Unravelling survival strategies of proteasome inhibitor (PI) resistant myeloma cells

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Abstract

The implementation of proteasome inhibitors (PIs) represents a milestone in the treatment of multiple myeloma (MM), the second most common hematological malignancy. Despite of these advances most patients suffer from refractory disease and run out of therapy options. Hence, MM remains an incurable disease. This limitation in outcome is chiefly linked to the occurrence of drug resistance during the course of the disease. The intrinsic apoptosis pathway is regulated by the BCL2 (B-cell lymphoma 2) protein family. A delicate interplay within a complex network of protein-protein interactions is key to navigate cell survival and cell death. There is evidence that this interplay is influenced by PI resistance, indicating that the dysregulation of the BH3 protein family is one of the key survival mechanism in PI resistant cells.

In this study, we aim to reveal and characterize central mechanisms of apoptosis evasion in response to the FDA-approved PIs carfilzomib and ixazomib. For this purpose, we generated multiple human myeloma cell lines with acquired carfilzomib (n=9) and ixazomib (n=4) resistance. Access to this unique cell line panel offers a significant advantage to delineate survival strategies of resistant cells and to link these findings with upstream drivers, genetic patterns and/or signaling pathways mediating PI resistance. Moreover, this enables us to decipher drug specific (ixazomib vs carfilzomib) as well as drug class (PIs) and genetic background dependent survival strategies via the BCL2 family protein members.

Introduction

The proteasome is a multicatalytic proteinase complex which is responsible for the degradation of a wide variety of protein substrates within normal and malignant cells. The implementation of compounds that specifically target the enzymatic sites of the proteasome - proteasome inhibitors (PIs) - represents a milestone in the treatment of multiple myeloma (MM), the second most common hematological malignancy which is characterized by the clonal accumulation of plasma cells in the bone marrow. The success of proteasome inhibition in MM is based on the high amount of immunoglobulin secretion in malignant plasma cells, making them especially prone to elevated numbers of unfolded or improperly folded proteins. However, despite the clinical success story of PIs in MM, the majority of patients ultimately relapse and present a modified tumour clone displaying resistance to prior PI-based therapy.

Previous studies already identified numerous mechanisms of resistance to the FDA approved PIs bortezomib, ixazomib and carfilzomib. The latest insights point to metabolic alterations or distinct signalling pathways such as EGFR/JAK1/STAT3 signalling suppression via tight junction protein 1 (TJP1) as key events of PI resistance (Zhang et al., 2015).

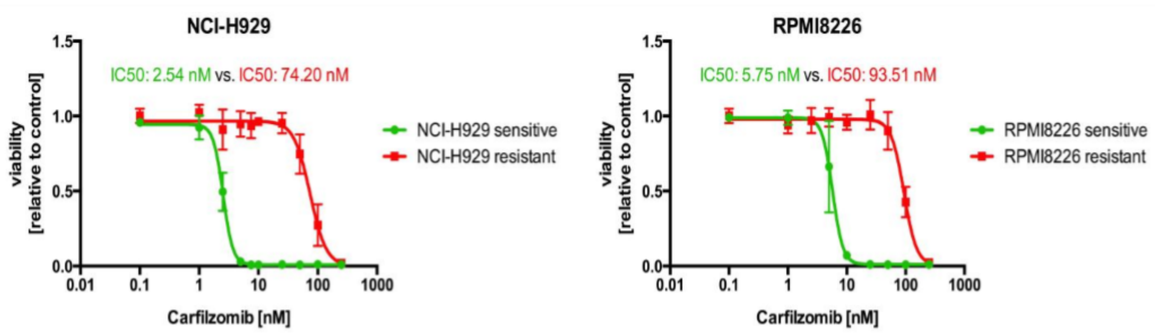
The intrinsic apoptosis pathway is regulated by the BCL2 (B-cell lymphoma 2) protein family. A delicate interplay within a complex network of protein-protein interactions is key to navigate cell survival and cell death. (Leber et al, 2007)

Amongst other known correlations between the BCL-2 family member and PI resistance, metabolic alterations in PI resistant cells were recently shown to affect the functionality of the BH3 mimetic Venetoclax (Besse et al., 2019). Higher sensitivity to this Bcl-2 inhibiting compound was observed in PI resistant as compared to PI sensitive cells, suggesting that the interrogation with key anti-apoptotic molecules disbalances the metabolic adaptations of PI resistant myeloma cells.

Results

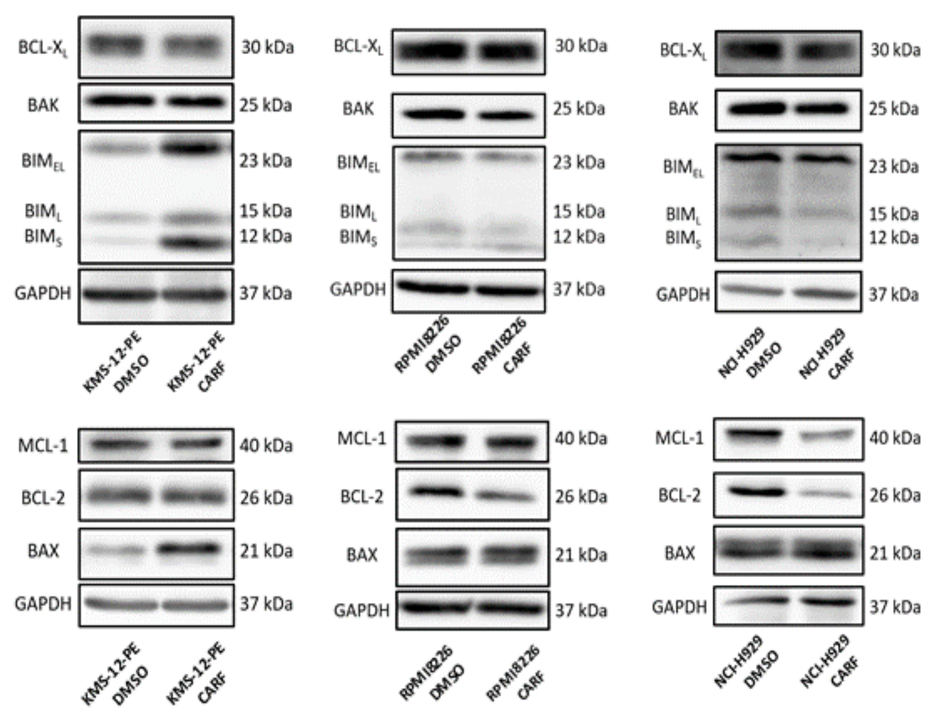
Exemplary dose response curves of carfilzomib sensitive and resistant human myeloma cell lines

Carfilzomib resistant variants of the NCI-H929 and RPMI8226 human myeloma cell lines were generated by continues exposure to increasing doses of carfilzomib for more than 1 year. This strategy led to a >10-fold shift in IC50 values in both cell lines. Graphs represent viability relative to control (DMSO) 72h post



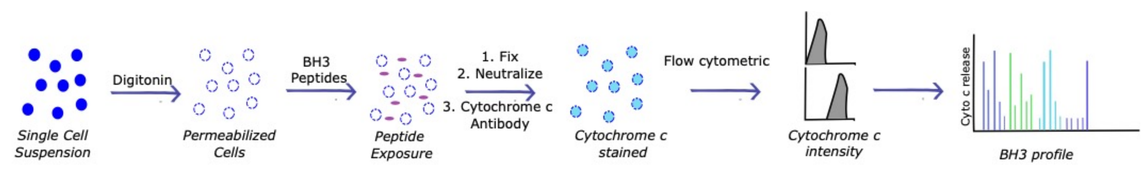
Baseline levels of BCL2 family members vary in carfilzomib sensitive vs resistant cell line variants

Protein expression analysis of BCL2 family members in naive (without drug exposure) carfilzomib sensitive and resistant cell line variants highlights cell line specific alterations of distinct pro- and anti-apoptotic proteins in KMS12PE (left panel), RPMI8226 (middle panel) and NCIH929 cells (right panel). Results are representative of three independent experiments. “DMSO” indicates sensitive, and “CARF” indicates the corresponding resistant cell line variants.



Methods

The unbiased flow cytometry-based profiling method iBH3 (Ryan et al. 2016) allows us to identify the relevance of distinct anti-apoptotic BH3 proteins in the cell line (or patient sample) of interest and enables us to reveal a potential switch of BH3 dependencies in PI resistant vs sensitive cells.



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