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The Role of EHMT1/2 in Colorectal Cancer

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HDAC inhibitors have shown disappointing results in clinical studies with colorectal cancer patients despite their unique mechanism of action and the upregulation of their targets in colorectal cancer. To harness their full potential, knockouts sensitizing colorectal cancer cells to HDAC inhibitor treatment have been identified in a previous screening experiment. In these screening efforts, EHMT1 and EHMT2, two isoenzymes that together methylate histones, were discovered to be synthetic lethal to HDAC inhibition. To assess the translational potential of this synthetic lethality, functional characterization of EHMT2 was performed in this thesis.

By analyzing immunohistochemical EHMT2 staining in cancer tissue sections of 1066 colorectal cancer patients, a correlation of low EHMT2 expression with worse overall and tumor-specific survival was discovered. By analysis of the combination of EHMT1/2 inhibitors BIX-01294, UNC0638, UNC0642, or A-366 with HDAC inhibitors vorinostat or panobinostat in vitro, synergistic effects could be detected in colorectal cancer cell lines and several patient derived organoids. By calculating four different synergy scores and applying an aggregate terminology, strong synergy could be identified in two out of the six tested compound combinations in HCT116 colorectal cancer cells and in three out of six combinations in HT29 cells. The remaining combinations did mostly induce weak synergy in the cell lines. In eleven tested patient derived organoid lines, the combination of UNC0638 with vorinostat induced strong synergy in one, and weak synergy in five organoid lines.

Mechanistically, the combination of UNC0638 with vorinostat induced several cell cycle and autophagy markers in a high throughput Western blot experiment (DigiWest). With conventional immunoblots, it was shown that vorinostat modulates the expression of cell cycle markers and that UNC0638 alters the expression of autophagy markers. Furthermore, the combination of UNC0638 with the late autophagy inhibiting compounds chloroquine and Lys05 synergistically reduced viability in HT29 and HCT116 cells, suggesting a vulnerability of UNC0638-treated colorectal cancer cells to inhibitors of autophagic flux. Flow cytometry studies could furthermore confirm a cell cycle shift of HCT116 cells treated with vorinostat alone or in combination with UNC0638, validating the cell cycle-modulating role of HDAC inhibition in the combination treatment. Even though apoptosis-related proteins were also amongst the upregulations detected by DigiWest, pre-treatment with a pan-caspase inhibitor did not rescue the viability-reduction detected upon combined UNC0638 and vorinostat treatment. This suggests that cell cycle and autophagy are the main mechanisms responsible for the viability effects of the combination therapy. Finally, Western blot experiments showed that the combination of vorinostat and UNC0638 reduces dimethylation of histone H3K9, driven mainly by UNC0638, and increases H3K9 acetylation, driven mainly by vorinostat.

Taken together, the results of this thesis show that colorectal cancer with low EHMT2 expression represents a high-risk cohort and that targeting of EHMT1/2 together with pan-HDAC inhibition can synergistically reduce viability of in vitro colorectal cancer models. In this combination, UNC0638 and vorinostat were shown to act together by affecting cell cycle and autophagy and changing the modification status of H3K9. These results confirm the translational potential of the synthetic lethal interaction between EHMT1/2 knockout and HDAC inhibitor treatment and encourage the further exploration of this combination, for example with in vivo experiments.