NOVEL ACTIVITY OF SELECTIVE INHIBITORS OF NUCLEAR EXPORT IN EPITHELIAL-TO-MESENCHYMAL TRANSITION MODELS

LB-185

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ABSTRACT

Epithelial-to-mesenchymal transition (EMT) that confers tumors an aggressive phenotype is a fine tuned process regulated by a number of molecules that are strategically distributed in the nuclear and cytosolic compartments of cancer cells. Major EMT regulating proteins and transcription factors (TFs), such as wnt/βcatenin, notch, TGF-β, Twist and Snail are recognized to undergo nuclear-cytosolic shuttling using specialized transporters; karyopherins (Fig1). The export of most of the EMT regulating TFs is mediated exclusively by Exportin1/XPO1/CRM1 through nuclear exclusion sequence (NES) recognition. These observations indicate that nuclear transport may play a critical role in the development of EMT (Fig2). However, to date there have been no studies evaluating the impact of nuclear export inhibition on EMT. We have earlier developed Specific Inhibitors of Nuclear Export (SINEs) that block XPO1 leading to nuclear retention of various tumor suppressor proteins (TSPs) and TFs. This is the first report showing that XPO1 inhibition by SINEs induce global re-organization of proteins leading to reversal of EMT in snail transduced immortalized mammary epithelial cells.

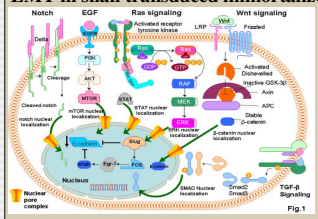


Fig1. Nucleus as the reservoir for EMT promoters

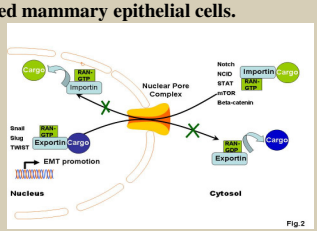


Fig2. Karyopherins as gatekeeper for EMT protein movement. Azmi et al., CCDT 2014

METHODS

Reagents: HMLE-SNAIL cells were provided by Dr. Robert Weinberg from Whitehead Institute. Cell growth inhibition, apoptosis, protein expression and localization was evaluated using MTT, Annexin V FITC, Histone ELISA, colonogenic, immunofluorescence and Western blotting respectively. For microarray expression profiling, RNAs from quadruplet samples were obtained post 24 hrs SINE treatment followed by Ingenuity systems analysis.

RESULTS

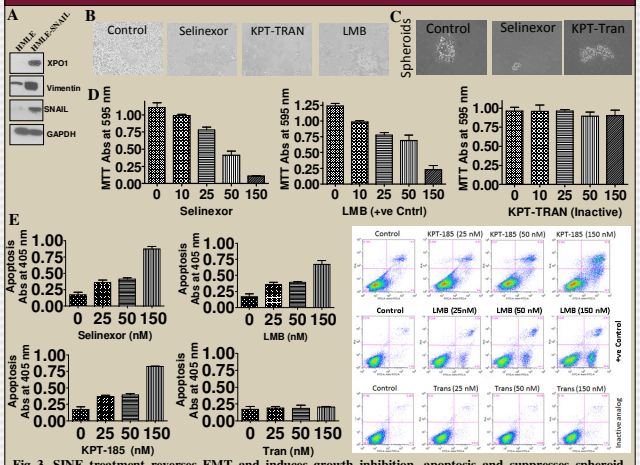


Fig 3. SINE treatment reverses EMT and induces growth inhibition, apoptosis and suppresses spheroid forming ability of HMLE-SNAIL cells. [A] HMLE-SNAIL cells show higher expression of XPO1 **[Right]** Selinexor (KPT-330), its inactive analog KPT-TRANS or Leptomycin B (LMB as +ve control) at clinically relevant concentration of 150 nM (24 hrs) resulted in reversal of cellular morphology from mesenchymal to epithelial. **[C]** sphere forming assay **[D]** growth inhibition MTT **[E]** Apoptosis Histone ELISA {left), Annexin (left).

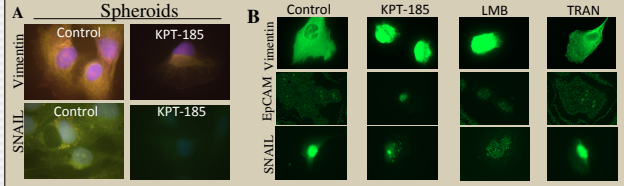
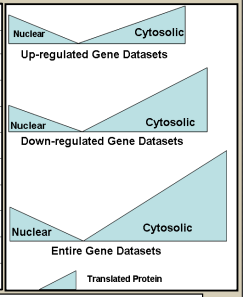


Figure 4. SINEs suppress EMT markers. [A] IF of SINE treated HMLE-SNAIL spheroids (150 nM) **[B]** IF of HMLE-SNAIL cells post SINE (150 nM) treatment for 24 hrs.

RESULTS

Network	Score	Focus Molecule	Function
1	40	28	Gene Expression, Protein Synthesis, Cancer
2	36	26	DNA Replication, Recombination, and Repair, Energy Production, Nucleic Acid Metabolism
3	34	26	Cell Death and Survival, Nervous System Development and Function, Cellular Movement
4	29	23	Cell Death and Survival, Nervous System Development and Function, Cellular Movement
5	28	22	Developmental Disorder, Hereditary Disorder, Metabolic Disease
6	27	22	Cell-To-Cell Signaling and Interaction, Cellular Movement
7	26	21	Cellular Development, Cellular Growth and Proliferation, Tissue Development and Function
8	26	22	Cell Death and Survival, Nervous System Development and Function
9	25	21	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry
10	24	20	Carbohydrate Metabolism, Molecular Transport, Small Molecule Biochemistry



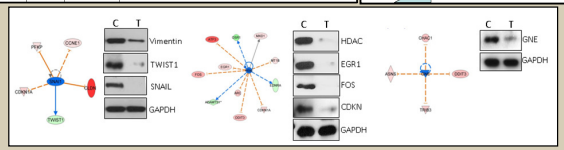


Figure 5. Systems analysis of SINE activity in HMLE-SNAIL cells [Left] Ingenuity identified top 10 networks modulated by KPT-185 **[Right]** DAVID and Genomatix tools were used to address the question whether there is enrichment in the dataset according to localization of translated protein. Three data sets were used in the analysis: up-regulated gene list, down-regulated gene list and entire genes (p=0.01) which shows cytosolic enrichment to a level of significance. **[Bottom Panel]** Biological validation of down-regulated networks.

CONCLUSIONS

Inhibition of nuclear exporter XPO1 reverses mesenchymal phenotype to epithelial

EMT reversal was found to be through nuclear retention and degradation of SNAIL protein along with suppression of Vimentin and EpCAM

Systems biology and pathway network analysis confirms that XPO1 inhibition induces global re-organization of EMT promoters shifting the balance towards MET

In view of our results, nuclear export inhibition can potentially be a new form of therapy for highly aggressive tumors that harbor EMT