# Novel role of XPO1 in regulating MicroRNAs related to pancreatic ductal adenocarcinoma invasion and metastasis

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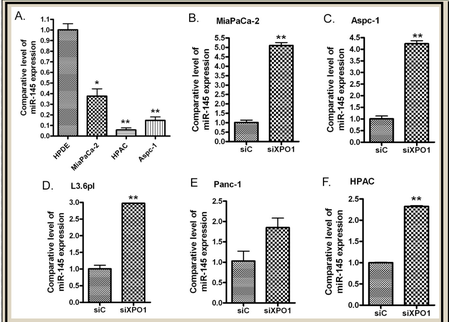
## ABSTRACT

There are no known reports on the role of exportin 1 (XPO1) in microRNA biology. In this study, we for the first time demonstrate that interfering with XPO1 machinery can influence miRNA signaling leading to suppression of pancreatic ductal adenocarcinoma (PDAC) proliferation, invasion and metastasis. Our molecular experiments showed that the inhibition of cell proliferation and migration by the XPO1 inhibitor, selinexor, is mediated through the up-regulation of miR-145 and down-regulation of its target genes including EGFR, MMP1, MT-MMP, c-Myc, Pak4 and Sox-2. Selinexor also regulates the expression of miR-34c, let-7d, and miR-205.

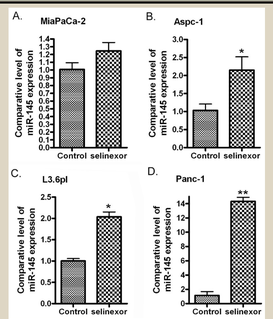
## METHODS

miRNA arrays and RT-PCR were performed on total RNA samples from PDAC cell lines (HPAC, MiaPaCa-2, AsPc-1 and L3.6pl) and normal human pancreatic ductal epithelial (HPDE) cells. PDAC cells were treated with selinexor or transfected with XPO1 siRNA or miR-145 mimic. The total RNA and protein from treated or transfected cells were subjected to real-time PCR or immunoblot analysis. The impact of selinexor on PDAC proliferation, invasion and migrations was also evaluated using MTT and scratch assay.

## RESULTS

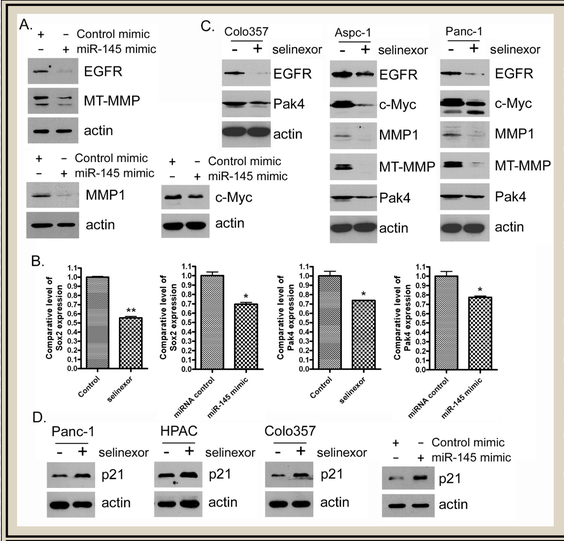



**miR-145 was significantly down-regulated in PDAC cells (A) and transfection of XPO1 siRNA induced the expression of miR-145 (B-F).** The total RNAs were subjected to real-time RT-PCR for detection of miR-145 expression (*: p<0.05; **: p<0.01).

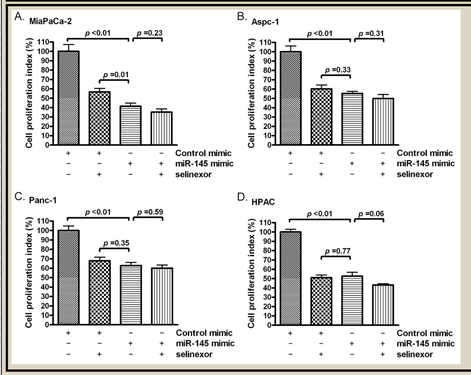



**Selinexor treatment increased the expression of miR-145.** PDAC cells were treated with 500nM selinexor for 48h. miR-145 expression was tested by real-time RT-PCR.

## RESULTS

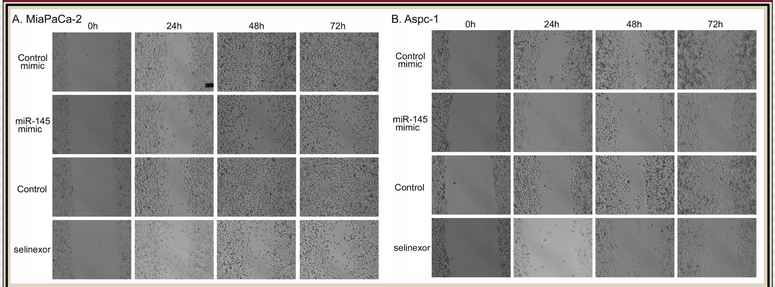



**Selinexor treatment or miR-145 mimic transfection inhibited the expression of miR-145 target or downstream genes at protein or RNA level.** MiaPaCa-2, Aspc-1, Panc-1, Colo357 and HPAC cells were treated with 500nM selinexor or transfected with miR-145 mimic or control mimic for 72 hours. Total protein was extracted from each sample and subjected to Western Blot analysis for detection of EGFR, MMP1, MT-MMP, c-Myc, Pak4 and $p21^{WAF1}$ expression at protein level. Total RNA was extracted and subjected to real-time PCR for detection of Sox-2 and Pak4.

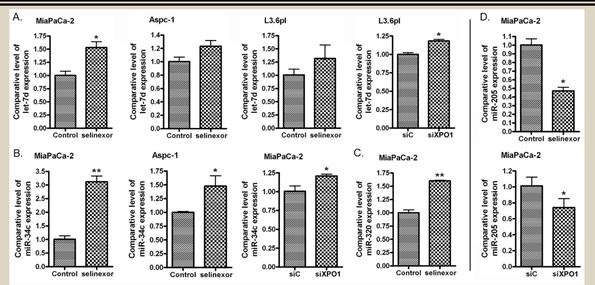



**Selinexor treatment or miR-145 mimic transfection inhibited the proliferation of PDAC cells.** MiaPaCa-2 (A), Aspc-1 (B), Panc-1 (C) and HPAC (D) cells were treated with 500 nM selinexor or transfected with miR-145 mimic or control mimic. The cell proliferation index was measured by MTT assay.

## RESULTS




**Selinexor treatment or miR-145 mimic transfection suppressed the migration activity of PDAC cells.** Selinexor treated or miR-145 mimic transfected MiaPaCa-2 (A) and Aspc-1 (B) PDAC cells were seeded in 6 well plate and subjected to wound healing assay for 3 days. The cells were photographed in each day.




**Selinexor treatment or miR-145 mimic transfection induced the expression of let-7d (A), miR-34c (B) and miR-320 (C), and reduced the expression of miR-205 (D).** MiaPaCa-2, Aspc-1 and L3.6pl PDAC cells were treated with 500 nM Selinexor or transfected with miR-145 mimic or control mimic for 48 hours. The total RNA from each sample was subjected to real-time RT-PCR for detection of let-7d, miR-34c, miR-320 and miR-205.

## CONCLUSIONS

**These results are the first to show that targeted inhibition of the nuclear exporter protein XPO1 by RNAi or chemically by selinexor could restore tumor suppressive miRNAs in PDAC.**

**Selinexor, a Phase II drug, could be used in combination with conventional chemotherapeutics for better treatment outcome in aggressive PDAC that warrants further clinical investigations.**