**ABSTRACT**

FBXL5 is an F-box protein and one of four subunits constituting the ubiquitin protein E3 ligase complex (SKP1-cullin-F-box). It has been shown to promote nuclear ubiquitination therefore down regulating snail induced epithelial-to-mesenchymal transition (EMT). However, cancer associated up regulation of the nuclear export Cm1/Exportin 1 (XPO1) expression results in nuclear exclusion of FBXL5 leading to snail stability and EMT. Here we demonstrate that XPO1 inhibition by Selective Inhibitors of Nuclear Export (SINE) results in nuclear retention of FBXL5, leading to nuclear degradation of Snail and to the reversal of EMT in HMLE-SNAIL models.

**METHODS**

Reagents: HMLE-SNAIL cell pairs were provided by Dr. Robert Weinberg from the Whitehead Institute. Cell growth inhibition, apoptosis, protein expression, localization and ubiquitination were evaluated using MTT, Annexin V FITC, Histone ELISA, co-precipitation, western blotting and Co-IPs respectively. For systems analysis microarray expression profiling RNA from quadruplet samples was performed at 24 hrs. Xenograft were developed by injecting 1x10^6 Ras-HMLE-snaill cells bilaterally at subcutaneous site in ICR SCID mice.

**RESULTS**

**XPO1 inhibition by Selective Inhibitors of Nuclear Export (SINE) reverses EMT.** [A] XPO1 expression (150 nM) of either KPT-330/Selinuxor or KPT-385 (~24 hrs) resulted in down regulation of Snail and reversal of EMT. [B] Western blotting of whole cell lysates (WCL) and nuclear lysates (NCL) under similar treatment conditions: [D] E3 ligase complex (SKP1-cullin-F-box). It has been shown to promote snail nuclear degradation of SNAIL and to the reversal of EMT in HMLE-SNAIL models.

**Role of FBXL5 in Snail turn-over and consequent reversal of EMT.** [A] FBXL5 (cytostatic (100 nM) of FBXL5 treated cells exposed to the experimental conditions of either control or SINE treated cells). Snail and ubiquitin antibody followed by Western blotting with snail specific antibody. [C,D,E,F] MTT and Apoptosis at 72 hrs post KPT-330/Selinuxor treatment in the absence or presence of siRNA for FBXL5 (100 nM). [H] Western blot showing FBXL5 siRNA silencing. [I] Western blot showing FBXL5 siRNA silencing. [J] Immunohistochemistry showing enhanced proliferation indicating its role in regulating snail expression.

**CONCLUSIONS**

Inhibition of the nuclear exporter XPO1 by Selinuxor reverses EMT

EMT reversal results from the nuclear retention of the F-BOX protein FBXL5 and the nuclear degradation of SNAI1.

Selinuxor leads to complete tumor inhibition (tumor free at 120 days) in ras transformed HMEL-snaill derived sub-cutaneous xenografts

This study demonstrates a novel strategy of targeting EMT at the nuclear pore using Selinuxor.