Group 4), as well as increased HDAC2 protein abundance in these molecular subgroups. Artificial reduction of HDAC2 protein levels in *MYC* amplified medulloblastoma cell lines by siRNA-mediated knockdown led to hyperacetylation of histone 4 protein, an increase in Caspase-3-like activity, an increase in the sub G0/G1 fraction, and a reduction of the number of viable cells, all indicative for induction of apoptosis. These findings for the first time demonstrate the functional dependency of a medulloblastoma cell lines to HDAC2 protein.

In contrast to *MYC* non-amplified medulloblastoma cell lines, *MYC* amplified medulloblastoma cell lines were shown to be highly susceptible to the enzymatic inhibition of class I HDACs 1, 2 and 3 with vorinostat or MS-275. The cytotoxic effects of vorinostat and MS-275 on *MYC* amplified medulloblastoma cells were observed at inhibitor concentrations well within the range of clinically achievable peak plasma concentrations, making this finding clinically highly relevant. MYC amplification is a potential positive predictive marker for the treatment of medulloblastoma with HDACis.

In a comparative analysis it could be shown for the first time that MS-275 in contrast to vorinostat has a slow binding kinetic to class I HDACs, possibly causing the observed longer half-life of the formed drug-target complex. The biological relevance of this finding could be demonstrated in metabolic activity assays and western blot analysis showing biological effects of class I HDAC inhibition even long after removal of MS-275 from the extracellular compartment.

For the first time the effect of novel class IIa HDAC small molecule inhibitors on medulloblastoma cells was investigated. The inhibitors effectively reduced the turnover rate

of class IIa HDAC substrates in an intracellular HDAC activity assay but had no effect on cellular metabolic activity suggesting that the enzymatic activity of class IIa HDACs is not of vital importance to medulloblastoma cells.

From these findings it can be concluded that HDAC2 is a valid drug target in Group 3 medulloblastoma. The inhibition of class I HDACs including HDAC2 appears to be a promising strategy for the treatment of high risk group 3 medulloblastoma. MYC amplification could be a positive predictive marker for the treatment with HDACis. Novel substances taking into account the compound's binding kinetics will possibly allow for effective HDAC inhibition *in vivo* which will be necessary to successfully translate these findings into the clinic.