Deconstructing protein and gene expression pathways to define the anticancer effects of XPO1 inhibition in ovarian cancer

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Abstract

Emerging evidence across multiple cancer types, including ovarian cancer (OVaC), has identified dysregulation in nuclear-cytoplasmic shuttling and transcription of exportin 1 (XPO1), a key regulator of nuclear transport, as being crucial to cancer aggressiveness, chemoresistance, and decreased patient survival. These findings and their OVaC target is a role in tumor suppressor and cell cycle regulatory genes, confirming the rationale for targeted therapeutic inhibition of XPO1. We have recently reported on the generation of a novel XPO1 small molecule inhibitor (KPT-185) that is capable of blocking XPO1 function.

Results

We now demonstrate that specific XPO1 expression levels, patient-derived OvaC cell lines (PD-OvaC) exhibit a broad-dose-dependent IC50 response to XPO1 inhibition. To delineate the mechanism of action underpinning sensitivity and resistance and better understand the role of differential nuclear-cytoplasmic shuttling in OvaC, we examined the global differences between pathway activation/inhibition following XPO1 inhibition using a combination of both conventional protein expression and gene expression platforms. Differentiation between protein and gene expression levels was achieved using a combination of biological and computational approaches. Next, we investigated the expression patterns of XPO1 inhibitors in clinical settings and found that tumors with higher XPO1 expression were more sensitive to XPO1 inhibition. This finding was confirmed using a series of preclinical and clinical OvaC cell lines and tumors.

Conclusion 

Our findings indicate that XPO1 is a key regulator of nuclear transport and has a critical role in cancer aggressiveness. This novel XPO1 inhibitor provides a promising therapeutic approach for the treatment of OvaC.

Methods

Patient samples were collected per our IRB and PD-OvaC cell lines were isolated and established as described by Liu et al. (2015). PD-OvaC cells were treated with KPT-185, IC50 (116.1 nM) for varying time points and cells were collected and fractionated into cytoplasmic and nuclear fractions. Western blot analysis of multiple protein expression was performed to determine the exact time point of maximal protein accumulation in the nuclear versus XPO1-inhibited fraction. Using the signatures generated in these experiments, we performed a supervised analysis using the Illumina, GeneChip, and ProtoArray expression analysis software to identify genes differentially expressed in response to XPO1 inhibition. To further validate these findings, we performed a unsupervised analysis using the RNA-seq data from OvaC cell lines treated with KPT-185.

Conclusions & Future Directions

- PD-OvaC and OvaC cell lines exhibit a differential sensitivity to XPO1 inhibition by KPT-185.
- XPO1 inhibition results in a unique transcriptomic and proteomic profile between the three OvaC cell lines examined.
- Ingenuity IPA analysis allowed for identification of potential targets that were validated by XPO1 analysis.
- The expression of specific pathways indicates that the expression profiles of these pathways may provide a potential biomarker for detection and monitoring of efficacy.
- XPO1 expression levels were found to be a key mediator of cellular proliferation and survival in OvaC.
- Further studies into the interaction between various pathways and their regulation in OvaC are needed to fully understand the mechanisms underlying therapeutic resistance.

References

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- XPO1 inhibition induces differential responses in PD-OvaC cells irrespective of XPO1 levels. (A) PD-OvaC cell viability was measured by MTS assay after the addition of KPT-185, IC50 (116.1 nM). IC50 inhibited cell viability was measured by MTS assay after the addition of KPT-185, IC50 (116.1 nM). IC50 inhibited cell viability was measured by MTS assay after the addition of KPT-185, IC50 (116.1 nM).

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