Selinexor or KPT-862 Mediated XPO1 Inhibition Synergizes with Dexamethasone to Repress Convergent Pathways in the mTORC1 Signaling Network and Drive Cell Death in Multiple Myeloma


ABSTRACT

Background: Dexamethasone (DEX), a synthetic glucocorticoid (GC), is administered to nearly all multiple myeloma (MM) patients as a single agent and in combination with other chemotherapeutic or targeted agents. DEX and other GCs bind to glucocorticoid receptors (GR) in the cytoplasm, induce nuclear translocation and regulate GC-dependent gene expression networks in the cell. Selective inhibition of XPO1 Export Sin3A/mSin3B compounds (selinexor and KPT-8620) exhibit potent anti-tumor activity in MM especially when combined with DEX. SINE compounds enhance nuclear localization of tumor suppressor proteins (TSPs) through inhibition of the nuclear export protein, XPO1. We discovered that inhibition of the mechanistic Target of Rapamycin Complex 1 (mTORC1) pathway is a primary driver of the combination effect. Here we further elucidate the molecular mechanism of action of the SINE-DEX system in MM.

Methods: GR- MM1S and GR+ MM1R MM cell lines were treated with SINE compounds and/or DEX for 24 hours. Whole cell lysates were subjected to SDS-PAGE and western blot analysis. Gene expression and GR transcriptional activity was analyzed using qPCR and ELISA, respectively. Cell viability was examined using the CellTiter-Fluor assay.

Results: We found that MM cell lines treated with SINE compounds (selinexor or KPT-8620) have increased basal GR protein levels. Consistent with these results, the SINE-DEX combination shows enhanced GR transcriptional activity. Several GR-DEX target genes are known to inhibit the GTPase RHEB. A rise homolog enriched in brain (RHEB), which is required for mTORC1 activation. We discovered that the SINE-DEX combination not only reduces RHEB protein but also induces the RHEB inhibitory pathways promoting REDD1 and the KLF15-BCT2 axis. Although SINE compound-mediated inhibition of mTORC1 (i.e. reduced phosphorylation of S6K and 4E-BP1) is GR independent. SINE-DEX inhibition is more robust in GR+ MM1S cell line when compared to the GR+ MM1R cells. This combination resulted in the selection IC50 in MM1S cells shifting from 40 to 11 nM in the presence of low dose DEX. As expected, DEX did not modulate the IC50 in MM1R cells.

Conclusion: We show that SINE compound inhibition of MM cell viability is enhanced by DEX. Our results indicate that this combination effect is due to convergent suppression of mTORC1 signaling by GR targets. The findings provide mechanism activation of the SINE-DEX combination in MM with suggestive biomarkers (RED2/D, KLF15, BCT2 and GR) that may predict better response to the combination. Therefore, these data may translate directly to the current clinical development of SINE compounds.

EXPERIMENTAL SECTION

Selinexor Plus Dexamethasone Mechanism

XPO1-Dependent Nuclear Export

Glucocorticoid Transcriptional Regulation

SINE Compounds Increase Expression of Glucocorticoid Receptor

*Selinexor Dexamethasone

Figure 2: MM15 (multiple myeloma) tumor cells were treated with 200 nM selinexor or KPT-8620 for 24 h. (A) Real-time PCR indicated that SINE compounds increased glucocorticoid receptor (GR) mRNA expression. (B) Immunoblot of whole cell lysates shows induction of GR protein levels following SINE compound treatment.

Selinexor Plus Dexamethasone Inhibition Causes A Synergistic Increase in GR Transcriptional Activity

Figure 3: MM1S and MM1R MM cell lines were treated with 1 µM selinexor and 100 nM dexamethasone (DEX) for 24 hours. (A) DEK treatment results in phosphorylation and induction of nuclear (active) glucocorticoid receptor. (B) The combination of selinexor plus DEX resulted in a 4.5-fold increase in basal GR activity, compared to 3-fold increase by DEX treatment alone. GR+ MM1R cells had no change in basal GR activity in the presence of either compound alone or in combination.

Selinexor Plus Dexamethasone Inhibition of the mTOR Pathway in GR+ and GR- MM Cell Lines

Figure 4: MM15 (GR+) and MM1R (GR-) cells were treated with 200 nM selinexor and 100 nM DEX for 24 hrs. In MM1S cells, selinexor alone had no effect on the mTOR pathway (p70S6K and pS6K). However, in combination with DEX, a significant reduction in p70S6K and 4E-BP1 phosphorylation resulted. In MM1R cells the effect of selinexor on the mTOR pathway was significant in the absence of DEX.

Selinexor Plus Dexamethasone Modulates the Expression of GR Downstream Targets That Negatively Regulate mTOR

Figure 5: To understand inhibition of the mTOR pathway, the expression of known GR regulated targets of mTOR activity were evaluated. DEX treatment alone induced the expression of all target genes to varying degrees in MM1S cells, while having no effect on MM1R cells. Interestingly, the combination with selinexor dramatically enhanced the expression of REDD1 in MM1S cells.

Selinexor Plus Dexamethasone Upregulates REDD1 and BCT2 in GR+ cells But Only BCT2 in GR- cells

Figure 6: MM1S and MM1R MM cell lines were treated with 200 nM selinexor and 100 nM DEX for 24 hours. In MM1S, BCT2 and REDD1, both downstream of KLF15 (a GR target) exhibited convergent increases. In MM1R, BCT2 was significantly reduced by selinexor treatment, although induction was not enhanced after adding DEX. DEX treatment induces the expression of REDD1 in MM1S only, which is enhanced in combination. RHEB expression was significantly reduced by SINE-DEX combination.

Selinexor Reduces BCAA Levels in GR+ and GR- cells

Figure 7: MM1S and MM1R (GR-) cells were treated with 200 nM selinexor and 100 nM DEX for 24 hours. In MM1S, BCAAT and REDD1, both downstream of KLF15 (a GR target) exhibited convergent increases. In MM1R, BCT2 was significantly reduced by selinexor treatment, although induction was not enhanced after adding DEX. DEX treatment induces the expression of REDD1 in MM1S only, which is enhanced in combination. RHEB expression was significantly reduced by SINE-DEX combination.

Selinexor S-selectors with mTOR inhibitors in GR+ and GR- cells

Figure 8: The effects of selinexor alone or in combination with rapamycin for 72 hours on cell viability of (A) MM1S and (B) MM1R cell lines using the MTT assay. Drug combination analysis using an isobologram confirmed synergistic interaction between selinexor and rapamycin in both the cell lines.

SUMMARY

- SINE compounds induce mRNA and protein expression levels of Glucocorticoid Receptor (GR) in MM cells.
- Selinexor plus dexamethasone shows marked cytotoxic synergy in cells expressing GR by positively regulating the levels of key members of the intracellular apoptosis pathway.
- Combination of selinexor and dexamethasone increases transcriptional activity of GR.
- The beneficial combinatory effect is mediated through mTOR inhibition as confirmed by Resveratrol Pair Polyphenol (RPPA) and microscopy.
- Among the known GR regulated modulators of mTOR, up-regulation of REDD1 (GR target gene) and BCT2 by the combination lead to mTOR inhibition.
- Selinexor-induced BCT2 protein lead to a reduction in cellular BCAA concentration.
- In GR+ MM 1R cells, selinexor as a single agent can negatively regulate the mTOR pathway by inducing BCT2 expression.
- Higher concentration of BCAA might make MM1R cells more susceptible to mTOR inhibition upon increase of BCAA levels.
- Selinexor synergizes with the mTOR inhibitor rapamycin, in both GR+ and GR- cells.
- Selinexor is currently being investigated in combination with DEX in a clinical study in refractory MM previously treated with/without to IMIDs, proteasome inhibitors and anti-CD38 monoclonal antibody (NCT02336815).

CONCLUSION

The current finding suggests that GR pathway status could be used as predictive marker to guide drug combination selection for MM patients. While selinexor combined with dexamethasone might be beneficial for GR+ patients, selinexor with mTOR inhibitors could prove advantageous for patients with GR- or GR tumors.

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