Overcoming Drug-resistance in Multiple Myeloma by CRM1 Inhibitor Combination Therapy

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Abstract

Significant progress has been made over the past several years in the treatment of multiple myeloma (MM). However patients eventually develop drug resistance and die from progressive disease. The incurable nature of MM clearly demonstrates the need for novel agents and treatments. The overall objective of this study was to investigate the use of CRM1 inhibitors (KPT330 and KOS2464) to sensitize de novo and acquired drug-resistant MM cell lines to the proteasome inhibitors bortezomib (BTZ) and carfilzomib (CFZ) and to doxorubicin (DOX). Sensitivity was measured by both cell viability (CellTiter-Blue) and apoptosis assay. CRM1 inhibitors, KPT-330 or KOS-2464 sensitized de novo and acquired drug-resistant MM cell lines to BTZ, DOX and CFZ as shown by apoptosis assay.

Methods

Drug resistant U266 and 8226 MM cell lines were developed at VCU (Steven Grant) and the Moffitt Cancer Center (Ken Shain) respectively by the incremental exposure to BTZ. Cells were treated in vitro with CRM1 inhibitors (KPT330 or KOS2464) +/- either BTZ, CFZ, or DOX. Sensitivity was measured by both cell viability (CellTiter-Blue) and apoptosis (activated caspase 3). U266 resistant cells were also used to challenge NOD/SCID-gamma mice which were then treated with KPT330 and KOS2464 to both BTZ and CFZ as shown by apoptosis assay. Resistance U266 and 8226 MM cell lines were developed at VCU (Steven Grant) and the Moffitt Cancer Center (Ken Shain) respectively by the incremental exposure to BTZ. Cells were treated in vitro with CRM1 inhibitors (KPT330 or KOS2464) +/- either BTZ, CFZ, or DOX and CD138/light chain positive MM cells isolated and treated with KPT330 or KOS2464 (300 nM) +/- BTZ, CFZ, or DOX. Sensitivity was measured by both cell viability (CellTiter-Blue) and apoptosis assay.

Results

BTZ selected U266 and 8226 MM cells were assayed and found to be resistant to BTZ, CFZ, and DOX as compared to parental cell lines. Both U266 and 8226 resistant MM cell lines were found to be 15-fold resistant to BTZ. Cell sensitivity was decreased synergistically when the CRM1 inhibitor KPT330 was used in combination with BTZ or DOX. Combinatorial index values were < 1.0 (synergistic) in both parental and drug resistant U266 and 8226 MM cell lines. Resistant U266 and 8226 MM cell lines were sensitized by the CRM1 inhibitors KPT330 and KOS2464 to both BTZ and CFZ as shown by apoptosis assay. CD138/light chain double positive MM cells derived from newly diagnosed and refractory (drug-resistant) MM patients were also used to challenge NOD/SCID-gamma mice which were then treated with KPT330 and KOS2464 (300 nM) +/- BTZ, CFZ, or DOX. Sensitivity was measured by both cell viability (CellTiter-Blue) and apoptosis assay.

Conclusions

• CRM1 inhibitors KPT-330 and KOS-2464 sensitized drug-resistant multiple myeloma to the proteasome inhibitors BTZ and CFZ.
• Studies were performed in BTZ resistant MM cell cultures.
• BTZ resistant MM animal models and ex vivo CD138+ light chain positive MM cells were examined for inducible apoptosis by flow cytometry and caspase assay. We found that double positive myeloma cells were sensitized by CRM1 inhibitors to doxorubicin, bortezomib and carfilzomib. CD138+ light chain negative MM cells were unaffected.

Background: Drug Resistance

Multiple myeloma (MM) is an incurable, progressive hematologic malignancy characterized by the accumulation of clonal plasma cells in the bone marrow, often with related skeletal fractures and bone pain. Despite advances in therapeutic approaches including proteasome inhibitors and novel immunotherapies, the overall survival remains poor, with most patients succumbing to progressive disease. One challenge in MM is the development of drug resistance, which may be due to the unique biology of the disease and its complex microenvironment. The CRM1 inhibitor KPT330 or KOS2464 was used to sensitize drug-resistant MM cell lines to the proteasome inhibitors bortezomib (BTZ) and carfilzomib (CFZ) and to doxorubicin (DOX). Sensitivity was measured by both cell viability (CellTiter-Blue) and apoptosis assay.