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Proteasome inhibition in the regulation of natural killer cell function and multiple myeloma cell apoptosis

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Abstract

Multiple myeloma (MM) is a hematologic malignancy mostly occurring in the elderly and characterized by an expansion of monoclonal plasma cells in the bone marrow and increased monoclonal immunoglobulin in plasma. The outcome of this disease has been greatly improved due to introduction of new drugs. The proteasome inhibitor bortezomib (velcade®) is one of the therapeutic drugs showing very pronounced efficacy in the treatment of MM. However, cytotoxic effects of bortezomib on immune-competent cells have also been observed. In the current thesis, we focus on studying regulatory effects of proteasome inhibition on natural killer (NK) cells and MM cell. We found that bortezomb induces apoptosis of NK cells at a clinically relevant dose, and this is mainly due to induction of reactive oxygen species (ROS). Additionally, bortezomib also decreased NK cell activating receptor NKp46 expression, resulting in impaired NKp46-mediated redirected killing activity. Bay 11-7082, a pharmacological inhibitor of NF-κB activation, also reduced NKp46 expression and suppressed redirected cytotoxicity, suggesting NF-κB was involved in the regulation of NKp46 expression. To further study the effects of bortezomib on NK cells, we used human interleukin (IL)-2 activated NK cells. Down regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression at both protein and mRNA level in IL-2 activated NK cells was observed after bortezomib and Bay 11-7082 treatment, suggesting that the proteasome is involved in the regulation of TRAIL expression through modulation of NF-κB activity. Moreover, perforin-independent killing activity of MM cell line RPMI8226 and U266 was also reduced after bortezomib treatment, and blocking cell surface-bound TRAIL impaired NK cell-mediated lysis of the TRAIL-sensitive MM cell line, RPMI8226 cells. Next we studied a novel inhibitor of proteasome deubiquitination, b-AP15. We noted accumulation of polyubiquitinated proteins in RPMI8226 and U266 cells after b-AP15 treatment. Moreover, pro-apoptotic effects of b-AP15 on MM cells were also seen by detecting phosphatidylserine (PS) exposure, processing of pro-caspase-3, and cleavage of poly (ADP-ribose) polymerase (PARP); apoptosis was shown to be caspase-dependent. Additionally, b-AP15 also induced apoptosis in NK cells. However, the pro-apoptotic effect of b-AP15 on NK cells was not as pronounced as the effect of bortezomib, highlighting that b-AP15 may have less adverse effects on the immunosurveillance of NK cells against tumors. Furthermore, we evaluated a multifunctional protein HS-1 associated protein X-1 (HAX-1), which is overexpressed in MM, and its potential role in the regulation of MM. Our data indicated that silencing of HAX-1 expression in the human U266 and RPMI8226 cells can not sensitize cells to bortezomib or b-AP15, nor to NK cell-mediated killing. However, the ability of U266 cells to migration was reduced after HAX-1 knockdown, indicating that HAX-1 could play a role in regulating tumor metastasis. In summary, our studies contributed a better understanding of proteasome inhibitors as anti-cancer drugs and have provided insights into possible adverse effects on immune-competent cells. Our studies also identified HAX-1 as a possible target in MM treatment.

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