

A novel dual HDAC6 and proteasome inhibitor elicits outstanding cytotoxicity against multiple myeloma

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Introduction:

Proteostasis is an important survival mechanism in multiple myeloma (MM) cells. Histone deacetylase 6 (HDAC6) is involved in the autophagy degradation pathway of malformed proteins. Our previous research highlighted indirubin-3-monoxime (I3MO) as a promising therapeutic agent for MM due to its ability to inhibit proteasome activity. Therefore, we synthesized a novel I3MO derivative, 8b, by coupling an HDAC6 inhibitor to the I3MO structure.

Methods:

The anti-MM effects of 8b both in vivo and in vitro were investigated. RNA-seq were performed to identify downstream pathway after 8b treatment. Autophagy and proteasome inhibition phenotypes were determined. Synergistic effect of 8b with bortezomib was tested both in vivo and in vitro.

Results:

The cytotoxicity of 8b was detected in both MM cell lines and MM patient samples. 8b also displayed cell cytotoxic effect on bortezomib (BTZ) resistance cell lines KMS11-BR. 8b treatment significantly induced the apoptosis and cell cycle arrest in MM cells in a dose and time dependent manner. Furthermore, treatment with 8b (6.25 mg/kg) caused a significant tumor reduction in myeloma murine model.

RNA-seq analysis showed that 8b treatment led to down regulation of both proteasome and autophagy pathway in all four MMCLs. Decreased proteasome activities, specifically chymotrypsin-like (CT-L) and caspase-like (C-L) activities, were observed in ARP1 and U266 cell lines following 8b treatment. At the same time, Confocal microscopy analysis and flow cytometry analysis revealed a decrease in aggresome formation in MM cells with 8b treatment. After aggresome formation, it will further undergo degradation through downstream autophagy pathways. Consistent with our RNA-seq data, 8b treatment led to a decreased fraction of autophagosomes and suppressed autophagy level in MM cells.

Furthermore, 8b significantly enhanced the sensitivity of MM cells to BTZ-induced apoptosis, indicating synergistic effects between 8b and BTZ in vitro. A xenograft myeloma murine model showed that the combination treatment group, consisting of 8b (6.25 mg/kg) and BTZ (0.5 mg/kg), synergistically suppressed tumor burden compared to treatment with either 8b or BTZ alone. Recent studies have suggested that bortezomib-induced autophagy is one of the

mechanisms responsible for acquired drug resistance in MM cells. Interestingly, treatment with 8b efficiently suppressed autophagy induced by bortezomib in MM cell lines. These findings suggest that 8b synergistically enhances PIs cytotoxicity against MM by inhibiting the proteasome activity and suppressing the induced autophagy triggered by PIs.

Conclusions:

Our study demonstrated that novel dual HDAC6 and proteasome inhibitor 8b is an agent triggering dual inhibition of proteasome and autophagy, which represents a promising therapeutic strategy to improve patient outcomes in MM.