Life without KRAS: profiling the KRAS-dependent kinome to identify novel therapeutic vulnerabilities in pancreatic cancer

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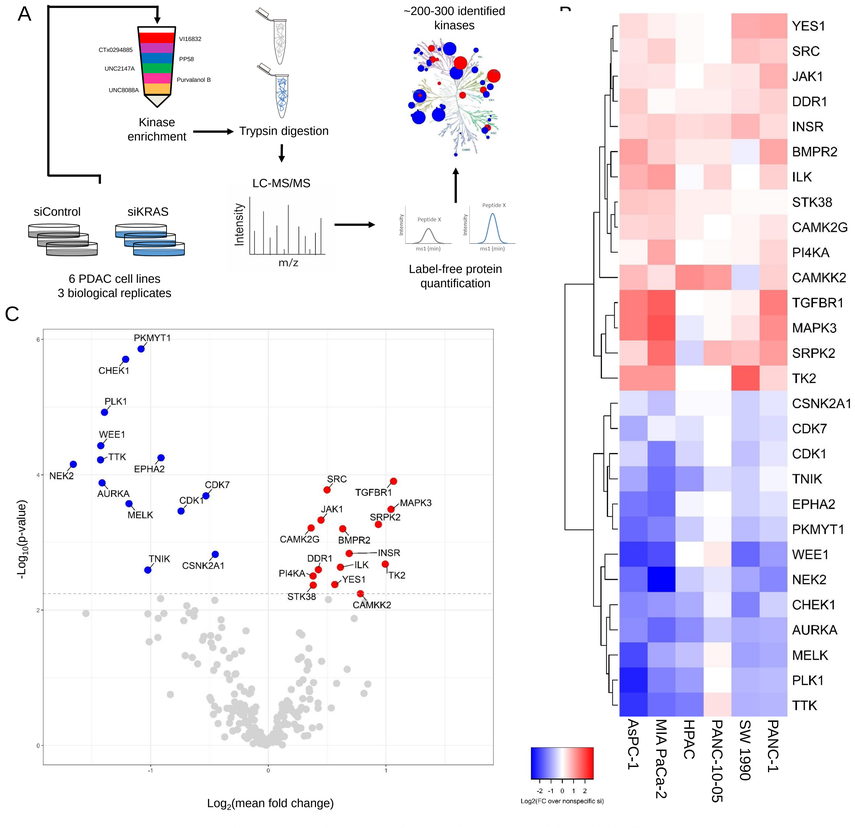
Abstract

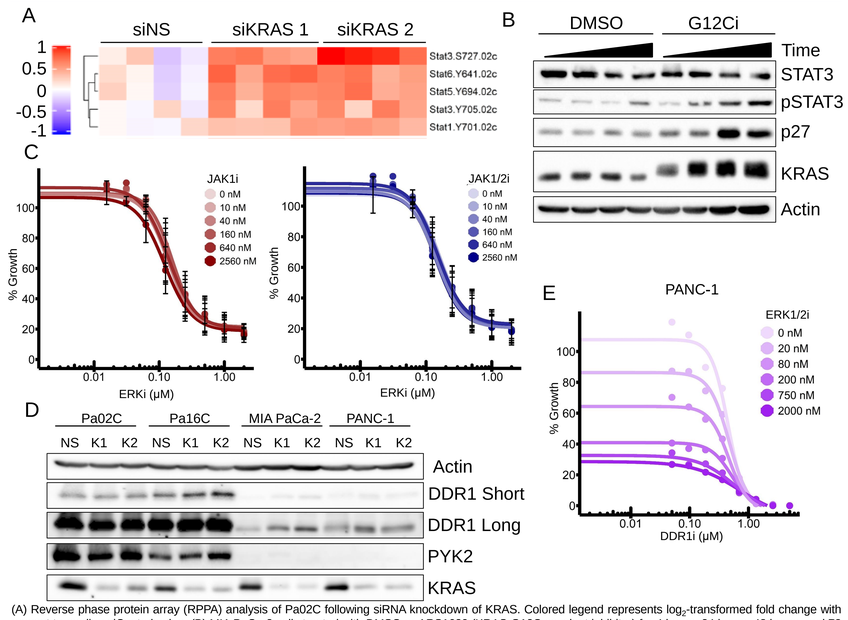
Oncogenic KRAS mediates signaling activities through a network of kinases (the kinome). To broadly characterize the KRAS-dependent kinome in pancreatic ductal adenocarcinoma (PDAC), we applied the Multiplexed Inhibitor Bead/Mass Spectroscopy (MIB/MS) chemical proteomics strategy to monitor kinome-wide changes in activity/expression of >200 kinases following 72 hours of siRNA-mediated knockdown of KRAS. We hypothesized that upregulated kinases may act as compensatory activities to overcome loss of KRAS function, whereas downregulated kinases may contribute to KRAS driver function. We identified 28 kinases that were significantly upregulated/downregulated in a panel of six PDAC cell lines. KRAS suppression-induced upregulated kinases included JAK1, DDR1, ERK1 and SRC family members YES1 and SRC. Reverse phase protein array (RPPA) pathway activation analysis confirmed activation of STAT transcription factors, reactivation of ERK1/2 signaling, and increased SRC signaling. Pharmacologic inhibition of SRC, but not JAK, suppressed PDAC growth, supporting SRC as a compensatory activity. Downregulated kinases included those involved in both the DNA damage response and cell cycle regulation, such as WEE1, AURKA, CHK1, CDK1, and PLK1. Western blotting verified loss of PLK1, WEE1, CHK1, and CDK1 as concurrent with loss of KRAS protein. To assess whether inhibition of these downregulated kinases conferred sensitivity to the ERK inhibitor SCH772984 (ERKi), we used the clinical candidate WEE1 inhibitor adavosertib (WEE1i). The combination of WEE1i and ERKi synergistically suppressed proliferation of PDAC cell lines and patient-derived organoids, and increased apoptosis. Together, our data suggest that concurrent inhibition of the DNA damage response pathway and the ERK MAPK cascade may be an effective treatment for KRAS-mutant pancreatic cancer.

Multiplexed inhibitory beads with mass spectrometry (MIB-MS) reveals KRAS-dependent kinase changes



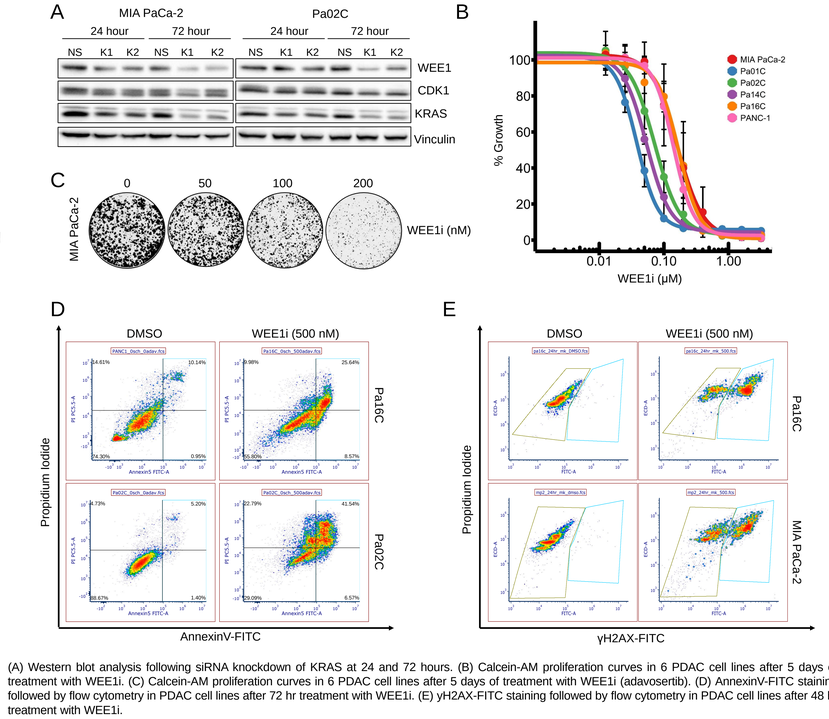
(A) MIB-MS method performed in 6 PDAC cell lines [SW 1990, MIA PaCa-2, PANC-1, HPAC, AsPC-1, PANC-10-05]. (B) Volcano plot showing results of significance testing after ANOVA [y-axis] with the log-transformed mean fold change in siKRAS over siControl. Significant kinases are colored red [increased] or blue [decreased] if p-value is significant following Benjamini-Hochberg correction. (C) Unsupervised clustering of log-transformed fold change values for significant kinases following ANOVA analysis.

DDR1, but not JAK1, is a therapeutic vulnerability in KRAS-driven PDAC



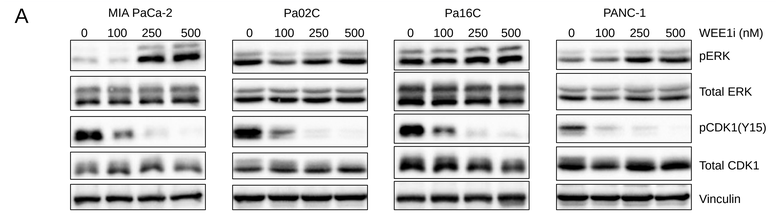
(A) Reverse phase protein array (RPPA) analysis of Pa02C following siRNA knockdown of KRAS. Colored legend represents $\log_2$-transformed fold change with respect to median siControl value. (B) MIA PaCa-2 cells treated with DMSO or ARS1620 (KRAS-G12C covalent inhibitor) for 4 hours, 24 hours, 48 hours, and 72 hours.(C) 2D proliferation analysis of MIA PaCa-2 cells (5 day) following treatment with Filgotinib (JAK1i) or Ruxolitinib (JAK1/2i) + SCH772984 (ERKi). (D) Western blot analysis (72 hour) of PDAC cell lines following siControl or siKRAS knockdown. (E) 2D proliferation analysis (5 day) following treatment with 7rh (DDR1i) + SCH772984 (ERKi).

Pharmacological inhibition of WEE1 induces apoptosis and γH2AX accumulation in PDAC



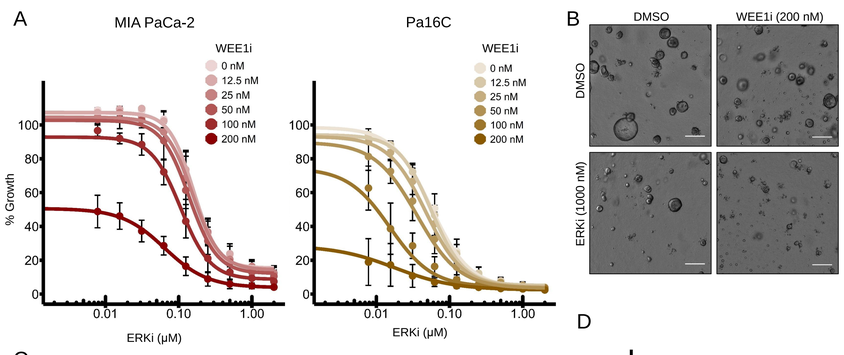
(A) Western blot analysis following siRNA knockdown of KRAS at 24 and 72 hours. (B) Calcein-AM proliferation curves in 6 PDAC cell lines after 5 days of treatment with WEE1i. (C) Calcein-AM proliferation curves in 6 PDAC cell lines after 5 days of treatment with WEE1i (adavosertib). (D) AnnexinV-FITC staining followed by flow cytometry in PDAC cell lines after 72 hr treatment with WEE1i. (E) γH2AX-FITC staining followed by flow cytometry in PDAC cell lines after 48 hr treatment with WEE1i.

Single-agent inhibition of WEE1 causes rebound ERK phosphorylation



(A) Western blots in 4 PDAC cell lines after 24 hour treatment with variable concentrations WEE1i [adavosertib].

Combination WEE1i/ERKi causes synergistic inhibition of proliferation in 2D and 3D organoid cultures



(A) Calcein-AM proliferation curves in MIA PaCa-2 cells [PDAC] after 5 days of growth in combinations of ERKi [SCH772984] with WEE1i [adavosertib]. (B) PDAC organoids [hM1A] seeded for 3 days in Matrigel and organoid maintenance factors followed by 10 days inhibitor treatment with ERKi [SCH772984] in combination with WEE1i [adavosertib]. Scale bar is equivalent to 200 μm. (C) BLISS synergy analysis following Annexin V staining and flow cytometry analysis of apoptotic cell populations. Inhibitors used include WEE1i (adavosertib) and ERKi (SCH772984). (D) Quantification of CellTiter-Glo 3D cell viability assay in organoids (n=4).

Conclusions

- Genetic knockdown of KRAS alters expression/activity of multiple kinases, including JAK1, DDR1, and WEE1.
- Genetic knockdown of KRAS causes WEE1 proteasomal degradation as well as decreased transcription of both CHK1 and WEE1.
- Pharmacologic inhibition of WEE1 effectively reduces 2D growth as well as induction of apoptosis and γH2AX accumulation.
- Combined inhibition of WEE1/ERK1/2 synergistically reduced growth of PDAC cells in both 2D and in organoids

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