Combination therapies with CDK4/6 inhibitors to treat *KRAS*-mutant pancreatic cancer

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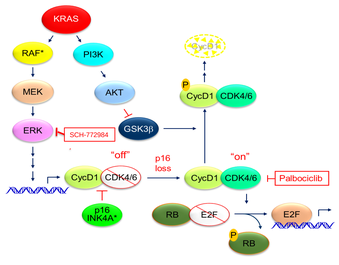
Abstract



Pancreatic ductal adenocarcinoma (PDAC) patients have a dismal five-year survival rate in the advanced metastatic setting; therefore, the development of targeted therapies is a significant unmet clinical need. The two most frequent genetic events in PDAC (mutational activation of KRAS and loss of the tumor suppressor *CDKN2A*) converge on activation of the kinases CDK4 and CDK6, which promote G1 cell cycle progression (A). We found that CDK4/6 inhibitors (CDK4/6i), which are pharmacologic mimics of p16^{INK4A} function, elicited single-agent activity in a subset of KRAS-mutant PDAC cell lines. However, applying Reverse Phase Protein Array (RPPA)-based pathway activation mapping analyses, we observed widespread CDK4/6i-induced compensatory signaling activity/expression changes leading to increased ERK-MAPK signaling. Concurrent treatment with the ERK1/2 inhibitor (ERKi) SCH772984 reversed this phenotype, synergistically reduced cell growth, and increased both apoptosis and G1 arrest in PDX cell lines and organoid models by a well-defined mechanism.

Next, we used a CRISPR/Cas9 druggable genome library loss-of-function screen to identify genes that modulated sensitivity to CDK4/6i. We identified a functionally diverse array of genes that enhanced growth suppression in combination with CDK4/6i, centered around distinct signaling nodes including cell cycle regulation and mitosis, PI3K-AKT-mTOR signaling, SRC family kinase signaling, cell metabolism and biosynthesis, and DNA damage and repair pathways, suggesting ways to overcome *de novo* or acquired CDK4/6 inhibitor resistance in the clinic. Identified synergistic combinations were validated using siRNA and small-molecule inhibitor-based approaches. Our observations suggest that CDK4/6 inhibitors alone, or in novel combinations, may benefit PDAC patients clinically.

PDAC cells resist CDK4/6 inhibition by activating ERK-MAPK, PI3K, and anti-apoptotic pathways

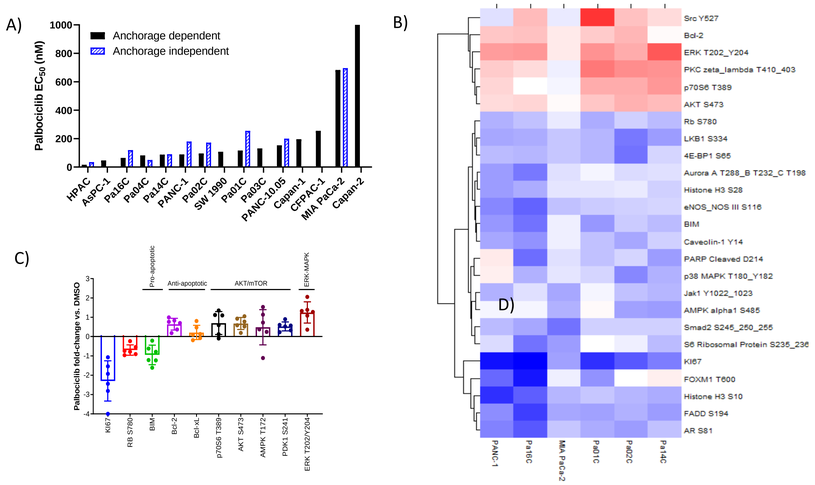


Figure 1: **(A)** EC_{50} for CDK4/6i (palbociclib) for PDAC cell lines in cell culture. **(B)** Reverse-phase protein array (RPPA) for palbociclib. Indicated proteins increase (red) or decrease (blue) expression with palbociclib compared to control. **(C)** Mean and distribution of indicated proteins and signaling pathways from (B). Largest compensatory change is increased activating phosphorylation of ERK.

CDK4/6i synergizes with ERKi in cell lines and PDAC organoids

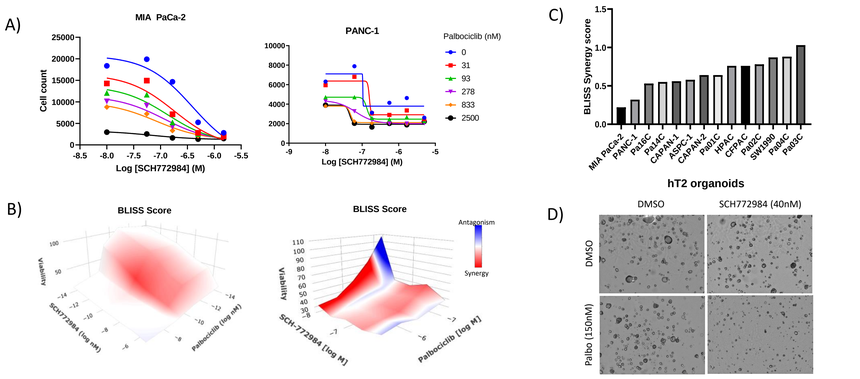


Figure 2 (A) Combined CDK4/6i (palbociclib) with ERKi (SCH772984) at indicated concentrations causes a strong loss of cell proliferation over 5 days. **(B)** BLISS synergy calculated from (A) mapped over the viability dose-response curve. Red = synergy, blue = antagonism. **(C)** Peak synergy scores for CDK4/6i + ERKi across a PDAC cell line panel. **(D)** Effect of CDK4/6i + ERKi on PDAC organoids ht2 (pictured). This combination is synergistic across a full dose-response matrix. Similar results were obtained from 7 additional PDAC organoids (data not shown).

Combined CDK4/6i and ERKi inhibition cooperatively induce apoptosis and enhances anti-proliferative signaling changes

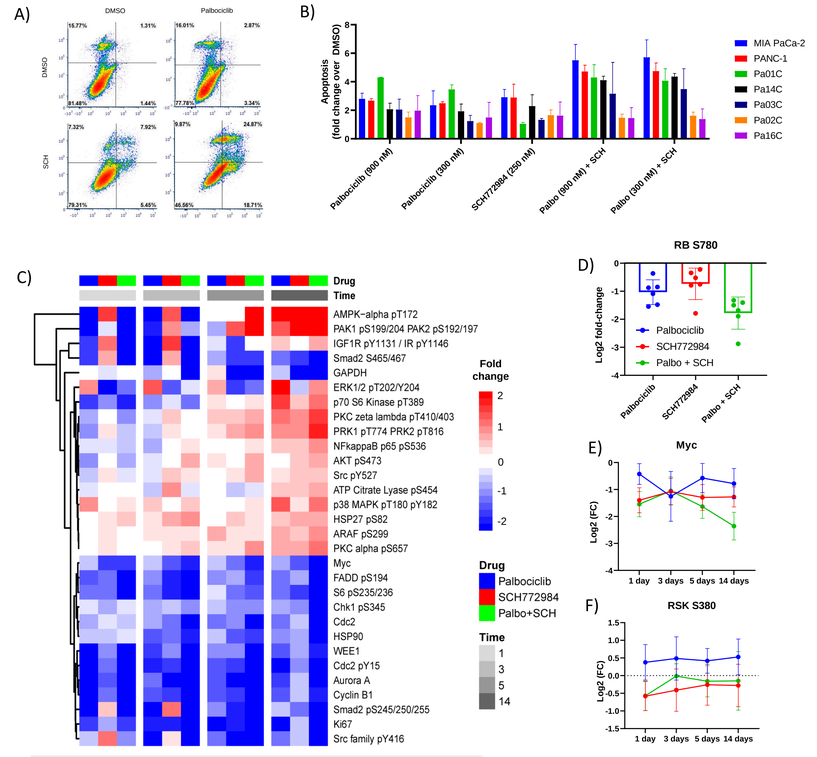


Figure 3 (A) CDK4/6i + ERKi induce apoptosis (FITC-Annexin V/PI staining, flow cytometry, MIA PaCa-2 cells exemplified). **(B)** Quantified apoptosis for drugs alone or in combination across multiple PDAC lines. **(C)** RPPA for CDK4/6i, ERKi, or the combination after 1, 3, 5, or 14 day continuous treatment. The median protein fold change (red=up, blue=down) is shown from six PDAC cell lines. **(D)** Log2 fold-change for RB (S780) phosphorylation after 14 days treatment. **(E-F)** Log2 fold-change over the 14 day timecourse for palbociclib, SCH772984, or the combination for RSK (S380 phosphorylation, **E**) and MYC **(F)**.

Loss-of-function CRISPR/Cas9 screen identifies novel potent combinations with CDK4/6i

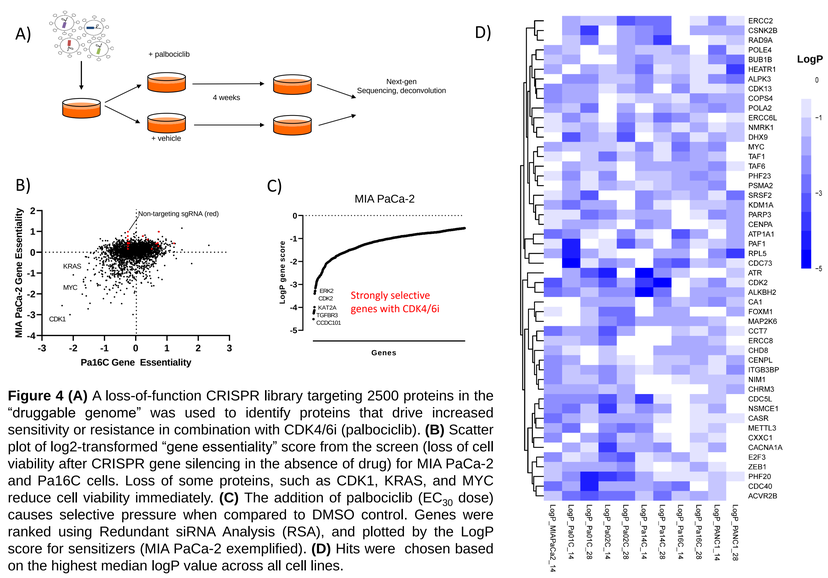


Figure 4 (A) A loss-of-function CRISPR library targeting 2500 proteins in the "druggable genome" was used to identify proteins that drive increased sensitivity or resistance in combination with CDK4/6i (palbociclib). **(B)** Scatter plot of log2-transformed "gene essentiality" score from the screen (loss of cell viability after CRISPR gene silencing in the absence of drug) for MIA PaCa-2 and Pa16C cells. Loss of some proteins, such as CDK1, KRAS, and MYC reduce cell viability immediately. **(C)** The addition of palbociclib (EC_{30} dose) causes selective pressure when compared to DMSO control. Genes were ranked using Redundant siRNA Analysis (RSA), and plotted by the LogP score for sensitizers (MIA PaCa-2 exemplified). **(D)** Hits were chosen based on the highest median logP value across all cell lines.

Diverse CRISPR screen hits validated by siRNA counter-screen to cause synergistic loss of viability with CDK4/6i

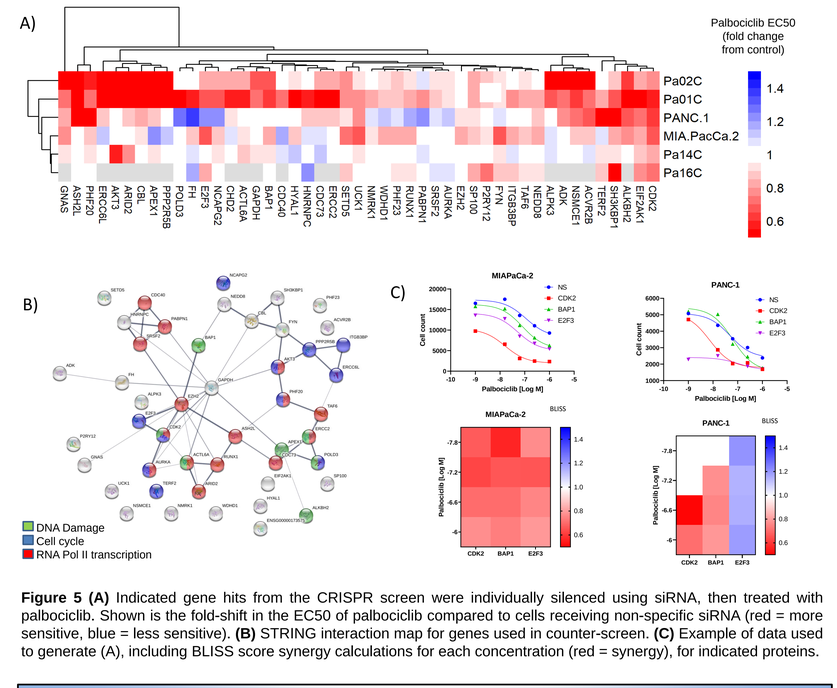


Figure 5 (A) Indicated gene hits from the CRISPR screen were individually silenced using siRNA, then treated with palbociclib. Shown is the fold-shift in the EC50 of palbociclib compared to cells receiving non-specific siRNA (red = more sensitive, blue = less sensitive). **(B)** STRING interaction map for genes used in counter-screen. **(C)** Example of data used to generate (A), including BLISS score synergy calculations for each concentration (red = synergy), for indicated proteins.

CRISPR screen hit CDK2 potently induces apoptosis in combination with CDK4/6i or ERKi

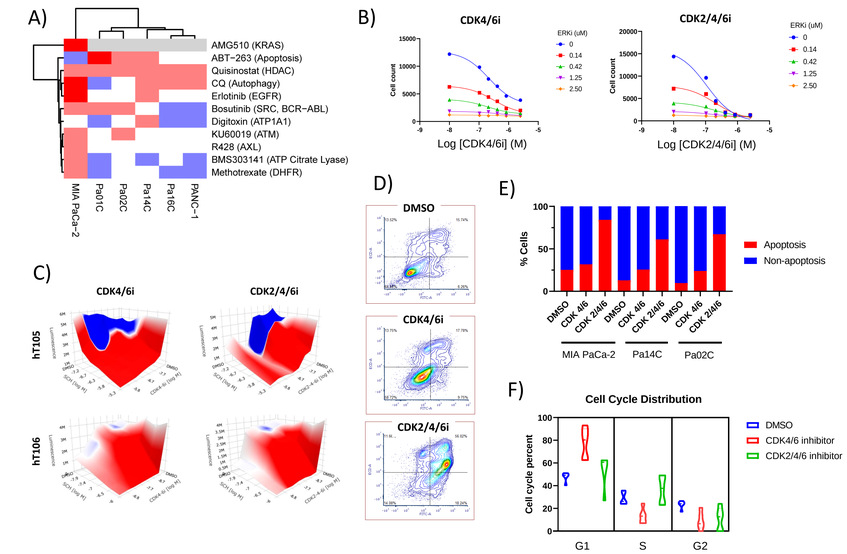


Figure 6 (A) Indicated drugs were tested against the panel of PDAC lines in the CRISPR screen; on average, drugs were synergistic (red), antagonistic (blue), or additive (white); G12C inhibitor was only tested in KRAS G12C lines. **(B)** Viability dose-response curves for a combination CDK2/4/6 inhibitor (PF-06873600) with ERKi. This combination is mildly synergistic (not shown). **(C)** Bliss synergy scores for the CDK2/4/6i + ERKi combination in organoids. Shown is synergy mapped over the dose-response curves (red = synergism, blue = antagonism). **(D)** Apoptosis for DMSO, CDK4/6i, and CDK2/4/6i (annexin V/PI staining, flow cytometry, MIA PaCa-2 exemplified). **(E)** Quantification of (D) and two additional PDAC lines. **(F)** Average cell cycle distribution for six PDAC cell lines after treatments for 72 hours.

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