Abstract

The B cell lymphoma-2 (BCL2) oncogene is highly expressed in Chronic lymphocytic leukaemia (CLL) and other lymphomas such as Follicular lymphoma and Diffuse large B cell lymphoma. Venetoclax (VEN; ABT-199), a BCL2 selective inhibitor, was identified as a efficacious compound to treat patients with high-risk CLL and is approved to treat patients with CLL and acute myeloid leukaemia. However, resistance mechanisms towards VEN are still not fully understood. Therefore, in order to offer treatment options after VEN-failure, it is crucial to understand how resistance against VEN is mediated. By analysing cell lines, primary CLL and other NHL patient samples with acquired VEN resistance on the genetic, epigenetic, and protein level, we provide evidence that resistance towards VEN is mediated mainly by downregulation of BAX and PUMA as well as by upregulation of MCL1. The results of this thesis uncover for the first time that acquired resistance towards VEN was mediated by a methylation event, identifying a regulatory CpG island within the putative *PUMA* promoter *via* pyro-sequencing. Its methylation results in decreased *PUMA* expression on both transcript and protein level. Epigenetic regulation of PUMA was identified in both cell lines and CLL patient samples. Interestingly, treatment with the demethylating drug 5´-AZA reverses the methylation and results in the re-expression of PUMA, restoring the sensitivity towards VEN. Additionally, using CRISPR/Cas9 *PUMA* KO cell lines we could show that loss of *PUMA* results in metabolic reprogramming with higher OXPHOS and ATP production. While loss of PUMA expression was specific for acquired resistance towards VEN, but not towards MCL1 inhibition, loss of BAX was a critical step for sensitivity towards both BCL2 and MCL1 inhibition. A compound mono screen in BAX-deficient cells revealed the extrinsic apoptosis pathway as a promising target. The connection to extrinsic apoptosis was confirmed *in vitro* by testing human anti-CD19 CAR T-cells. Altogether, the data in this thesis were able to verify and expand the knowledge about acquired VEN resistance and identified treatment options for patients.