P857 BORTEZOMIB- AND CARFILZOMIB-RESISTANT MYELOMA CELLS SHOW INCREASED ACTIVITY OF ALL THREE ARMS OF UNFOLDED PROTEIN RESPONSE AND ENRICHMENT OF SIRTUIN SIGNALING PATHWAY

**Topic:** 13. Myeloma and other monoclonal gammopathies - Biology & Translational Research

Tadeusz Kubicki¹, Kinga Bednarek², Magdalena Kostrzewska-Poczekal², Magdalena Łuczak³, Zuzanna Kanduła¹, Krzysztof Lewandowski¹, Lidia Gil⁴, Małgorzata Jarmuż-Szymczak², Dominik Dytfeld¹

¹ Department of Hematology and Bone Marrow Transplantation, Poznan University of Medical Sciences, Poznan, Poland; ² Institute of Human Genetics, Polish Academy of Science, Poznan, Poland; ³ Institute of Bioorganic Chemistry, Polish Academy of Science, Poznan, Poland

**Background:**

Proteasome inhibitors (PIs) are among most potent and widely-used class of drugs in multiple myeloma (MM) treatment. One of the main challenges in MM therapy is acquired resistance to drugs, PIs are no exemption from this phenomenon. Several theories were proposed to describe mechanisms responsible for resistance to most commonly used PIs – bortezomib and carfilzomib. One of the most promising explanations involves modulation of unfolded protein response (UPR) pathway. The pathway is initiated by conformational changes in three sensor proteins (PERK, IRE1α, ATF6) in response to overaccumulation of misfolded proteins. Recent study (Sarin et al, Leukemia 2020) suggests that MM cell lines used for in vitro experiments differ substantially in resemblance to patients’ tumors, partially explaining difficulties in translation of results from laboratory models to MM treatment. Here we evaluated MM1S cells, that are among best fitted to resemble actual MM patients’ biology.

**Aims:**

The study aimed at describing functional differences between PI-sensitive MM1S cells (MM1S WT) and their daughter cells, resistant to either bortezomib (MM1S/R BTZ) or carfilzomib (MM1S/R CFZ), as well as between both resistant cell lines.

**Methods:**

Resistant cell lines were generated by continuous culture with increasing concentrations of drugs. Subsequently proteomic profiling of sensitive and resistant cells was performed. To functionally analyze proteomic results proteasome activity and generation of reactive oxygen species (ROS) were measured. Finally, changes in UPR activation were assessed by Western blot analysis of key proteins involved in this pathway.

**Results:**

BTZ- and CFZ-resistant cell lines were successfully generated. IC₅₀ values were 3-fold higher for the resistant cells (MM1S WT IC₅₀=15.2 nM for BTZ, IC₅₀=8.3 nM for CFZ; MM1S/R BTZ IC₅₀=44.5 nM; MM1S/R CFZ IC₅₀=23.0 nM). When exposed to different PI than during resistance generation period MM1S/R BTZ cells were resistant to CFZ (IC₅₀=43.5 nM) whereas MM1S/R CFZ cells were similarly sensitive to BTZ as MM1S WT (IC₅₀=24.0 nM). Unsupervised principal component analysis revealed that MM1S/R BTZ and MM1S/R CFZ differ significantly from MM1S WT cells and also from each other. Canonical pathway analysis showed similar pathways enriched in both comparisons – MM1S WT vs MM1S/R CFZ and MM1S WT vs MM1S/R BTZ, however important differences were present in terms of statistical significances of particular pathways (Figure). Sirtuin signaling was identified as the most enriched pathway in BTZ-resistant cells (top-4 in CFZ-resistant) and EIF2 signaling in CFZ-resistant cells. In functional studies, both PIs continued to block chymotrypsin-like proteasome activity in resistant cells. The relative reduction in the activity was similar for resistant and sensitive cells – 65% for MM1S/WT in BTZ, 45% for MM1S/R BTZ, 96% for MM1S/WT in CFZ, 77% for MM1S/R CFZ. Baseline activity of all 3 catalytical domains of proteasome was significantly higher in the resistant cells. MM1S/R BTZ cells generated lower amount of ROS in comparison to MM1S WT (64%), while there were no differences in similar comparison for MM1S/R CFZ cells. Both baseline and drug-induced activity of UPR was higher in PI-resistant cells then in MM1S WT and included all three
arms of UPR - IRE1α/XBP1s, ATF6 and EIF2α/ATF4 (downstream effectors of PERK).

Image:

Summary/Conclusion:

Contrary to some previous reports, performed mainly on other MM cell lines, PI-resistant MM1S cells show upregulation of UPR. This reflects heterogeneity of MM and prompts further studies on UPR role and its interplay with sirtuin signaling.