Selinexor, a Selective Inhibitor of Nuclear Export (SINE) Compound, Inhibits NF-κB Activity by Sequestering IκB-α in the Nucleus and Blocking IκB-α Degradation

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Abstract

Background: Selinexor is a small-molecule therapeutic that inhibits XPO1 mediated nuclear export, resulting in nuclear accumulation of tumor suppressor proteins (TSPs) and subsequently cancer cell death while sparing normal cells. It has been previously demonstrated that the inhibitor of NF-κB, IκB-α, is localized to the cytoplasm by XPO1 in several cancer cell lines and that treatment of cancer cells with selinexor reduces NF-κB transcriptional activity. The mechanism of NFκB inhibition by selinexor, however, is not fully understood. We hypothesized that nuclear retention of IκB-α and down-regulation of IκB-α kinase (IKK) in response to selinexor treatment would inhibit NF-κB transcriptional activity.
Results: TNFα induced the phosphorylation of NFκB p65 subunit on serine 536 and IκB-α on serine 32/36 through IKK. This resulted in the dissociation of IκB-α from NF-κB and led to IκB-α degradation via the 26S proteasome. Uninhibited NF-κB migrated to the nucleus and initiated transcriptional activation supporting tumorigenicity and inflammation. The IKK kinase is a complex made of two kinases (IKKα and IKKβ) and one regulatory subunit, NEMO/IKKγ. We found that selinexor treatment blocked IKK activity through the down regulation of the IKK (IKKγ) gamma subunit protein levels. This inhibition was dose dependent and prevented IκB-α phosphorylation, thereby protecting IκB-α from degradation. The protection of intact IκB-α from degradation and its forced nuclear accumulation through XPO1 inhibition enabled inhibition of NFκB transcriptional activity even in the presence of TNFα. Selinexor did not alter the protein levels of IKKα or IKKβ.
Conclusions: Selinexor blocks TNFα induced degradation of IκB-α by reducing the levels of IKKγ. In addition, selinexor increased nuclear IκB-α levels through the inhibition of nuclear export. This blocked NFκB activity and enhanced cancer cell death. We are currently investigating the beneficial effects of combining selinexor with proteasome inhibitors, which are known to prevent IκB-α degradation.

Selinexor Inhibits XPO1 and Locks Tumor Suppressor Proteins Including IκB-α in the Cell Nucleus

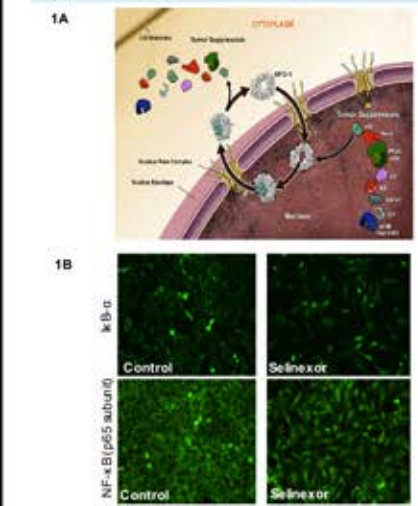


Figure 1: A. Mechanism of Selinexor Action. Selinexor inhibits XPO1-mediated nuclear-cytoplasmic transport locking tumor suppressor proteins in the cell nucleus and inducing apoptosis in cancer cells. **B.** Immunofluorescence staining of IκB-α and NF-κB p65's ubunit shows induced nuclear accumulation after treatment with selinexor.

IκB-α Binds to NF-κB Subunits both in the Cytoplasm and Nucleus

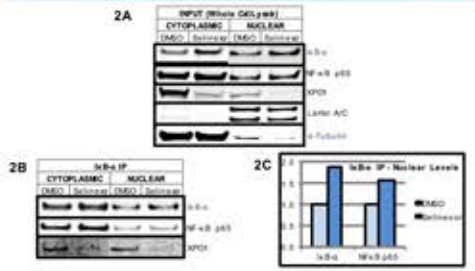


Figure 2 A: Cellular fractionation of U-2 OS (Osteosarcoma) cells shows increased nuclear levels of complexes of IκB-α - NFκB p65 after selinexor treatment. Lamin A/C was used as nuclear protein marker; α-Tubulin as a cytosolic protein marker. **B.** IκB-α immunoprecipitation (IP) of cytoplasmic and nuclear fractions shows that IκB-α binds to NF-κB and to XPO1 both in the nucleus and cytoplasm. **C.** Densitometry analysis of IκB-α and NF-κB levels in nuclear fractions following IκB-α IP shows increased levels after selinexor treatment.

Selinexor Inhibits NF-κB Transcriptional Activity

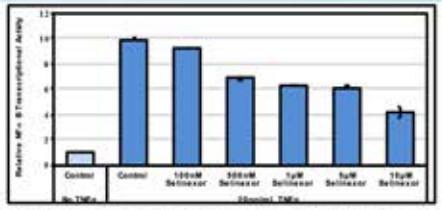


Figure 3: TNFα exposure induces NF-κB p65 subunit transcriptional activity by 10-fold. Selinexor inhibits the transcriptional activity in a dose dependent manner (in U-2 OS cells).

Selinexor Resistance Correlates with Low Levels of IκB-α and High NF-κB Transcriptional Activity

4A

U2OS cells	Selinexor IC50 (μM)	Fold Change
No transfection	0.027	1
Control siRNA	0.023	0.85
IκB-α siRNA	1.85	65.5

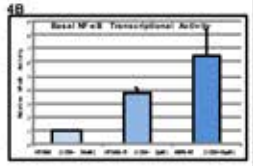


Figure 4: A. Knockdown of IκB-α in U-2 OS reduces sensitivity to selinexor by 65-fold, whereas control siRNA shows no effects. **B.** Parental sensitive fibrosarcoma, HT1080, selinexor-resistant HT1080 and clival and soft part sarcoma, ASPS-KY cells; IC50 50 nM, 2 μM and >1 0μM respectively having NF-κB transcriptional activity that directly correlates with resistance to selinexor.

Selinexor Blocks IκB-α Degradation by Inhibiting Phosphorylation of IκB-α and NF-κB

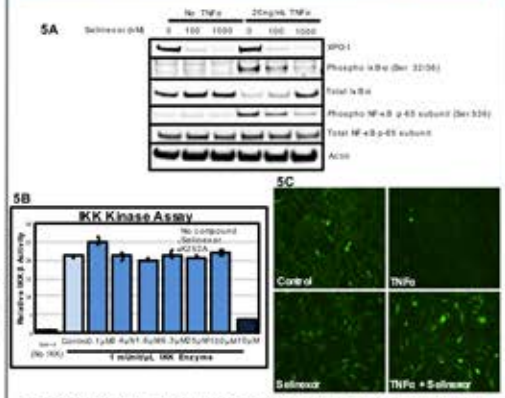


Figure 5: A. TNFα stimulation leads to phosphorylation of IκB-α and NF-κB resulting in IκB-α degradation and NF-κB pathway activation. Selinexor blocks the pro-inflammatory effects of TNFα in U-2 OS cells. **B.** The kinase activity of IκB-α kinase (IKK), which phosphorylates IκB-α and NF-κB, was analyzed using recombinant IKKβ. Selinexor has no direct inhibitory effect on IKK β kinase activity. Pan kinase inhibitor K252A was used as a positive control. **C.** Selinexor induces nuclear localization of IκB-α in the presence or the absence of TNFα.

Selinexor Reduces Protein Levels of IKKγ (NEMO), Not Mediated via Proteasome Degradation

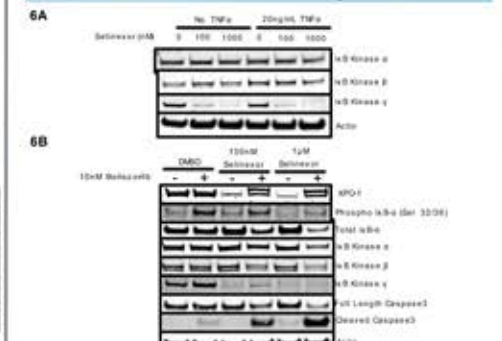


Figure 6: A. Selinexor reduces the levels of the γ subunit of IKK, but not the α and β subunits in U-2 OS cells that were treated with selinexor for 24 hours with or without TNFα stimulation. **B.** Selinexor induces the reduction of IKKγ levels in a dose dependent manner in the presence of proteasome inhibitor, bortezomib. Interestingly, accumulation of phosphorylated IκB-α seen with proteasome inhibitor treatment, which is abolished in the presence of both bortezomib and selinexor.

Selinexor Inhibits Expression of NEMO by Inducing Autophagy

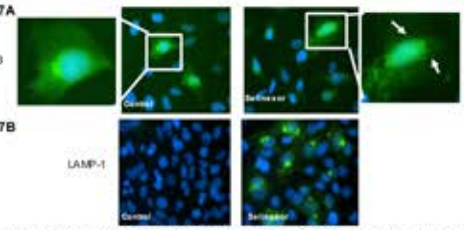
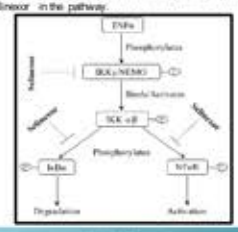


Figure 7: Selinexor induces lysosomal degradation. U-2 OS cells were **A.** transfected with Premo™ Autophagy sensor LC3B-GFP (green) or **B.** were transduced with Cell Light® Lysosomes-GFP (fusion construct of LAMP-1 and emGFP) and treated with 1 μM selinexor for 24 hours. The cells were fixed with paraformaldehyde and counterstained with Dapi. Selinexor leads to LC3B cleavage, resulting in foci formation (arrows). Lysosomal-associated membrane protein 1 (LAMP-1) is expressed only after selinexor treatment marking fusion of lysosome and autophagosome.

Summary

- Selinexor binds to XPO1 and sequesters key tumor suppressor proteins and cell cycle regulators in the cell nucleus.
- Selinexor inhibits NF-κB transcriptional activity even in the presence of TNFα. Selinexor increases the nuclear localization of both NF-κB p65 subunit and IκB-α, but IκB-α blocks the ability of NF-κB transcription factors to bind to DNA.
- Upregulation of NFκB transcriptional activity is directly correlated with selinexor cell resistance. Similarly, IκB-α silencing results with selinexor resistance.
- Selinexor blocks the TNFα induced phosphorylation of IκB-α in a dose dependent manner and restores the levels of IκB-α protein.
- Selinexor dramatically reduces the protein levels of IKKγ, but not IKKα and IKKβ.
- Selinexor induces autophagy which we predict, is responsible for IKKγ degradation.
- The diagram below summarizes the steps involved in NF-κB transcription activation and the intervention of selinexor in the pathway.



Conclusions

- **In the past we demonstrated that the combination treatment of selinexor with proteasome inhibitors is synergistic and that the treatment leads to NF-κB inhibition.**
- **Here we explain mechanistically how selinexor inhibits the NF-κB pathway.**

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