Targeting p130Cas- and microtubule-dependent MYC regulation sensitizes KRAS mutant pancreatic cancer to ERK MAPK inhibition

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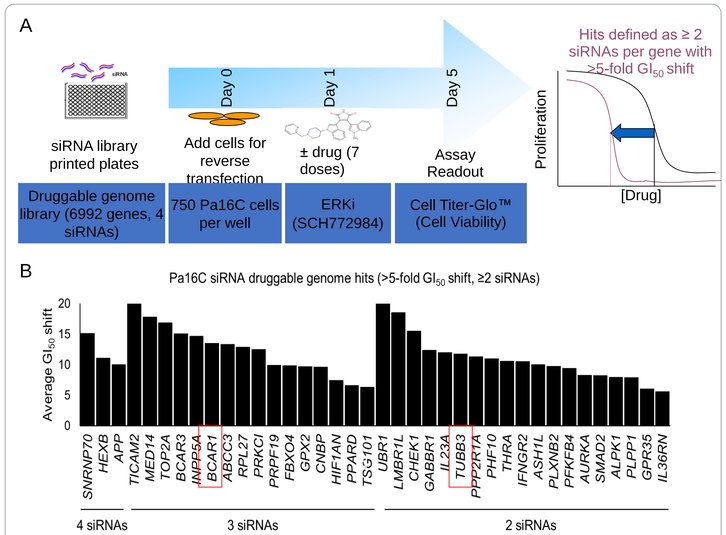
Abstract

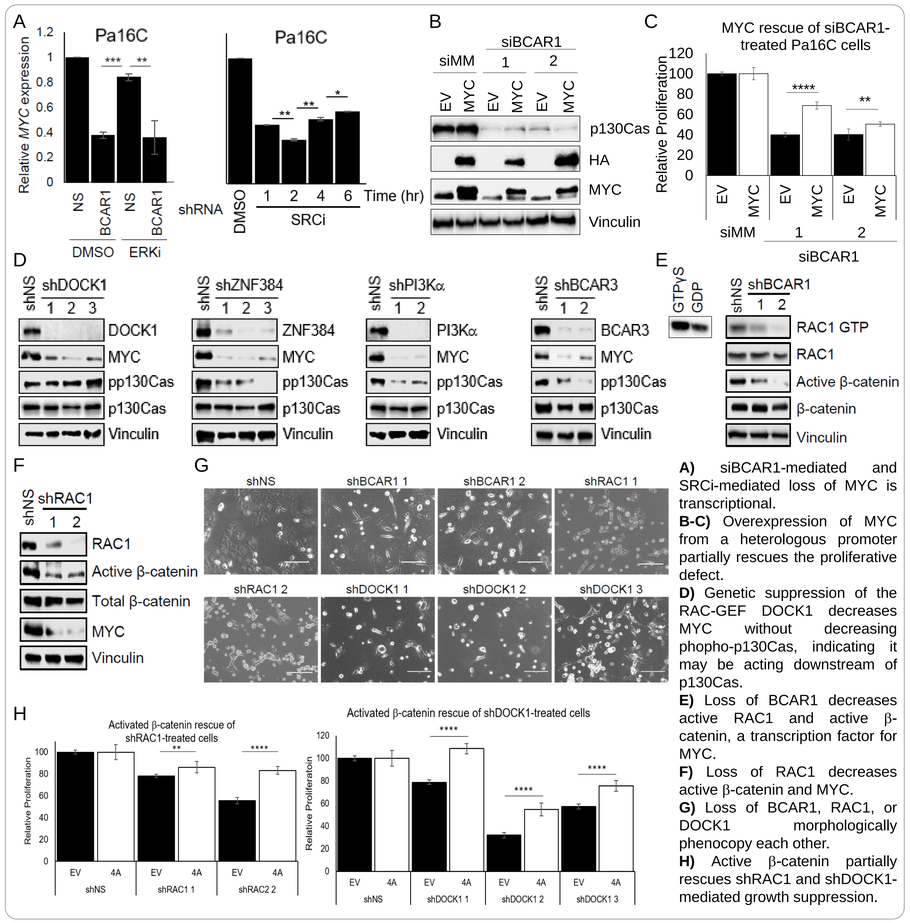
To identify therapeutic targets for *KRAS*-mutant pancreatic cancer, we conducted a druggable genome siRNA screen and determined that suppression of *BCAR1* sensitizes pancreatic cancer cells to ERK inhibition. Integrative analysis of genome-scale CRISPRCas9 screens also identified *BCAR1* as a top synthetic lethal interactor with mutant KRAS. *BCAR1* encodes the SRC substrate p130Cas. We determined that SRC inhibitor-mediated suppression of p130Cas phosphorylation impairs *MYC* transcription through a DOCK1-RAC1-β-catenin dependent mechanism. Additionally, genetic suppression of *TUBB3*, encoding the βIII-tubulin subunit of microtubules, or pharmacological inhibition of microtubule function, decreased levels of MYC protein and potently sensitized pancreatic cancer cells to ERK inhibition. Accordingly, the combination of a dual SRC/tubulin inhibitor with an ERK inhibitor cooperated to reduce MYC protein and to synergistically suppress the growth of *KRAS*-mutant pancreatic cancer. Thus, we demonstrate that mechanistically diverse combinations with ERK inhibition suppress MYC to impair pancreatic cancer proliferation.

siRNA screen identifies *BCAR1* and *TUBB3* as sensitizers to ERKi with SCH772984



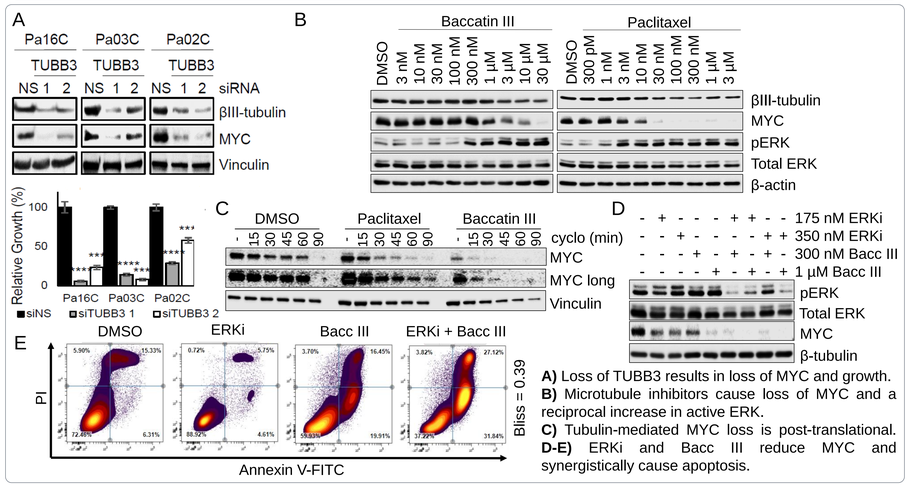
A) Description of siRNA druggable genome screen.
B) Ranked hits stratified first by siRNA hit rate and second by hit strength.
C-D) ERKi and siBCAR1 treatment reduce MYC and synergistically cause apoptosis in PDAC.

BCAR1 transcriptionally regulates MYC levels through a SRC-p130Cas-DOCK1-RAC1-β-catenin pathway



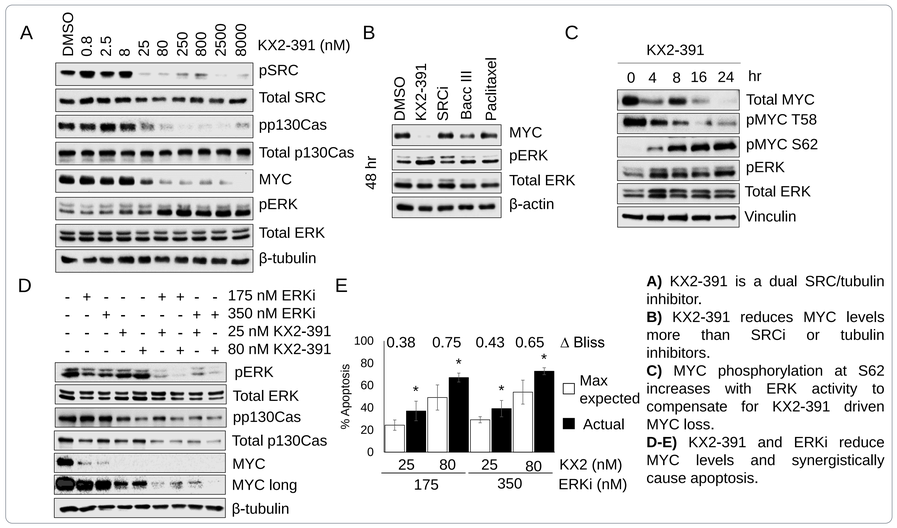
A) siBCAR1-mediated and SRCi-mediated loss of MYC is transcriptional.
B-C) Overexpression of MYC from a heterologous promoter partially rescues the proliferative defect.
D) Genetic suppression of the RAC-GEF DOCK1 decreases MYC without decreasing phopho-p130Cas, indicating it may be acting downstream of p130Cas.
E) Loss of BCAR1 decreases active RAC1 and active β-catenin, a transcription factor for MYC.
F) Loss of RAC1 decreases active β-catenin and MYC.
G) Loss of BCAR1, RAC1, or DOCK1 morphologically phenocopy each other.
H) Active β-catenin partially rescues shRAC1 and shDOCK1-mediated growth suppression.

Tubulin inhibition drives MYC loss and synergistically causes apoptosis when combined with ERKi



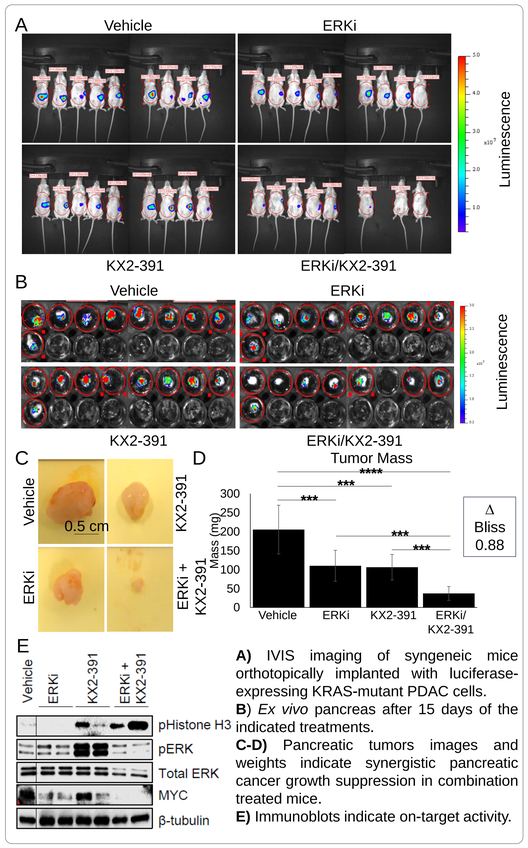
A) Loss of TUBB3 results in loss of MYC and growth.
B) Microtubule inhibitors cause loss of MYC and a reciprocal increase in active ERK.
C) Tubulin-mediated MYC loss is post-translational.
D-E) ERKi and Bacc III reduce MYC and synergistically cause apoptosis.

Combination KX2-391 and ERKi treatment causes synergistic MYC suppression and apoptosis



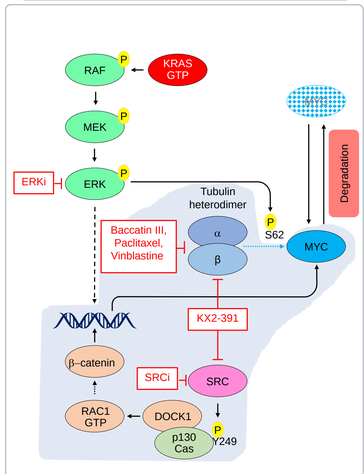
A) KX2-391 is a dual SRC/tubulin inhibitor.
B) KX2-391 reduces MYC levels more than SRCi or tubulin inhibitors.
C) MYC phosphorylation at S62 increases with ERK activity to compensate for KX2-391 driven MYC loss.
D-E) KX2-391 and ERKi reduce MYC levels and synergistically cause apoptosis.

Combination KX2-391 and ERKi treatment synergistically reduces PDAC tumorigenic growth



A) IVIS imaging of syngeneic mice orthotopically implanted with luciferase-expressing KRAS-mutant PDAC cells.
B) *Ex vivo* pancreas after 15 days of the indicated treatments.
C-D) Pancreatic tumors images and weights indicate synergistic pancreatic cancer growth suppression in combination treated mice.
E) Immunoblots indicate on-target activity.

Model



-ERKi causes MYC protein degradation and reduces MYC transcript.
-SRCi/p130Cas suppression reduces MYC transcript through a SRC->p130Cas->DOCK1->RAC1-β-catenin pathway.
-Tubulin inhibition decreases MYC protein half-life.
-KX2-391 combines SRCi and tubulin inhibitor activities.
-Combining ERKi with KX2-391 synergistically kills PDAC cells in cell culture and *in vivo*.

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