Abstract

In the works presented here, we studied molecular changes associated with drug resistance in human mantle cell lymphoma (MCL) cells using proteomics. Our analyses allowed us to identify causal and/or secondary changes in protein expression associated with the development of resistance to the experimental drug TRAIL and the clinically used antimetabolites cytarabine and fludarabine. Resistance of MCL cells to the recombinant proapoptotic cytokine TRAIL was associated with downregulation of key enzymes of purine metabolism. This pathway potentially represents a molecular “weakness”, which could be used as a therapeutic target for selective elimination of such resistant cells.

Resistance to the pyrimidine analog drug cytarabine was associated with cross-resistance to other antinucleosides. Proteomic and transcriptomic analyses showed pronounced downregulation of deoxycytidine kinase (dCK), which activates both purine and pyrimidine antinucleosides. This change explains the cross-resistance and is the causal mechanism of resistance to cytarabine. Our observations suggest that MCL patients, who do not respond to cytarabine-based therapy, should be treated with non-nucleoside drugs.

MCL cells resistant to purine-derived antinucleoside fludarabine were cross-resistant to all tested antinucleosides and also to ibrutinib, inhibitor of Bruton tyrosine kinase (BTK). Our proteomic analysis using a metabolic labeling approach (SILAC) showed marked downregulation of dCK and BTK among the differentially expressed proteins. Further, we detected upregulation of the anti-apoptotic protein Bcl-2, and demonstrated increased sensitivity of fludarabine-resistant MCL cells to the Bcl-2 inhibitor ABT199.

These “proof of concept” studies demonstrated the potential of proteomic analysis for personalized therapy of resistant malignancies. Proteomics, however, still has its limitations: the second section of this thesis deals with integral membrane proteins (IMPs). IMPs are underrepresented in conventional proteomic analyses, primarily due to their amphipathy, low digestibility with trypsin, and low expression levels. These properties call for specific approaches. We introduced an improved and simplified method for IMP analysis that targets transmembrane segments of IMPs. We used this method to characterize the membrane proteome of a MCL cell line. We identified over 800 IMPs including several so-called “missing proteins”, that had not previously been observed on the protein level.

Key words: proteomics, mantle cell lymphoma, drug resistance, drug targets, integral membrane proteins, mass spectrometry