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Targeted Therapy of Neuroblastoma with HDAC8 Inhibitor and ALK Inhibitor

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Neuroblastoma is the most common extracranial solid cancer in childhood, and the most common cancer in infancy. Despite continuous intensification of therapy, the long term survival rate of high risk patients is still below 50%. In addition, the therapy-related immediate and long-term toxicities exert considerable impact on the life quality of these young patients. Targeted therapy, which is more specific and causes less side effects, are urgently demanded for neuroblastoma. However, deep sequencing of neuroblastoma has identified only few gene mutations, which suggested that epigenetic regulation of gene expression could play more important roles. Compounds targeting epigenetic mechanisms, such as histone acetylation and methylation, could better benefit neuroblastoma patients. As The expression of histone deacetylase 8 (HDAC8) is correlated with poor survival and high stage of neuroblastoma and HDAC8 inhibitors show anti-neuroblastoma effects in both in vitro and in vivo experiments, HDAC8 is proposed as a promising new neuroblastoma drug target. Through a kinome-wide siRNA screening, HDAC8 inhibitors were found to exert synthetic lethal effects with siRNA against anaplastic lymphoma kinase (ALK). Mutations of ALK are responsible for more than half of familial neuroblastoma, and were also identified in more than 10% sporadic neuroblastoma patients. The tumorigenic roles of ALK have been validated by transgenic animal models. Based on these findings, the aim of this thesis was to develop a novel combination therapy for neuroblastoma with HDAC8 inhibitors and ALK inhibitors, and to unravel the underlying mechanisms. The combination of HDAC8 inhibitors and ALK inhibitors forced neuroblastoma cells to die, irrespectively of the ALK status of the cells.

Treatment of the cells with both inhibitors resulted in synergistic effects. Further mechanistic experiments identified apoptosis as the responsible kind of cell death induced by the combination treatment. In contrast, the combination treatment did not affect the viability of untransformed, proliferative fibroblasts. In neuroblastoma cells, HDAC8 inhibition decreased ALK activation (phosphorylation) and expression. Furthermore, an additional resistance mechanism of the tumor cells towards HDAC8 inhibitor treatment was identified. Both HDAC8 inhibition as well as depletion increased the phosphorylation and activation of EGFR. The application of an EGFR inhibitor clearly enhanced the therapeutic effects of HDAC8 inhibitors in cellular experiments. In the combination treatment group, HDAC8-inhibitor-caused EGFR phosphorylation was counteracted by ALK inhibitor crizotinib. Further experiments showed that crizotinib inhibited EGFR activation in a dose-dependent manner. All together, these findings shed more light on the malignancy of neuroblastoma, on resistance mechanisms of neuroblastoma cells to HDAC8 inhibitor treatment, and demonstrated that combined treatment with HDAC8 inhibitors and ALK inhibitors was effective against a panel of neuroblastoma cell lines.