Selective Inhibitor of Nuclear Export (SINE) compounds prevent migration and proliferation of Triple Negative Breast Cancer (TNBC) cells by restoring expression of ARRDC3

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Introduction

TNBC is the most aggressive types with worst clinical outcomes among the four district sub-types (luminal A, lumi nal B, HER2-positive and TNBC) classified by gene express ion profiles.
Currently, there is no approved targeted therapy for either early or late stage TNBC patients as a majority of TNBC lac ks therapeutically targetable hormone receptors (estrogen a nd progesterone) and HER2 . Some TNBC-targeted therape utics including cetuximab (anti-EGFR monoclonal antibody), imatinib (c-KIT tyrosine kinase inhibitor), iniparib (PARP inhi bitor) and cisplatin are currently undergoing preclinical/clinic al investigation, but the trials of these agents have failed to demonstrate clinical efficacy. For this reason, discovering eff ective molecular targets and associated therapies for TNBC is an urgent issue.

ARRDC3 (arrestin-related domain-containing protein-3), one of 6 human a-arrestin families, is a negative regulato r of the b2-adrenergic receptor (b2AR) and integrin b4 (ITG b4) by mediating ubiquitination and subsequent degradation of phosphorylated form of these receptors. A negative regul ation of b2AR and ITG b4, whose roles in breast cancer pro gression are established, indicates the role of ARRDC3 as a potential metastatic suppressor. Our previous studies demo nstrated that epigenetic silencing of ARRDC3 is linked to ag gressive nature of TNBC cells, suggesting that ARRDC3 co uld be a novel therapeutic target of TNBC.

Selective Inhibitors of Nuclear Export (SINE) comp ounds, are small molecule inhibitors of Exportin 1 (XPO-1,c alled as chromosome region maintenance 1, CRM1). Expre ssion of XPO-1 is up-regulated in several types of cancers and its overexpression is linked to poor prognosis. Inhibition of XPO-1 by SINE compounds results with nuclear retention and activation of tumor suppressor proteins such as p53, Ik B, and FOXO. In the following study we used two SINE com pounds: KPT-185 and selinexor, a clinical SINE compound which is being evaluated in multiple later stage clinical trials in patients with relapsed and/or refractory hematological an d solid tumor malignancies.

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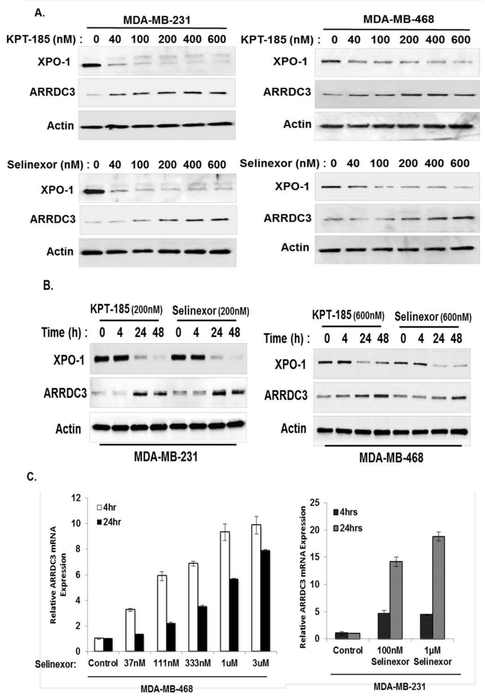
Objective

The main aim of this project is to investigate the hypothesis that small molecule compounds restoring ARRDC3 level could potentially be a novel therapeutic option for TNBC.

Questions to be investigated;
1. Can SINE compounds induce anti-cancer effects in TNBC model?
2. If yes, then does ARRDC3 mediate the anti-cancer effects of SINE compounds?

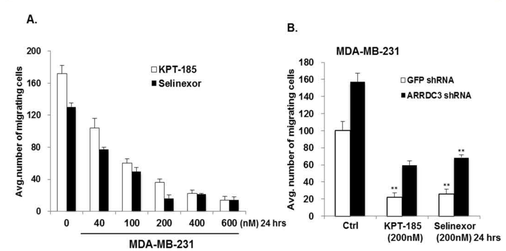
Results

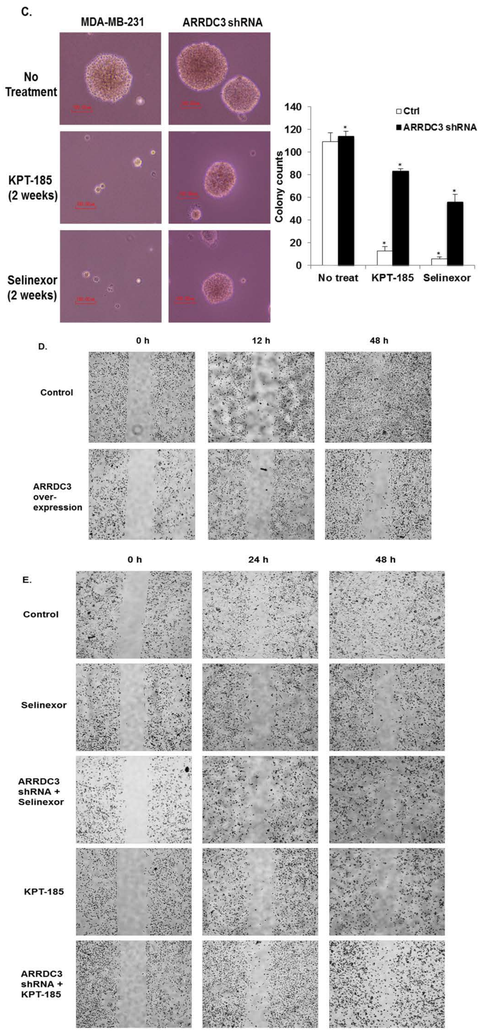
SINE compounds restore ARRDC3 expression in TNBC cell lines



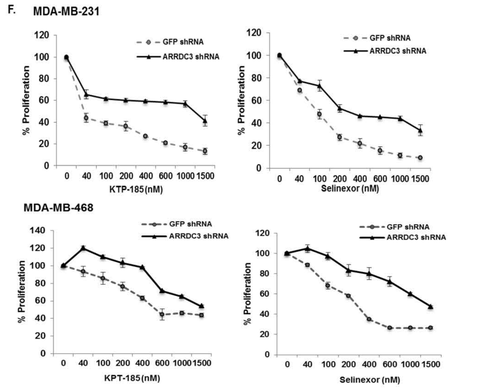
(A) MDA-MB-231 and MDA-MB-468 cells were treated with or without various concen trations of KPT-185 and selinexor for 24hr before lysis by RIPA buffer. Whole cell lysat es were analyzed for expression of ARRDC3, XPO-1 and actin by Western blotting. (B) MAD-MB-231 cells treated with 200 nM of the compounds and MDA-MB-468 treat ed with 600 nM of compounds were incubated at the indicated times. Protein levels were determined by Western blot analysis. (C) Cells were treated with Selinexor at th e indicated concentrations for 4hr and 24hr. Purified total RNA was subjected for qRT-PCR.

SINE compounds inhibits TNBC functions important for progression in an ARRDC3 dependent manner.



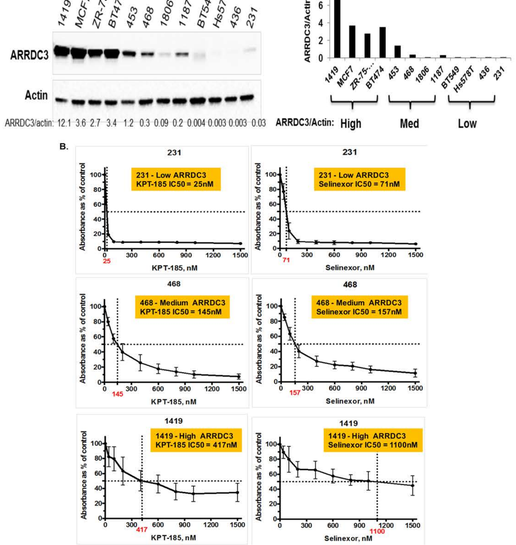


(A) MDA-MB-231 cells were treated with various concentrations of KPT-185 and selin exor. The ability of cells to migrate toward 100 nM LPA was measured using a transw ell cell motility assay after 24hr treatment. Migration was quantified by counting the ce lls that migrated to the lower surface of the membrane per square milliliter using brigh t-field optics. (B) MDA-MB-231 cells were stably infected with either GFP (as control) or ARRDC3 shRNA. These cells were treated with 200 nM of KPT-185 and Selinexor for 24hr and subjected to transwell cell motility assay. (C) MDA-MB-231 cells expressi ng GFP or ARRDC3 shRNA were cultured in soft agar containing growth medium with KPT-185 and selinexor for two weeks as described in materials and methods. Left pa nel shows images of colony conformation, which is captured at x10 magnification. Rig ht graph shows quantification of colony numbers. (D) MDA-MB-231 cells overexpress ing GFP or GFP-ARRDC3 lenti-vector were split into the chambers (Ibidi's culture-ins ert in μ-dish) (E) The MDA-MB-231 cells expressing shRNA against GFP and ARRDC 3 were loaded into the chambers and allowed to adhere overnight. The chambers wer e removed and then SINE compounds (KPT-185; 1uM and selinexor; 1uM) diluted in medium were added to dish. Wound-healing assay was carried out in triplicate. Snap shots at specific time points were used as representative image.



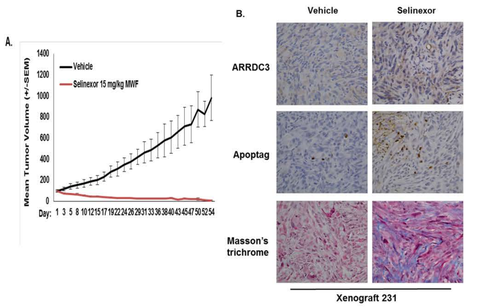
(F) MDA-MB-231 and MDA-MB-468 cells expressing GFP or ARRDC3 shRNA were treated for 72hr and 48hr with different concentrations of KPT-185 and selinexor. Proliferation of these cells was measured by MTT assay.

Sensitivity of SINE compounds inversely correlates with basal levels of ARRDC3 expression in breast cancer cell lines



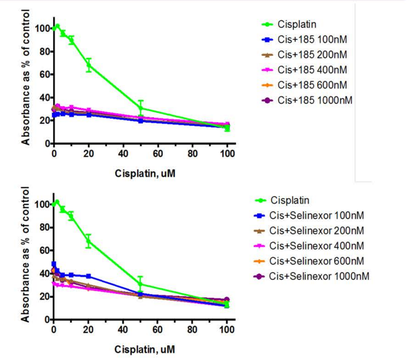
(A) Whole cell lysates were prepared from the indicated cell lines. Equal amounts of extracts from each sample were used for Western blot analysis by using anti-AR RDC3 antibody. β-Actin was used as loading control. Densitometric analysis was p erformed to measure the relative intensity of the bands from Western blotting anal ysis.(B) MDA-MB-231 (Mesencymal-like TNBC), MDA-MB-468 (Epithelial-like TNB C) and HCC-1419 (Luminal Breast cancer) cells were seeded in 96-well plates an d then treated with various concentrations of KPT-185 and Selinexor for 72hr. Cell viability was measured by MTT assay. The absorbance was normalized against th e control (as 100%). Dose-response curve were plotted. IC50 values were determi ned by using GraphPad Prism 6.

Selinexor effectively restores ARRDC3 expression and inhibits in vivo tumor growth of MDA-MB231 xenograft



(A) Mice bearing MDA-MB231 xenograft tumors were treated with vehicle or seline xor (15mg/kg; PO, QOD: Monday, Wednesday and Friday. Tumor size was measu red at the indicated days for 54 days. Error bar represents SEM (P=0.0002) (B) At the end of treatment, tumor tissues were excised. ARRDC3 and apoptosis were an alyzed by immunohistochemically and tumor stroma by Mason's Trichrome.

SINE compounds synergize with cisplatin to inhibit TNBC cell proliferation



MDA-MB-231 cells were treated with indicated concentrations of SINEs (KPT-185 and Selinexor) with or without cisplatin for 48hr. Cell growth inhibition was measur ed by MTT assay. The absorbance was normalized against the control (as 100%). The curve were plotted by using GraphPad Prism 6.

Conclusions

1. SINE compounds have potent inhibitory effects in TN BC model *in vitro* and *in vivo*.
2. SINE compounds restore ARRDC3 expression and re storation of this expression shows important therapeut ic effects in TNBC.
3. SINE compounds and specifically selinexor could be an effective therapeutic option for TNBC with down-regulated ARRDC3 expression.