Targeting nuclear transport pathways to overcome endocrine resistance and recurrence

Eylem Kulkoyluoglu[1], Kinga Wrobel[1], Yiru Chen[1], Jamie Holloway[2], Yosef Landesman[3], Partha S. Ray[4,5,6,7], Alexander E. Lipka[8], Rebecca L. Smith[9], Zeynep Madak-Erdogan[1]

[1] Department of Food Science and Human Nutrition, UIUC, Urbana, IL
[2]Arlington, MA
[3]Karyopharm Therapeutics Newton Massachusetts, USA
[4,5,6,7] Onconostic Technologies, Urbana, IL, Department of Surgery, Department of Bioengineering, Interdisciplinary Health Sciences Institute
[8]Departmen of Crop Sciences, UIUC, IL
[9] Department of Pathobiology, Collage of Veterinary Medicine, UIUC, Urbana, IL

ABSTRACT

Currently, around 75% of patients with breast tumors test positive for estrogen receptor alpha (ERα) and are treated with endocrine therapies, such as tamoxifen. One-third of the breast tumors eventually become refractory, reducing the survival rate for affected patients. A combination of alternative endocrine therapies and kinase inhibitors is currently used in such patients. However, after an initial period of therapy response, these tumors relapse in a more aggressive form. Further, the alternative therapies are not optimal in terms of pharmacological properties, are poorly tolerated, and have side-effects that severely decrease quality of life of the patient. Thus, there is a critical need for novel, targetable, mechanism-based therapeutic strategies that 1) re-sensitize ERα (+) tumors to endocrine therapies, and 2) include diagnostic methods to select patients likely to benefit from this approach.

Our objective in this study is to validate a group of nuclear transport genes as biomarkers for endocrine resistance, and to evaluate their inhibition as a novel means to enhance the effectiveness of endocrine therapies. Our central hypothesis is that high expression of these genes in ERα (+) tumors serve as a viable biomarker for risk of endocrine therapy failure. We focused on XPO-1, the main nuclear export protein, which exports ERK5 from the nucleus to the cytoplasm and we used selinexor (KPT-330), the inhibitor of XPO-1, which is already used in clinical trials for solid and hematological cancers. Our experiments show that estradiol induces nuclear localization of ERK5, which otherwise would contribute to increased invasiveness and metastatic potential in the cytoplasm. Selinexor (KPT 330) increases ERK5 nuclear localization in tamoxifen resistant breast cancer cell lines. Our hypothesis is that sequestering ERK5 in the cell nucleus and blocking its recycle into the nucleus by selinexor is directly associated with the improved transcriptional response to endocrine therapies. The nuclear export pathways have not previously been implicated in the development of endocrine resistance, and given the need for better strategies for selecting patients to receive endocrine reagents and improving therapy response of relapsed ERα (+) tumors, our findings show high and significant promise for uncovering the role of these pathways and demonstrating their use in reducing cancer recurrences.

RESULTS

Nuclear transport gene signature is upregulated in BT474 cells (Luminal B type) compared to MCF-7 cells (Luminal A type).

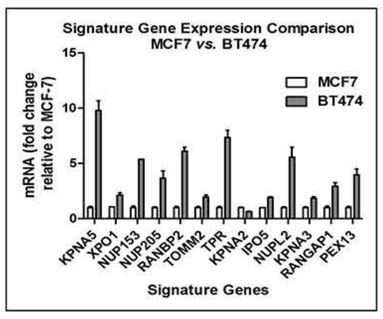


Figure 1: mRNA expression levels of 13-signature genes in both MCF-7 and BT474 cells.

In clinical tumor samples, XPO-1 mRNA is higher in Luminal B subtype breast tumors.

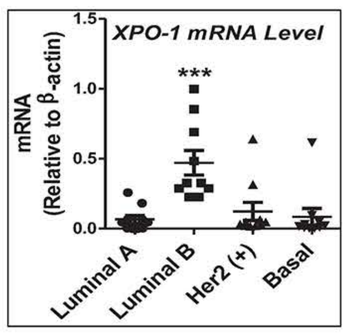


Figure 2: mRNA is obtained from 10 tumor samples per subtype and XPO1 mRNA expression is analyzed by Q-PCR. One way ANOVA, Bonferroni post-test, ***, *P*-value<0.0001

Overexpression of XPO1 in Tamoxifen responsive MCF-7 cells increases activation of pro-proliferative kinase signaling pathways and cell proliferation.

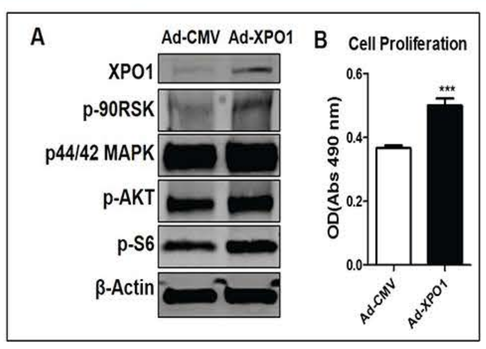


Figure 3: (A) MCF-7 cells were transduced with XPO-1 Adenovirus and pro-proliferative kinase protein levels were determined. (B) The impact of XPO-1 transduction on MCF-7 cell proliferation was determined by WST1 assay.

XPO-1 inhibitor, KPT 330 (Selinexor), inhibit cell proliferation (A) and abrogate 4-OH-Tamoxifen induced cell cycle progression in BT474 cells (B).

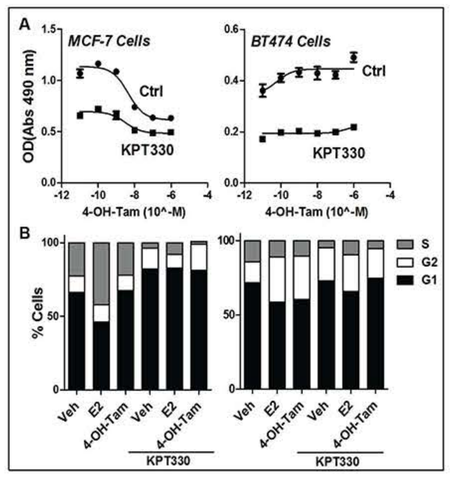


Figure 4: (A) Cells were treated with increasing doses of 4-OH-Tamoxifen in the presence and absence of 1 μm KPT 330. Cell numbers were examined using MST assay at day 6. Values are the mean ±SEM from at least 2 independent experiments. (B) Cells were treated with VEH or 10^{-7} M 4-OH-Tamoxifen in the presence and absence of 1 μm KPT 330. Cell numbers were analyzed with FACS analysis.

XPO-1 is required not for ERα mediated gene transcription in response to estrogen, but required for 4-OH-Tamoxifen preferential gene expression.

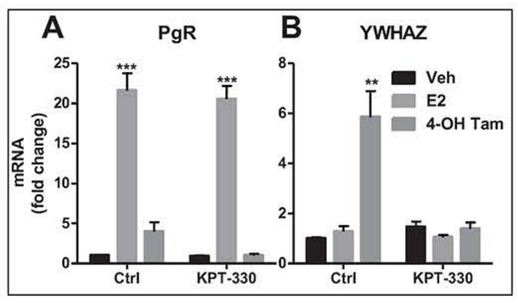


Figure 5: XPO-1 inhibitor, KPT 330 (Selinexor) blocks 4-OH-Tamoxifen gene expression (A), but not E2 preferential gene expression (B) in MCF-7 cells. Cells were treated with 10^{-6} M E2 and 10^{-7} M 4-OH-Tamoxifen in the presence and absence of 1 μm KPT 330. Values are the mean ±SEM from at least 2 independent experiments.

XPO-1 inhibitor KPT 330 (Selinexor) increases E2 and 4-OH-Tamoxifen mediated PgR expression in Tamoxifen resistant BT474 cells.

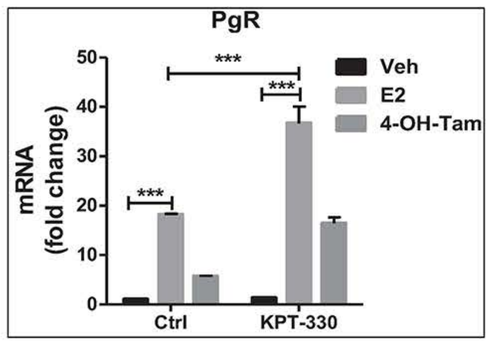


Figure 6: In the presence of XPO-1 inhibitor, E2 stimulation of PgR, which is a good prognostic factor, is increased. It suggests that XPO-1 inhibition might improve tumor outcomes. BT474 cells were treated with 10^{-6} M E2 and 10^{-7} M 4-OH-Tamoxifen in the presence and absence of 1 μm KPT 330. Values are the mean ±SEM from at least 2 independent experiments.

XPO-1 inhibitor KPT 330 (Selinexor) decreases cell proliferation in combination with 4-OH-Tamoxifen in both Tamoxifen sensitive MCF-7 cells and Tamoxifen resistant BT474 cells.

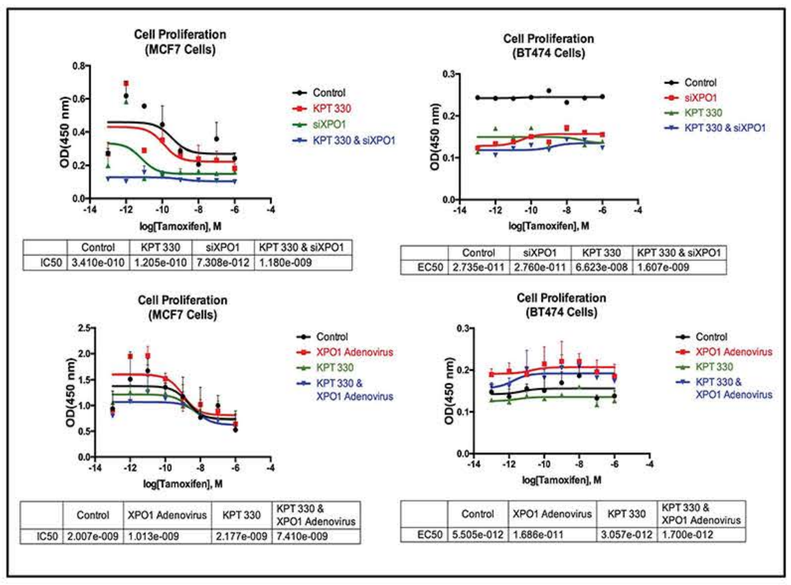


Figure 7: MCF-7 and BT474 cells were treated with increasing doses of 4-OH-Tamoxifen in the presence and absence of 1 μm KPT 330. Cell numbers were examined using WST1 assay at day 6. Values are the mean ±SEM from at least 2 independent experiments.

KPT 330 (Selinexor) increases ERK5 nuclear localization of ERK5 in tamoxifen resistant BT474 cells.

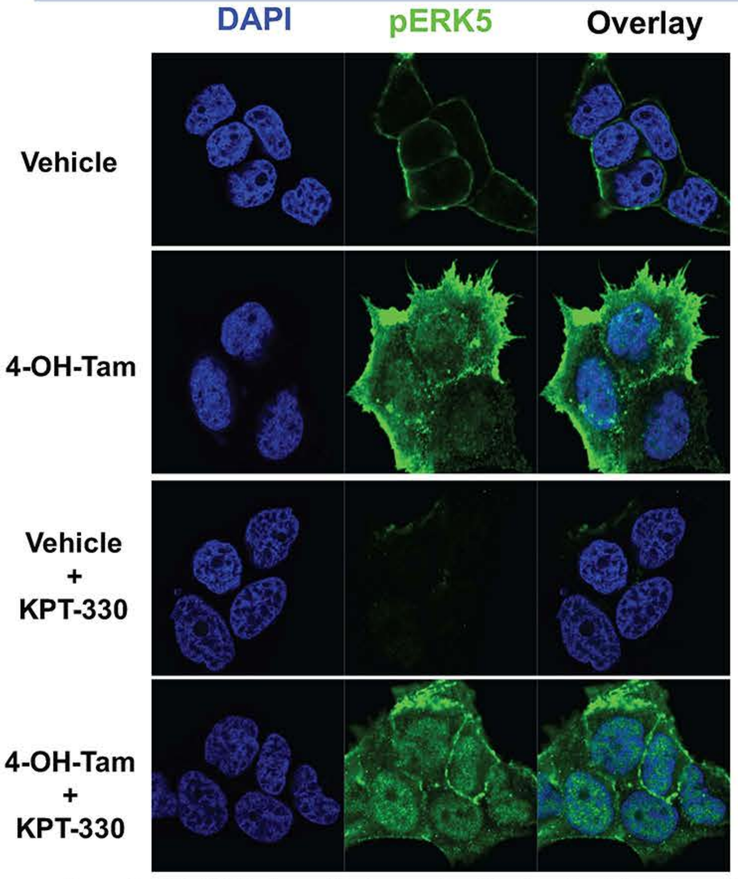


Figure 8: KPT 330 (Selinexor) induces relocalization of pERK5 into nucleus in BT474 cells. Immunofluorescence microscopy was performed after 45 minutes of 10^{-6} M 4-OH-Tamoxifen and 30 minutes KPT 330 (Selinexor) treatment in BT474 cells with an antibody specific to pERK5. Nuclei were stained with DAPI.

XPO-1 inhibitor KPT 330 (Selinexor) blocks 4-OH-Tamoxifen (TAM) stimulated tumor growth in BT474 xenograft model.

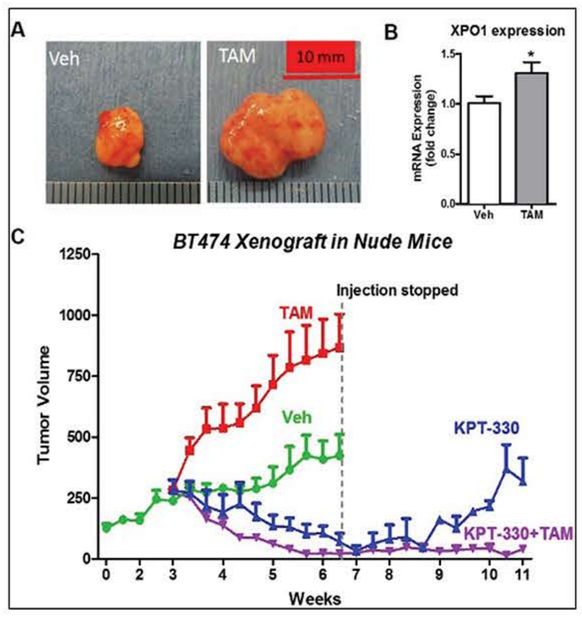
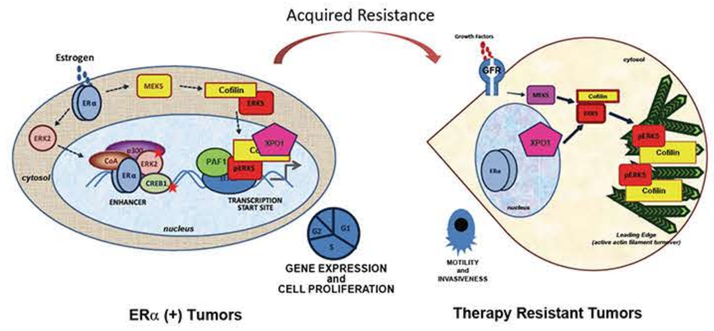


Figure 9: (A) 4-OH-Tamoxifen induces growth of BT474 xenografts in nude mice. (B) 4-OH-Tamoxifen treatment increases XPO-1 expression in tumors. (C) KPT 330 resensitizes BT474 xenografts to Tamoxifen treatment.

CONCLUSION



Overall, results of our studies should validate XPO1 as a target whose inhibition should enhance the effectiveness of endocrine therapies in breast cancer subtypes that are more refractory to endocrine therapies. Validation of XPO1 inhibition as a cancer treatment will lead us to devise, in the future, clinical trials for combination of two clinical therapeutic agents, 4-OH-Tamoxifen and KPT 330 (Selinexor) for therapy resistant breast cancers.

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