Activation of Oncogene-Induced Senescence in Autochthonous *Kras* Lung Tumors by Inhibition of *Twist1*

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Introduction

- *Kras* mutant adenocarcinomas of the lung are refractory to targeted agents and have been labeled “undruggable”.
- The identification of additional molecular targets in *Kras* mutant lung tumors that when inhibited result in a clinical response are needed.
- Oncogene-induced senescence (OIS) is a failsafe program that prevents normal cells from progressing towards malignancy following introduction of a mutant form of an oncogene such as *Kras*.
- OIS results in proliferative arrest of pre-malignant cells and *Twist1* has been shown to suppress OIS *in vitro*.
- We hypothesized *Twist1* may serve as a therapeutic target in *Kras* mutant lung tumors and upon inactivation of *Twist1,* OIS may activate and result in a clinical response

Results

- We have developed a novel inducible *Twist1* transgenic lung autochthonous tumor mouse model that recapitulates *KRAS* mutant human non-small cell lung adenocarcinomas (Fig 1A).
- *Twist1* accelerates *Kras* mutant lung tumor development, promotes progression from benign adenomas to adenocarcinomas and suppresses *Kras*-induced senescence (Fig 1B-F).

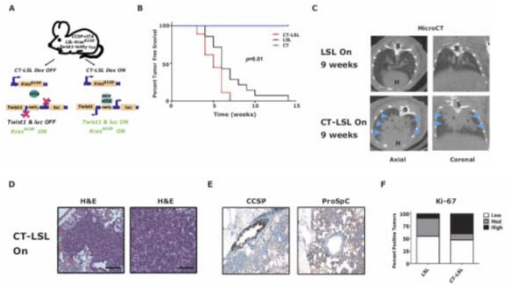

Figure 1. *Twist1* accelerates *KrasG12D*-induced lung tumorigenesis. (A) Crosses to produce *CCSP-rtTA/ Twist1-tetO7-luc /LSL-KrasG12D* (CT-LSL) mice. (B) Kaplan-Meier tumor free survival of F1 littermates with CT, LSL and CT-LSL genotypes. (C) Lung tumor burden is increased at 9 weeks post-AdCMVCre in CT-LSL versus LSL mice by microCT. Blue arrowheads denote lung tumors. H – heart; and S – spine. (D) H&E CT-LSL lung tumors. (E) IHC phenotyping of CT-LSL lung tumors indicate a type II pneumocyte cell of origin. (F) Ki-67 IHC of LSL versus CT-LSL lung tumors. Low - <5%; Med – 5-25%; and High - >25%.

- Most importantly, we show by inactivating *Twist1* genetically this can activate a latent *Kras*-induced senescence program in autochthonous adenocarcinomas as shown by tumor stasis using small animals imaging and histopathologically by markers for senescence.

Results (cont.)

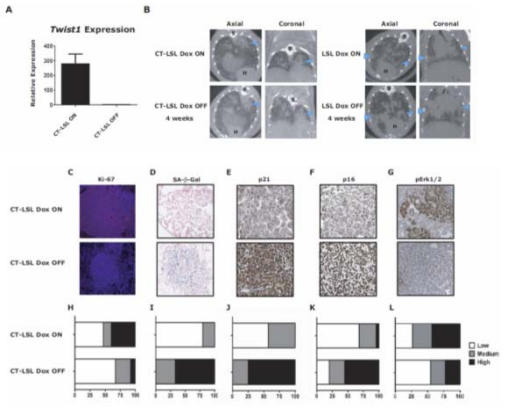

Figure 2. Activation of *Kras*-induced senescence by down regulation of *Twist1* in autochthonous *KrasG12D*/*Twist1*-induced lung tumors. (A) *Twist1* mRNA levels are reduced following doxycycline withdrawal. (B) CT-LSL OFF lung tumors are static following single *Twist1* inactivation by microCT. H – heart; and S – spine. (C-G) CT-LSL OFF lung tumors demonstrate markers consistent with senescence. (C) Reduction in proliferation by Ki-67 IHC, (D) increased lung tumors positive for SA-B-gal staining, increased levels of (E) p21 and (F) p16 by IHC. (G) pErk1/2 levels reduced moderately following *Twist1* inactivation in CT-LSL OFF tumors. (H-L) Quantification of (C-G) .

- Moreover, *TWIST1* was found to be commonly overexpressed in primary human lung cancers.

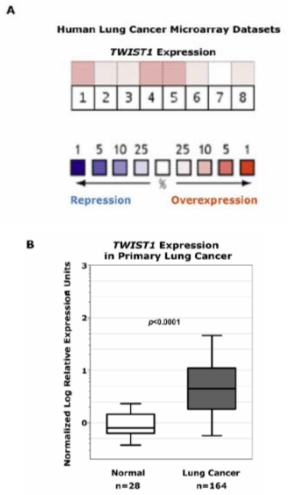

Figure 3. *TWIST1* is overexpressed in human primary lung cancers. (A) Human non-small cell lung cancer samples (n=394) compared against normal lung (n=159) from seven independent microarray datasets for *TWIST1* expression using Oncomine (*TWIST1* is overexpressed, p=0.04). (B) *TWIST1* mRNA is overexpressed in human lung cancer compared to normal lung, p<0.0001 by Mann-Whitney t-test.

Results (cont.)

- *TWIST1* knock down can also activate a latent senescence program in human lung cancer cells *in vitro*.

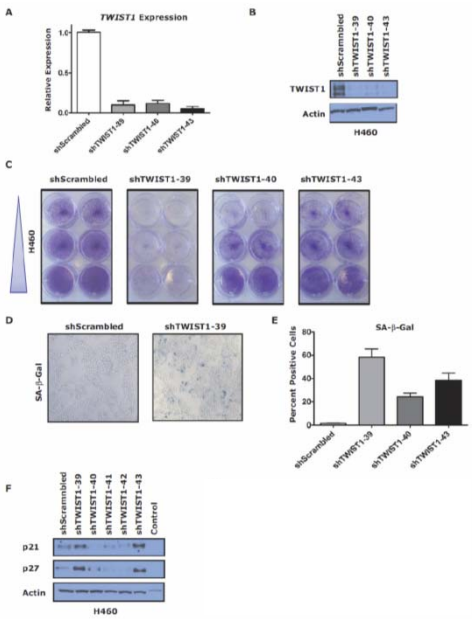

Figure 5. *TWIST1* knockdown activates senescence *in vitro* in human non-small cell lung cancer cells. Knockdown of *TWIST1* mRNA levels results in decreased TWIST1 protein in H460 cells by (A) qPCR and (B) TWIST1 Western blotting (C) Representative duplicates of crystal violet staining of H460 cells following *TWIST1* knockdown. (D) Representative photomicrographs of increased SA-B-gal staining of cells following *TWIST1* knockdown. (E) Quantification of (D). (F) *TWIST1* knockdown in H460 results in the upregulation of some additional markers of senescence, p21 and p27 as shown by Western blotting.

Conclusions

- Our work incorporates highly penetrant spontaneously arising autochthonous murine lung tumors with the ability to simulate targeted therapy *in situ* and analyze the results with traditional molecular techniques as well as non-invasive tumor imaging.
- These model systems serve as more clinically relevant bridge for studies transitioning from the bench to the clinic and can also be used to probe basic questions of OIS.
- Furthermore, our preclinical studies suggest *TWIST1* as a promising therapeutic target for the treatment of human *KRAS* mutant lung cancers.