Gain of function cDNA screen identifies SIK3 activation as a therapeutic strategy for STK11 mutant cancer

Poster # C105

James Tepper, Silvia Fenoglio, Samuel R Meier, Ashley H Choi, Shangtao Liu, Binzhang Shen, Douglas A. Whittington, Yi Yu, Xuewen Pan, Jannik N Andersen, and Teng Teng

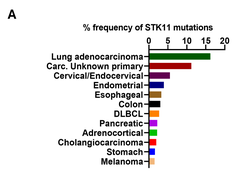
INTRODUCTION

STK11 (also known as LKB1) is a master serine/threonine kinase that regulates a diverse array of cellular functions via phosphorylation of downstream kinases. STK11 is frequently mutated in human cancers, and we hypothesized that reactivation of its downstream effectors could reverse the tumor suppressive role of STK11.

Using pooled gain-of-function cDNA screens of known STK11 substrates in a 3D cell culture format, we identified the constitutive activation of the salt-inducible kinases (SIKs) as the top effector molecules driving the progression of STK11 mutant cancers. Follow-up in vivo minipool screens, focusing on the SIK1/2/3 kinases, confirmed that phosphorylation mimetic mutations, which model constitutive activation of the SIK kinases, produced dropout phenotypes comparable to STK11 re-expression.

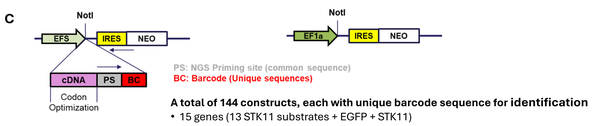
Single gene validation studies further confirmed that expression of a kinase activation mutant form of SIK3 was sufficient to significantly suppress tumor growth of the STK11-mutant A549 xenograft model. In contrast, expression of kinase-dead SIK3 mutant cDNA enhanced tumor growth. Mechanically, tumor RNAseq analysis revealed that genetic activation of SIK3 mimicked the transcriptional effects of STK11 re-expression. Altogether, our functional genomics screen and genetic validation highlight SIK3 activation as a promising therapeutic strategy for STK11-mutant cancers.

1. cDNA LIBRARY DESIGN TO FUNCTIONALIZE DOWNSTREAM EFFECTOR MOLECULES OF STK11



STK11 is frequently mutated in human cancers

STK11 is a master regulator of multiple kinases

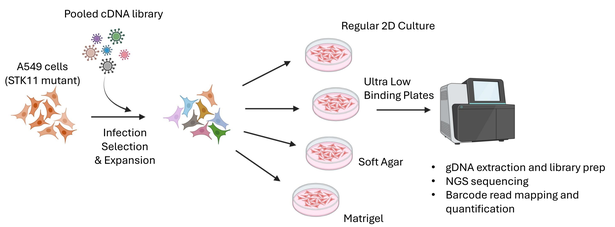


A total of 144 constructs, each with unique barcode sequence for identification

- 15 genes (13 STK11 substrates + EGFP + STK11)
- Both human and mouse cDNA sequences
- Both WT gene and constitutive active and/or catalytic dead mutants
- Two different promoter strengths

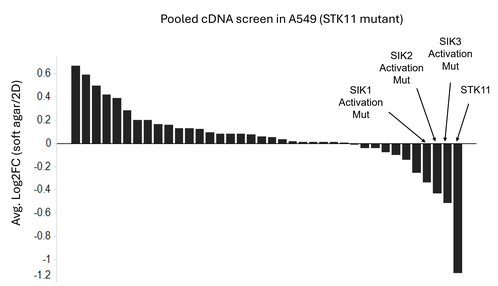
Barcoded cDNA library design and target composition across 144 cDNA constructs

2. DESIGN OF POOLED cDNA LIBRARY SCREEN IN 2D AND 3D FORMAT



Graphic illustration of barcoded cDNA library screen design under different cell culture conditions

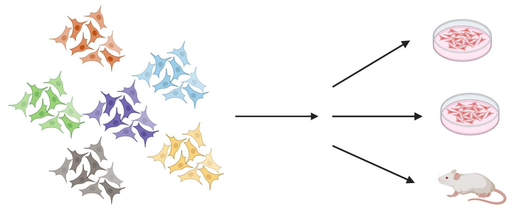
3. SIK1/2/3 ACTIVATION MUTANTS ARE TOP HITS IN 3D SCREEN



Activation mutants of SIK1/2/3 scored as top hits in addition to STK11 expression in 3D-culture

4. IN VIVO VALIDATION SCREEN USING abcDNA MINIPOOL SCHEME

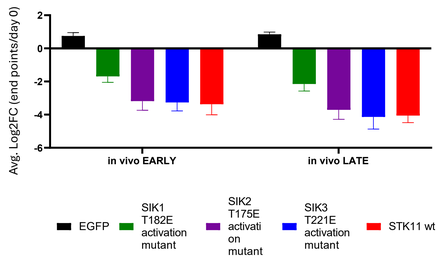
- Individually engineered and expanded A549 cell lines (14 total)



- EGFP as negative control
- WT STK11 as positive control
- WT, constitutive active, kinase dead, and double mutants for SIK1/2/3

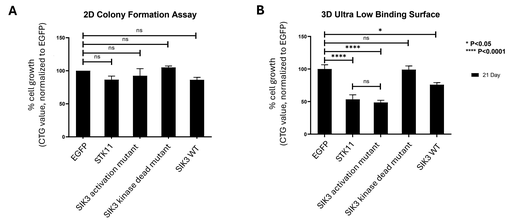
Graphic illustration of validation minipool screen scheme in vitro and in vivo

5. ACTIVATION MUTANTS OF SIK1/2/3 PHENOCOPY STK11 EXPRESSION



Constitutively active SIK mutant cDNAs drop out in vivo similarly to re-expression of STK11

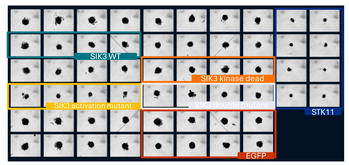
6. CONSTITUTIVELY ACTIVE SIK3 PHENOCOPIES STK11 RE-EXPRESSION IN VITRO



SIK3 activation mutant does not affect A549 cell growth in 2D format

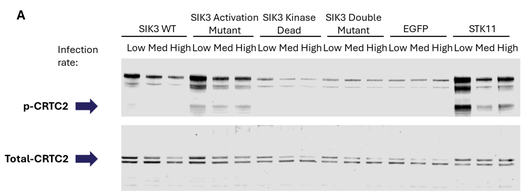
SIK3 activation mutant reduces A549 cell growth in 3D format

C

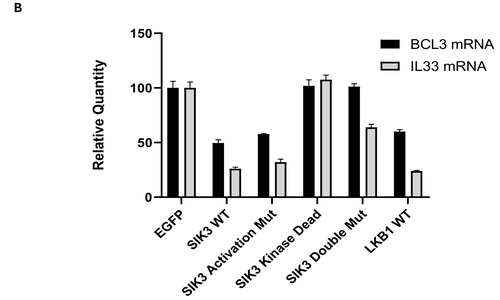


Representative images of SIK3 mutant cell line growth on 3D ultra low binding surface

7. CONSTITUTIVELY ACTIVE SIK3 AFFECTS STK11 DOWNSTREAM PATHWAYS

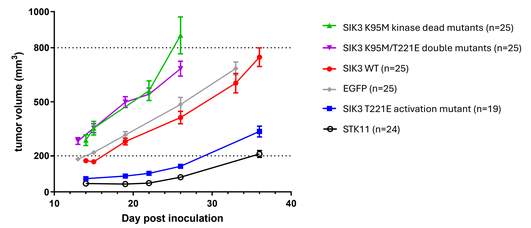


SIK3 activation mutant leads to phosphorylation of CRTC2, mimicking STK11 re-expression



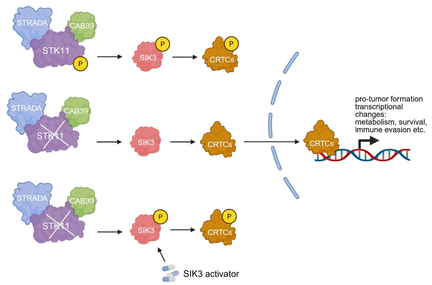
SIK3 activation mutant leads to decreased BCL3 and IL33 expression, mimicking STK11 effects

8. CONSTITUTIVELY ACTIVE SIK3 PHENOCOPIES STK11 RE-EXPRESSION IN VIVO



Expression of SIK3 activation mutant represses tumor growth comparable to STK11 re-expression in the STK11-mutant A549 CDX model

9. SIK3 ACTIVATION IS POTENTIAL THERAPEUTIC STRATEGY TO TARGET STK11 MUTANT CANCER



Graphic illustration of therapeutic hypothesis of SIK activation in STK11 mutant cancer

Summary

- **Barcoded pooled cDNA library screen enables evaluation of STK11 downstream effector molecules in 2D and 3D cultures and in vivo models**
- **SIK activation mutants emerge as top hits mimicking STK11 re-expression**
- **SIK3 activation suppress STK11-mutant cancer cell growth under 3D culture conditions and and in vivo and repress downstream CRTC2 signaling**
- **Kinase-dead SIK3 enhances tumor growth, confirming dependence on kinase activity**
- **SIK3 activation represents a potential strategy to therapeutically reverse the consequences of STK11 tumor suppressor gene loss**

Acknowledgements

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References

- The results shown here are in whole or part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga.
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