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

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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/b-catenin, 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.

METHODS

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 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 spherical forming ability of HMLE-SNAIL cells. [A] HMLE-SNAIL cells show higher expression of XPO1 (Right) Sclerostin (KPT-185), its inactive analog KPT-TRAN or Leporinyn 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] Graph showing Spheroids forming assay [B] Growth inhibition MTT and [E] Apoptosis by Annexin V-FITC (left), Apoptosis by ELISA (right).

CONCLUSIONS

Inhibition of nuclear export 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 E-cad.

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.